British Journal of Nutrition (2012), **108**, 1034–1041
doi:10.1017/S0007114511006192
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Effects of ethanol consumption on the B-group vitamin contents of liver, blood and urine in rats

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(Submitted 11 July 2011 – Final revision received 14 October 2011 – Accepted 17 October 2011 – First published online 15 December 2011)

Abstract

Several studies have shown that blood vitamin levels are lower in alcoholic patients than in control subjects. Acute ethanol exposure enhances the release of vitamins from liver cells *in vitro*. The aim of the present study is to confirm the effects of ethanol consumption on vitamin contents *in vivo*. We compared the contents of B-group vitamins in the liver, blood and urine between ethanol-fed and control rats fed a diet containing a sufficient- and low-vitamin mixture. The experimental rats were fed a 15 % ethanol solution freely for 28 d, and then 24 h urine samples were collected, after which the animals were killed. The B-group vitamin contents in the liver, blood and urine were measured. No differences in liver, blood and urine contents were observed between the control and ethanol-fed rats fed a diet containing a sufficient-vitamin mixture. On the contrary, in rats fed a diet containing a low-vitamin mixture, consumption of ethanol caused a decrease in the contents of vitamins B_1, B_2 and pantothenic acid in the liver; however, the contents of the other vitamins did not decrease. In the blood, the contents of vitamins B_1, B_2, B_6 and pantothenic acid were lower in the ethanol-fed rats than in the controls. Urinary excretion of the B-group vitamins, except for niacin, was lower in the ethanol-fed rats. These results show that ethanol consumption affects the absorption, distribution and excretion of each of the vitamins in rats fed a diet containing a low-vitamin mixture.

Key words: Vitamins: Urine: Blood: Liver: Ethanol

Numerous studies have shown that vitamin status of alcoholic patients differs from non-drinking subjects[1–7], and the majority have shown that blood vitamin levels are lower in alcoholic patients than in controls[8–10]. In addition, several reports have suggested that chronic alcohol feeding may lead to a significant inhibition of carrier-mediated thiamin[11,12] and folate[13–19] uptake in the intestine and kidney. This phenomenon is observed only in alcoholic patients who drink ethanol chronically. On the contrary, a reduction in circulating levels of B-complex vitamins often occurred without clinical evidence of hypovitaminosis[20]. Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol caused the release of all B-vitamins except biotin from the liver stores. Israel & Smith[22] reported that acute ethanol feeding to rats inhibited the conversion of pantothenic acid to CoA. These studies in animal models suggested that acute ethanol intake results in an increased hepatic release of vitamins and an impaired utilisation, which means increased levels of free forms of vitamins in the liver which can in turn permeate the cell membranes[21,22]. This might lead to increases in blood vitamin contents and in urinary excretion. Although there are many reports concerning the effects of ethanol on the absorption and metabolism of vitamins, the conclusion concerning the controversy remains elusive. The reason might be that there is no study regarding the simultaneous measurement of vitamin contents of liver (as a biomarker of the storage amount of vitamins), blood (as a biomarker of the circulation amount of vitamins) and urine (as a biomarker of the reabsorption ability of kidney and an extra amount of vitamins).

In the present study, we examined the effects of ethanol consumption on the contents of B-group vitamins of the liver, blood and urine in rats fed two kinds of diets containing either a sufficient- or a low-vitamin mixture.

Materials and methods

Chemicals

Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries. Maize oil was purchased from Ajinomoto. Gelatinised maize starch, a mineral mixture (AIN-93G mineral mixture)[23] and a vitamin mixture (nicotinic acid-free AIN-93 vitamin mixture containing

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Abbreviations: 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide.

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British Journal of Nutrition

https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press

25 % choline bitartrate)[23] were obtained from Oriental Yeast Company, Limited.

Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl; molecular weight 337·27), riboflavin ($C_{17}H_{20}N_4O_6$; 376·37), pyridoxine hydrochloride ($C_8H_{11}NO_3$-HCl; 205·63), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$; 1355·40), nicotinamide ($C_6H_6N_2O$; 122·13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca; 476·54), folic acid ($C_{19}H_{19}N_7O_6$; 441·40) and D(+)-biotin ($C_{10}H_{16}N_2O_3S$; 244·31) were purchased from Wako Pure Chemical Industries. 4-Pyridoxic acid ($C_8H_9NO_4$ = 183·16) was made by ICN Pharmaceuticals and obtained through Wako Pure Chemical Industries.

N^1-Methylnicotinamide chloride ($C_7H_9N_2O$-HCl; 159·61) was purchased from Tokyo Kasei Kogyo. N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$ 152·15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$ 152·15) were synthesised by the methods of Pullman & Colowick[24] and Shibata *et al.*[25], respectively. All other chemicals used were of highest purity available from commercial sources.

Animals and treatment

The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. The animals were maintained under controlled temperature (22°C), 60 % humidity and light conditions (12 h light–12 h dark cycle).

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a sufficient-vitamin mixture (Expt 1)

Male Wistar rats (3 weeks old) obtained from CLEA Japan were fed freely with a conventional purified diet, consisting of 20 % vitamin-free milk casein, 0·2 % L-methionine, 46·9 % gelatinised maize starch, 23·4 % sucrose, 5 % maize oil, 3·5 % AIN-93-G mineral mixture[14] and 1 % AIN-93 vitamin mixture[14] containing chorine bitartrate, but without nicotinic acid, to acclimatise for 7 d. Nicotinic acid had not been added to this diet because it is supplied enough from tryptophan in casein[26], and a dietary fibre-free diet was used because it is a tradition not to use dietary fibre in our laboratory which is not essential for normal growth[27].

The rats were divided into two groups (*n* 5 each). Group 1 was fed with a diet containing the 1 % vitamin mixture (a sufficient-vitamin diet) and allowed to drink water for 28 d. Group 2 was fed with a diet containing the 1% vitamin mixture (a sufficient-vitamin diet) and forced to drink a 15 % ethanol solution instead of water for 28 d. The 24 h urine samples were collected in amber bottles containing 1 ml of 1 M-HCl at 09.00–09.00 hours of the last day and were stored at −25°C until required. The rats were killed at about 09.00 hours; blood was collected and tissues were taken to measure the weights and the contents of B-group vitamins in the liver, blood and urine. Liver samples were preserved at −25°C until required.

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a low-vitamin mixture (Expt 2)

A preliminary experiment revealed that the body-weight gain of young rats was the same when fed a diet containing the 1 % AIN-93 vitamin mixture and the 0·3 % AIN-93 vitamin mixture, whereas the body-weight gain was lower in rats fed a diet containing the 0·2 % AIN-93 vitamin mixture than in those fed a diet containing the 1 or 0·3 % diets. Thus, we determined tentatively whether the diet containing the 0·3 % AIN-93 vitamin mixture could supply a minimum amount of vitamins for the growing rats.

Male Wistar rats (3 weeks old) obtained from CLEA Japan were fed freely with the conventional purified diet (mentioned above) to acclimatise for 7 d. The rats were then divided into two groups (*n* 5 each). Group 1 was fed a diet containing the 0·3 % vitamin mixture and allowed to drink water for 28 d. Group 2 was fed a diet containing the 0·3 % vitamin mixture and forced to drink a 15 % ethanol solution instead of water for 28 d. The 24 h urine samples and tissues were collected. Levels of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase were measured at Mitsubishi Chemical Medience (Tokyo, Japan).

Measurement of B-group vitamins in urine and blood

Preparation and measurement of the extracts of the B-group vitamins from the urine and blood are described as follows[28].

Vitamin B_1

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 5 % ice-cold TCA and homogenised with a Digital Homogenizer Hom (Iuchi). The acidified homogenate was centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatant was retained and used for the measurement of vitamin B_1[29].

