Characteristics of Under- and Over-Reporters of Energy Intake among Young Japanese Women

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Summary Evidence on factors associated with misreporting of energy intake is limited, particularly in non-Western populations. We examined the characteristics of under- and over-reporters of energy intake in young Japanese women. Subjects were 3,956 female Japanese dietetic students aged 18–20 y (mean body mass index: 20.9 kg/m^2). Energy intake was assessed using a comprehensive self-administered diet history questionnaire. Estimated energy requirement was calculated based on self-reported information on age, body height and weight, and physical activity with the use of an equation from the US Dietary Reference Intakes. Under-, acceptable, and over-reporters of energy intake were identified based on the ratio of energy intake to estimated energy requirement, according to whether the individual's ratio was below, within, or above the 95% confidence limits of the expected ratio of 1.0 (<0.70, 0.70–1.30, and >1.30, respectively). Risk of being an under- or over-reporter of energy intake compared to an acceptable reporter was analyzed using multiple logistic regression. The percentage of under-, acceptable, and over-reporters of energy intake was 18.4, 73.1, and 8.4%, respectively. Under-reporting was associated with overweight or obesity, perception that one's own weight was too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a metropolitan area). Over-reporting was associated with sedentary lifestyle only. This study of lean young Japanese women showed that energy intake misreporting, particularly under-reporting, was common and differential among populations. Particularly, perceived weight status was associated with under-reporting of energy intake, independent of actual weight status.
Key Words energy intake, under-reporting, body weight, young women, Japan

Although accurate assessment of habitual dietary intake is a prerequisite to studies of diet and health, the difficulty of obtaining dietary data that accurately represents what people usually eat is now generally recognized (1). Misreporting of dietary intake is a common phenomenon that appears to occur non-randomly (1–4) and to be selective for different kinds of foods and nutrients (5–9). The resulting potential for differential errors in dietary data complicates the interpretation of studies on diet and health and, at worst, might produce spurious diet-health relationships (1, 3, 7). Increasing our understanding of this serious issue therefore requires the identification of different characteristics associated with different kinds of misreporting of dietary intake.

Energy intake is the foundation of the diet, because all other nutrients must be provided within the quantity of food needed to fulfill the energy requirement. Reported energy intake is therefore a surrogate measure of the total quantity of food intake (1). In fact, under-reporting of energy intake has long been considered a serious problem in almost all dietary surveys (1–4, 6–18). In particular, overweight and obese people tend to under-report energy intake to a greater extent than lean people (1–4, 6–18). Moreover, recent studies have shown that, in addition to under-reporting, over-reporting of energy intake also needs to be taken into account, in some populations at least, such as those with low body mass index (BMI) (3, 10, 12, 14). Most of these studies have been conducted in Western countries (1–3, 5–8, 10–16), however, and research in non-Western countries such as Japan is sparse (4, 9, 17, 18). Because the ways people interpret and respond to dietary assessment may differ between Western countries and Japan, mainly due to large differences in dietary habits and body size, the accuracy of reported dietary intake may also differ, hampering the extrapolation of findings in Western countries to Japanese populations.

Here, to better understand the serious problem of dietary misreporting, the objective of this study was to

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examine differences in dietary and non-dietary characteristics between under-, acceptable, and over-reporters of energy intake in a group of young Japanese women. A characteristic of young Japanese women is their relatively low BMI, which is nevertheless accompanied by excessive weight concerns and a strong desire for thinness (19, 20), a combination seldom observed in other countries. In particular, we investigated the hypothesis whether actual and perceived weight statuses were independently associated with energy intake misreporting in this unique population.

MATERIALS AND METHODS

Study population. The present study was based on data from the Freshmen in Dietetic Courses Study II, a cross-sectional, self-administered questionnaire survey among dietetic students (n=4,679) from 54 institutions in 33 of 47 prefectures in Japan. A detailed description of the study design and survey procedure has been published elsewhere (21–24). Briefly, a set of two questionnaires on dietary habits and other lifestyle behaviors during the preceding month was distributed to all students at orientation sessions or early lectures for freshman students who entered dietetic courses in April 2005, in almost all institutions within 2 wk after the course began. In accordance with the survey protocol, answered questionnaires were checked at least twice for completeness by trained survey staff (mostly registered dietitians) and, when necessary, forms were reviewed with the subject to ensure the clarity of answers.