Vitamin B_2

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) and homogenised with a Teflon/glass homogeniser (Nikko Hansen). To 0·1 ml of the homogenate, 0·44 ml of water and 0·26 ml of 0·5 M-H_2SO_4 were added and then kept at 80°C for 15 min. After cooling, 0·2 ml of 10 % TCA were added and centrifuged at 10 000 ***g*** for 3 min at 4°C. From the supernatant obtained, 0·2 ml was withdrawn and added to 0·2 ml of 1 M-NaOH. The alkalised mixture was irradiated with a fluorescent lamp for 30 min and then 0·02 ml of glacial acetic acid were added to the mixture. The neutralised mixture was passed though a 0·45 μm microfilter and the filtrate was directly injected into the HPLC system for measuring lumiflavin[30].

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https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press

Vitamin B_6

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to 90 ml of 55 mM-HCl and homogenised with a Waring blender. The homogenate was autoclaved at 121°C for 3 h. After cooling, the mixture was adjusted to pH 5·0 with 1 M-NaOH and then made up to 100 ml with water. The solution was filtered with qualitative filter no. 2 (ADVANTEC MFS, Inc.). The filtrate was used for measuring vitamin B_6 as described previously[31].

Vitamin B_{12}

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to 2·5 ml of 0·57 M-acetic acid–sodium acetate buffer (pH 4·5) plus 5 ml of water and 0·1 ml of 0·05 % potassium cyanide (KCN). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was then put into a boiling water-bath for 5 min. After cooling, 0·15 ml of 10 % metaphosphoric acid were added and made up to 10 ml with water. The solution was filtered with qualitative filter no. 2 (ADVANTEC MFS, Inc.). The filtrate was used for measuring vitamin B_{12} as described previously[32].

Nicotinamide

Frozen liver samples, about 0·6 g, were thawed, minced, and then added to five volumes of 0·1 g/ml isonicotinamide. The suspension was homogenised with a Teflon/glass homogeniser. The homogenate (1 ml) was withdrawn and added to 4 ml of water, and then autoclaved at 121°C for 10 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained and the precipitated materials were extracted again with 5 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring nicotinamide as described previously[25].

Pantothenic acid

Frozen liver samples, about 0·2 g, were thawed, minced, and then added to ten volumes of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was incubated at 37°C overnight to convert free pantothenic acid from the bound type of pantothenate compounds. The reaction was stopped by putting it into a boiling water-bath for 5 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained and the precipitated materials were extracted again with 2 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring pantothenic acid as described previously[33].

Folate

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 0·1 M-KH_2PO_4–K_2HPO_4 buffer (pH 6·1). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was autoclaved at 121°C for 5 min. After cooling, 2·5 ml of pronase (5 mg/ml; Pronase MS; Kaken Pharmaceutical Company, Limited) were added and then incubated at 37°C for 3 h. The reaction was stopped by putting it into a boiling water-bath for 10 min. After cooling, 0·5 ml of conjugase (extract from porcine kidney acetone powder, Type II; Sigma-Aldrich) were added and incubated at 37°C overnight. The reaction was stopped by putting it into a boiling water-bath for 10 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained, and the precipitated materials were extracted again with 3 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring folate as described previously[34]. The conjugase solution was made as follows: 60 ml of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) were added to 20 g porcine kidney acetone powder and stirred for 30 min at 4°C. The suspension was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was dialysed against a large amount of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) to remove endogenous folate of the kidney acetone powder. The dialysed conjugase solution was used.

Biotin

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to two volumes of 2·25 M-H_2SO_4 and then homogenised with a Waring blender. The suspension was hydrolysed by autoclaving for 1 h at 121°C. After cooling, the suspension was centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatant was used for measuring biotin[35].

Analyses

The measurements of the B-group vitamins except for vitamin B_6 were described previously[19]. The urinary excretion of 4-pyridoxic acid, a catabolite of vitamin B_6, was measured according to the method of Gregory & Kirk[36].

Statistical analysis

Mean values between the treatment groups were compared using the Mann–Whitney U two-tailed t test. $P<0·05$ was considered to be statistically significant. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software).