In total, 4,394 students (4,168 women and 226 men) completed both questionnaires (response rate: 93.9%). For the present analysis, we selected female participants aged 18–20 y (n=4,060). We then excluded women who were in an institution where the survey was not conducted within 2 wk of entry (n=98) and those with missing information on the variables used (n=8). As some participants were in more than one exclusion category, the final analysis sample consisted of 3,956 women.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the National Institute of Health and Nutrition, Japan. Written informed consent was obtained from all subjects; in this survey, the signature of the student on both of the questionnaires was considered to constitute informed consent by both the student and her parent(s)/caregiver(s).

Dietary intake. Dietary habits during the preceding month were assessed using a comprehensive self-administered diet history questionnaire (DHQ) (4, 25–28). Details of the DHQ's structure and method of calculating dietary intake have been published elsewhere (4, 25–28). Briefly, the DHQ is a structured 16-page questionnaire which asks about the consumption frequency and portion size of selected foods commonly consumed in Japan, as well as general dietary behavior and usual cooking methods (25, 28). Estimates of daily intake for foods (150 items in total), energy, and selected nutrients

were calculated using an ad hoc computer algorithm for the DHQ (25, 28) based on the Standard Tables of Food Composition in Japan (29). Values of nutrient and food intake were energy-adjusted using the density method (i.e., percentage of energy for energy-providing nutrients and amount per 1,000 kcal of energy for other nutrients and foods) (9).

Validity of the DHQ with respect to commonly studied nutritional factors has been investigated (4, 25–28). Briefly, Pearson correlation coefficients were 0.48 for energy, 0.37–0.75 for energy-providing nutrients, and 0.38–0.68 for other nutrients between the DHQ and 3-d estimated dietary records in 47 women (25); 0.23 for sodium and 0.40 for potassium between the DHQ and 24-h urinary excretion in 69 women (26); 0.66 between the DHQ and serum phospholipid concentrations for marine-origin n-3 polyunsaturated fatty acids (sum of eicosapentenoic, docosapentaenoic, and docosahexaenoic acids) in 44 women (27); and 0.56 between the DHQ and serum concentrations for carotene in 42 women (27). Further, Pearson correlation coefficients between energy intake derived from the DHQ and total energy expenditure measured by doubly labeled water were 0.34 in 67 men and 0.22 in 73 women (4).

Non-dietary factors. Body weight and height were self-reported as part of the DHQ. BMI (kg/m²) was calculated as body weight (kg) divided by the square of body height (m). Weight status was defined according to World Health Organization recommendations as follows (30): underweight (BMI: <18.5 kg/m²), normal (BMI: ≥18.5 to <25 kg/m²), overweight (BMI: ≥25 to <30 kg/m²), and obese (BMI: ≥30 kg/m²).

In a 12-page questionnaire on nondietary lifestyle during the preceding month, subjects reported self-perceived weight status (too heavy, somewhat heavy, just about right, somewhat light, or too light), whether currently trying to lose weight (no or yes), residential status (living with family, living alone, or living with others), and smoking status (never, former, or current). Dietary consciousness was assessed in the lifestyle questionnaire using the following question: 'How often do you think about diet or nutrients to maintain your health?' and classified into five categories (always, often, sometimes, seldom, or never). Residential areas, reported in the lifestyle questionnaire, were grouped into six regions (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; and Kyushu) and into three municipality levels (ward (i.e., metropolitan area); city; and town and village).

Subjects also reported on the lifestyle questionnaire the time they usually got up and went to bed, which was used to calculate sleeping hours, and the frequency and duration of high-intensity activities (e.g., carrying heavy loads; bicycling, moderate effort; jogging; and singles tennis), moderate-intensity activities (e.g., carrying light loads; bicycling, light effort; and doubles tennis), walking, and sedentary activities (e.g., studying; reading; and watching television) during the preceding month. For subjects whose recorded total hours were <24 h, unrecorded hours were assumed to be spent on