Results

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a sufficient-vitamin mixture (Expt 1)

There were no differences in body-weight gain and liver weights between the groups. No differences in the levels of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, nicotinamide, pantothenic acid, folate and biotin were observed in the liver

British Journal of Nutrition

https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press

and blood. Although the 24 h urinary excretion of some of the vitamins was slightly lower in the ethanol-treated group than in the control, the differences were not significant (data not shown). Thus, ethanol consumption did not affect the B-group vitamin contents in the liver, blood and urine when the rats were fed a diet containing sufficient amounts of the vitamins.

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a low-vitamin mixture (Expt 2)

As shown in Table 1, body-weight gain, food intake and liver weights were lower in the ethanol-fed group than in the controls. The overall food intake was lower in the ethanol-fed group than in the controls, but energy intake was almost the same because of ethanol intake.

The effects of ethanol consumption on the activities of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase in plasma are shown in Table 2. No significant effects of ethanol consumption were observed for these indices of liver function.

The effects of ethanol consumption on the B-group vitamin contents of the liver are shown in Table 3. The contents of the vitamins in liver are measured as storage amounts of the vitamins, thus are expressed as mol/liver. The contents of vitamin B_1, vitamin B_2 and pantothenic acid were lower in the ethanol-fed group than in the controls, whereas the contents of vitamin B_6, vitamin B_{12}, nicotinamide, folate and biotin were not significantly different.

The effects of ethanol consumption on the B-group vitamin contents of the blood are shown in Table 4. The contents of vitamin B_1, vitamin B_2, vitamin B_6 and pantothenic acid were lower in the ethanol-fed group than in the controls, whereas the contents of vitamin B_{12}, nicotinamide, folate and biotin were not significantly different.

The effects of ethanol consumption on the 24 h urinary excretion of the B-group vitamins are shown in Table 5. The excretion of vitamin B_1, vitamin B_2, 4-pyridoxic acid (a catabolite of vitamin B_6), vitamin B_{12}, pantothenic acid, folate and biotin was lower in the ethanol-fed group than in the controls, whereas the contents of nicotinamide (sum of the contents of nicotinamide and its catabolites such as N^1-methylnicotinamide, 2-Py and 4-Py) were not significantly different.

Food intake was different in the two groups, so that urinary excretion ratios of the vitamins were calculated. As shown in Table 5, the excretion ratios of all vitamins except for vitamin B_{12} were lower in the ethanol-fed group.

Table 1. Effects of ethanol consumption on rat body-weight gain, food intake, ethanol intake, water intake, energy intake, food efficiency ratio and liver weight (Expt 2)

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

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Initial body weight (g)	36	1	36	1
Final body weight (g)	204	7	164*	8
Body-weight gain (g/28 d)	168	7	128*	3
Food intake (g/28 d)	363	14	258*	6
Ethanol intake† (g/28 d)	–		45	3
Water intake (ml/28 d)	396	26	–	
Energy intake‡ (kcal/28 d)	1488	58	1396	56
Energy intake‡ (kJ/28 d)	6230	242	5845	234
Food efficiency ratio§	0·46	0·01	0·50	0·00
Energy efficiency ratio‖	0·113	0·020	0·092	0·006
Liver weight (g)	9·70	0·55	8·47	0·36

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).
† The value is expressed in g of pure ethanol and not as the volume of 15 % ethanol.
‡ Energy of 1 g ethanol was calculated as 29·3 kJ (7 kcal)/g.
§ (Body-weight gain/food intake) × 100.
‖ (Body-weight gain/energy intake) × 100.

Table 2. Effects of ethanol consumption on the activities of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase in plasma

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

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Alanine aminotransferase (IU/l)	22·4	1·9	24·8	2·0
Aspartate aminotransferase (IU/l)	157	11	136	10
γ-Glutamyltranspeptidase (IU/l)	3·2	0·9	3·2	0·9

Discussion

An ordinary diet for rats generally contains sufficient amounts of nutrients including vitamins[23]. Under well-nourished conditions, rats are generally little affected by factors such as ethanol consumption. In fact, the present study proves that ethanol consumption did not affect the body-weight gain or the vitamin contents in the liver and blood when rats were fed a diet containing sufficient amounts of vitamins. On the other hand, when rats were fed a diet low in vitamins, body-weight gain was lower in the ethanol-fed group than in the control group and some vitamin contents of the liver and blood, and urinary excretion were decreased. These results show that chronic ethanol consumption affects

Table 3. Effect of ethanol consumption on liver B-group vitamin contents (Expt 2)

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

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1 (nmol/liver)	127	6	100*	4
Vitamin B_2 (nmol/liver)	686	62	422*	16
Vitamin B_6 (nmol/liver)	229	16	281	23
Vitamin B_{12} (nmol/liver)	0·39	0·03	0·38	0·02
Niacin (μmol/liver)	18·2	1·8	16·6	1·3
Pantothenic acid (μmol/liver)	3·16	0·19	2·42*	0·18
Folate (nmol/liver)	70·0	9·7	73·6	9·3
Biotin (nmol/liver)	9·31	1·10	9·65	0·46

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).

British Journal of Nutrition

https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press

Table 4. Effect of ethanol consumption on blood B-group vitamin contents (Expt 2)

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

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1 (pmol/ml)	159	4	139*	6
Vitamin B_2 (pmol/ml)	177	5	142*	4
Vitamin B_6 (nmol/ml)	0·49	0·04	0·34*	0·02
Vitamin B_{12} (pmol/ml)	1·55	0·03	1·41	0·01
Niacin (nmol/ml)	127	6	117	2
Pantothenic acid (nmol/ml)	1·13	0·04	0·89*	0·04
Folate (pmol/ml)	149	4	138	10
Biotin (pmol/ml)	30·4	3·4	25·9	1·0

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).

absorption, distribution and excretion of vitamins, as reported previously[1–19]. The present findings are not consistent with the *in vitro* perfusion of rat liver with ethanol, which caused the release of all B-vitamins except biotin from the liver stores[23]. This phenomenon was not observed in the present whole-body experiment, because the vitamin contents of the blood were not increased by ethanol consumption. In the present *in vivo* experiment, any vitamins released from the liver were quickly absorbed by non-hepatic tissues. In humans, the typical dietary vitamin intakes are generally around the minimum requirements. Thus, the nutritional status of rats fed a diet low in vitamins was similar to that of humans. Ethanol consumption was 45 g over 28 d, so that daily average ethanol consumption was about 1·6 g/d, which corresponds to an energy intake of 46·9 kJ (11·2 kcal)/d. The energy intake in the ethanol-fed group, including ethanol energy, was 5845 kJ (1396 kcal) over 28 d (about 209 kJ (50 kcal)/d). Thus, ethanol accounted for 20 % of dietary energy. Under these conditions, liver functions in rats were not injured. If humans were to consume 10 467 kJ (2500 kcal)/d, the equivalent ethanol consumption would be about 70 g/d, which corresponds to 1 litre of typical beer.

Vitamin depletion, common in malnourished alcoholic patients[10], can occur despite vitamin supplementation. Vitamin malabsorption[37], exacerbated by malnutrition, contributes to this depletion[38]. Also, in alcoholic patients, the impaired ability of the liver to bind and store vitamins might contribute to this depletion. This may probably be due to the hepatotoxicity of ethanol, which impairs not only the vitamin-binding capacity but also the vitamin storage of the liver. In the present study, a diet containing 20 % casein supplemented with methionine was used, which is an excellent protein source from a nutritional standpoint. This suggest the reasons why ethanol consumption did not cause any severe damage, such as an extremely low food intake and body-weight gain and roughness of fur for the rats, even when they were fed a low-vitamin diet.

Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol caused the release of all vitamins from the liver stores, especially thiamin. It is generally considered that this phenomenon causes increased urinary excretion of vitamins, but in the present *in vivo* experiments, ethanol consumption did not cause increased urinary excretion, but rather decreased it. This discrepancy between the expected and the actual findings may be attributed to the difference between the *in vitro* and *in vivo* experiments. Moreover, there are differences in short-term and long-term adjustment mechanisms for ethanol toxicity. The protein nutritional status was high in the present study because the diet used 20 % casein supplemented with methionine. Protein plays a pivotal role in vitamin absorption and storage in hepatocytes. Protein malnutrition causes malabsorption, reduced storage and impaired utilisation of vitamins. Thus, an adequate intake of vitamins, and also protein, is essential for preventing ethanol toxicity.

In the present study on the low-vitamin diet, vitamin B_1, vitamin B_2 and pantothenic acid contents in the liver and blood were lower in the ethanol-fed group than in the controls, even when rats were fed a high-protein diet. Furthermore, the total urinary excretion and excretion ratios of all three vitamins were also lower in the ethanol-fed group. Thus, ethanol consumption reduced the intestinal absorption of these vitamins, as reported by Subramanya *et al.*[12], Hamid *et al.*[13,14,16,17] and Wani & Kaur[19]. Vitamins such as

Table 5. Effect of ethanol consumption on urinary B-group vitamin excretion (upper row) and urinary excretion ratio (lower row) for each of the vitamins (Expt 2)†

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

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1				
nmol/d	3·5	0·1	1·8*	0·1
%	3·4	0·2	2·7*	0·2
Vitamin B_2				
nmol/d	3·6	0·3	0·15*	0·04
%	3·8	0·2	0·24*	0·05
4-PIC‡				
nmol/d	29·4	1·9	7·3*	0·5
%	15·6	0·5	4·5*	0·3
Vitamin B_{12}				
pmol/d	9·1	0·4	6·7*	0·2
%	8·9	0·3	9·1	0·2
Niacin§				
µmol/d	2·00	0·16	1·82	0·24
%	–‖		–‖	
Pantothenic acid				
nmol/d	24·3	2·4	6·3*	0·3
%	6·5	0·5	2·4*	0·2
Folate				
nmol/d	1·85	0·19	0·77*	0·11
%	7·3	0·7	4·4*	0·6
Biotin				
nmol/d	0·21	0·02	0·09*	0·01
%	5·0	0·4	3·0*	0·25

4-PIC, 4-pyridoxic acid.

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).

† Percentage urinary excretion ratio was calculated using the following equation: (24 h urinary excretion (mol/d)/intake of the vitamin during urine collection (mol/d)) × 100.

‡ A catabolite of vitamin B_6.

§ Niacin content was calculated as the sum of the nicotinamide content and its catabolites such as N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide.

‖ Urinary excretion ratio was not calculated as niacin was derived from tryptophan.

https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press
British Journal of Nutrition

https://doi.org/10.1017/S0007114511006192 Published online by Cambridge University Press

vitamin B_1, vitamin B_2 and pantothenic acid might be directly and/or indirectly involved in the metabolism of ethanol, indicating that the vitamin catabolites increased and were excreted into the urine. Of these three vitamins, only the catabolic fate of vitamin B_1 is relatively well known. It has been reported that the excretion of vitamin B_1 metabolites usually exceeds by far the excretion of intact vitamin B_1 using radioactive tracer experiments[39]. The major metabolites of vitamin B_1 in rat urine are 2-methyl-4-amino-5-pyridinecarboxylic acid[40], 4-methylthiazole-5-acetic acid[41] and thiamine acetic acid[42]. Pearson[39] reported that the sum of the metabolites accounted for about 50 % of the total urinary excretion of vitamin B_1 and its catabolites from radioactive tracer experiments. Although we cannot measure the catabolites of vitamin B_1, these metabolites might increase in the urine of the ethanol-fed rats. It is likely that a similar phenomenon would apply for the fates of vitamin B_2 and pantothenic acid.