sedentary activities. For subjects whose recorded total hours were >24 h, the total number of hours spent daily was proportionately decreased to equal 24. Each activity was assigned a metabolic equivalent value from a previously published table (0.9 for sleeping, 1.5 for sedentary activity, 3.3 for walking, 5.0 for moderate-intensity activity, and 7.0 for high-intensity activity) (31). The number of hours spent per day on each activity was multiplied by the metabolic equivalent value of that activity, and all metabolic equivalent-hour products were summed to produce a total metabolic equivalent-hour score for the day. These were then divided by 24 h to give a physical activity level (PAL) value, and classified into four categories (sedentary (PAL: <1.4), low active (PAL: ≥1.4 to <1.6), active (PAL: ≥1.6 to <1.9), and very active (PAL: ≥1.9)) according to the US Dietary Reference Intakes (32).

Identification of misreporting of energy intake. We calculated each subject's estimated energy requirement (which is equal to total energy expenditure during weight stability) based on self-reported information on age, body height and weight, and physical activity, with the use of the following equation from the US Dietary Reference Intakes (32).

Estimated energy requirement (i.e., total energy expenditure during weight stability) [kcal/d]

$$= 387 - 7.31 \times \text{age [y]} + \text{physical activity coefficient}$$
[1.00 for sedentary, 1.14 for low active, 1.27 for active, and 1.45 for very active] $\times (10.9 \times \text{body weight [kg]} + 660.7 \times \text{body height [m]})$

This equation was developed for use in lean to obese women (≥19 y) from a meta-analysis of methodologically sound studies using doubly labeled water as the criterion measure of total energy expenditure (n=433, SE fit: 229.1, R^2: 0.79) (32). An investigation using two equations for normal weight women and for overweight women (32) provided similar results (data not shown), while an investigation among 18-y-old women (n=3,574) using two equations for normal weight girls (9–18 y) and for overweight girls (32) provided similar results (data not shown). In this paper, we present the results derived from all 3,956 women aged 18–20 y using the first-mentioned equation, which had a maximum number of subjects and a minimum number of different sources of error.

Subjects were identified as acceptable, under-, or over-reporters of energy intake based on their ratio of reported energy intake to estimated energy requirement, according to whether the individual's ratio was within, below, or above the 95% confidence limits of the expected ratio of 1.0. The 95% confidence limits (± 2 standard deviation (SD) cut-offs) were calculated according to the following equation (33–35).

95% confidence limit

$$= \pm 2 \times \sqrt{(CV^2_{rEI}/d + CV^2_{pER} + CV^2_{mTEE})}$$

CV_{rEI} is the within-person coefficient of variation in reported energy intake, d is the number of days of dietary assessment, CV_{pER} is the error in predicted energy requirement equation, and CV_{mTEE} is day-to-day variation in total energy expenditure measured by dou-

bly labeled water (33–35). The values used were 23 for CV_{rEI} (36, 37), 30 for d (i.e., 1 mo), 11.5 for CV_{pER} (32), and 8.2 for CV^2_{mTEE} (38). The obtained 95% confidence limit was ±29.5 (%). Thus, acceptable reporters were defined as having a ratio of energy intake to estimated energy requirement in the range 0.70–1.30, under-reporters as a ratio <0.70, and over-reporters as a ratio >1.30.

Statistical analyses. All reported p values are 2-tailed, and p values of <0.05 were considered statistically significant. Mean differences in dietary characteristics between under-, acceptable, and over-reporters of energy intake were tested with one-way analysis of variance (ANOVA). When the overall p from ANOVA was <0.05, the post hoc Bonferroni test was performed. The chi-square test was used to test differences in proportions across categories of energy intake reporting.

The risk of being classified as an under-reporter of energy intake compared to an acceptable reporter, or as an over-reporter compared to an acceptable reporter, was estimated using logistic regression. First, crude odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of being classified as an under- or over-reporter were calculated for each category of factors which are possibly associated with energy intake misreporting, namely weight status (reference: normal), self-perceived weight status (reference: just about right), whether currently trying to lose weight (reference: no), dietary consciousness (reference: always), physical activity (reference: sedentary), smoking status (reference: never), residential status (reference: living with family), region (reference: Hokkaido and Tohoku), and municipality level (reference: ward (i.e., metropolitan area)). Multivariate-adjusted ORs and 95% CIs were then calculated by entering all variables simultaneously into the regression model to assess the genuine effect on risk. All statistical analyses were performed using SAS statistical software (version 9.1, 2003, SAS Institute Inc, Cary, NC, USA).