The content of vitamin B_6 in the blood was lower in the ethanol-fed group, but the content of vitamin B_6 in the liver was slightly higher in the ethanol-fed group than in the control. The urinary excretion of vitamin B_6, determined from its catabolite 4-pyridoxic acid, was much lower in the ethanol-fed group than in the control. Probably ethanol consumption resulted in an increased storage of vitamin B_6 in the liver.

Other B-group vitamin contents in the liver and blood, such as vitamin B_{12}, nicotinamide, folate and biotin, were not affected by ethanol consumption. The lack of any effect of ethanol consumption on the niacin content in this experiment was probably because nicotinamide was synthesised from tryptophan, which was present in the diet as casein and was supplied adequately[43]. For rats, NAD precursors such as nicotinic acid and nicotinamide are not essential. In fact, the urinary excretion of nicotinamide did not differ between the two groups. Concerning the effect of ethanol consumption on biotin, Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol did not cause the release of biotin, but caused the release of vitamin B_{12} first. In the present experiment, a similar phenomenon was observed for biotin, but not for vitamin B_{12}. Frank *et al.*[44] reported that the first vitamin released into the circulation during hepatic insult by ethanol is vitamin B_{12}. This disparity between the reported and the present findings might also arise from the difference in protein nutritional status.

There are many reports concerning how ethanol consumption affects folate absorption and metabolism[13–18,45–53]. Some studies have reported that ethanol consumption increased the urinary excretion of folates[46,47,50–53] and caused decreased serum folate levels. Romanoff *et al.*[53] reported that acute ethanol exposure inhibits the apical transport of 5-methyltetrahydrofolate in cultured human proximal tubule cells, and in subchronic ethanol studies, increasing concentrations of ethanol resulted in an up-regulation of folate transporters. Furthermore, Romanoff *et al.*[53] reported that both the folate receptor and reduced folate carrier transporter proteins were up-regulated in rats receiving an ethanol diet. On the contrary, Hamid *et al.*[13,14,16,17] and Wani & Kaur[19] reported that ethanol reduced the intestinal uptake of folate by altering the binding and transport kinetics of the folate transport system and also the expression of folate transporters in the intestine. In addition, Hamid & Kaur[15] reported that ethanol consumption reduces folate re-uptake in the renal absorption system by the decreased expression of transporters. The present data for folate are not consistent with previous reports[13–18,45–53]; the contents of folate in the liver and blood were not affected by ethanol consumption, and the urinary excretion of folate and the excretion ratio were decreased markedly. A study[52] reported that urinary folate excretion increased in ethanol-fed rats consuming folate-containing diets, but not in rats fed folate-deficient diets. In the present study, the urinary excretion of folate did not increase, but decreased. This was because the diet was low in folate. In the present study, the urinary excretion of folate was lower in the ethanol-fed group than in the non-ethanol group, suggesting that ethanol consumption and the feeding of a low-folate diet up-regulated the folate receptor and reduced folate carrier transporter proteins. This up-regulation was probably a compensatory response to counteract the effects of ethanol in inhibiting the reabsorption of folate. Therefore, the effects of ethanol would depend on the dose and duration of treatment.

In summary, these results show that ethanol consumption affects the absorption, distribution and excretion of each of the vitamins in rats fed a diet containing a low-vitamin mixture. On the other hand, when rats were fed a 20 % casein diet containing a sufficient amount of vitamins, ethanol consumption did not affect any factors that we measured.

Acknowledgements

This study was part of the project ‘Studies on the Dietary Reference Intakes for Japanese’ (principal investigator, K. S.), which was supported by a Research Grant for Comprehensive Research on Cardiovascular and Life-Style Related Diseases from the Ministry of Health, Labour and Welfare of Japan. The authors’ responsibilities are as follows: A. M. designed the study, performed the experiments and prepared the manuscript; M. S. and T. F. helped in the study design, performed the experiments and assisted with the data analysis; K. S. contributed to the study design and supervised the study. The authors declare that they have no conflict of interest.

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