RESULTS

Mean values of physical characteristics were as follows: 18.1 (SD: 0.3) y for age, 1.58 (SD: 0.05) m for height, 52.3 (SD: 7.7) kg for weight, and 20.9 (SD: 2.8) kg/m^2 for BMI. Dietary characteristics across categories of reporting status of energy intake are shown in Table 1. Mean value of the ratio of energy intake to estimated energy requirement was 0.93 (SD: 0.28). The percentage of under-, acceptable, and over-reporters of energy intake was 18.4, 73.1, and 8.4%, respectively. Energy-adjusted intake of most nutrients and foods differed among the categories of energy reporting status. For nutrients, under-reporters had the highest intake of carbohydrate and the lowest intake of protein, fat, cholesterol, potassium, calcium, and vitamin A. Over-reporters had the highest intake of protein, fat, alcohol, potassium, iron, and vitamin A and the lowest intake of carbohydrate. For foods, under-reporters had the highest intake of rice and noodles and the lowest intake of confectioneries, fats and oils, fish and shellfish, meats, and soft drinks. Over-reporters had the highest intake

Table 1. Dietary characteristics across categories of reporting status of energy intake.

	All (n=3,956)		Under-reporters (n=729; 18.4%)		Acceptable reporters (n=2,893; 73.1%)		Over-reporters (n=334; 8.4%)		p (ANOVA)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Ratio of energy intake to estimated energy requirement	0.93	0.28	0.60[a]	0.08	0.94[b]	0.15	1.56[c]	0.32	<0.0001
Energy intake (kcal/d)	1,827	551	1,235[a]	196	1,840[b]	327	3,009[c]	650	<0.0001
Estimated energy requirement (kcal/d)	1,984	194	2,065[a]	222	1,969[b]	184	1,931[c]	164	<0.0001
Nutrient intake									
Protein (% of energy)	13.3	2.1	12.9[a]	2.2	13.4[b]	2.1	13.6[c]	2.5	<0.0001
Fat (% of energy)	29.5	6.0	26.5[a]	5.9	29.8[b]	5.5	33.9[c]	6.6	<0.0001
Carbohydrate (% of energy)	55.7	6.9	59.0[a]	6.8	55.4[b]	6.4	51.3[c]	7.7	<0.0001
Alcohol (% of energy)	0.3	1.6	0.3[a]	1.5	0.3[a]	1.4	0.6[b]	2.8	0.01
Dietary fiber (g/1,000 kcal)	6.5	2.1	6.5	2.4	6.5	2.0	6.6	2.1	0.88
Cholesterol (mg/1,000 kcal)	163.8	64.1	151.9[a]	71.8	165.8[b]	62.0	172.4[b]	61.4	<0.0001
Sodium (mg/1,000 kcal)	2,117	556	2,098	617	2,123	536	2,108	578	0.51
Potassium (mg/1,000 kcal)	1,079	286	1,047[a]	340	1,079[b]	269	1,142[c]	297	<0.0001
Calcium (mg/1,000 kcal)	266.6	99.7	256.2[a]	112.3	268.0[b]	97.0	276.5[b]	91.1	0.003
Iron (mg/1,000 kcal)	3.7	0.9	3.6[a]	1.0	3.7[a]	0.9	3.8[b]	0.9	0.002
Vitamin A (μg retinol equivalents/1,000 kcal)	290.7	248.9	265.2[a]	287.0	292.0[b]	234.2	335.6[c]	275.0	<0.0001
Folate (μg/1,000 kcal)	152.2	55.1	156.9[a]	69.3	151.2[b]	51.3	151.0[a,b]	51.0	0.04
Vitamin C (mg/1,000 kcal)	48.1	22.7	49.0[a,b]	26.6	47.5[a]	21.5	51.6[b]	23.0	0.004
Food intake (g/1,000 kcal)									
Rice	159.2	70.1	185.0[a]	79.4	157.8[b]	65.2	114.5[c]	64.1	<0.0001
Bread	28.3	21.8	29.2[a]	24.6	28.5[a]	21.2	24.8[b]	19.8	0.005
Noodles	36.8	32.7	43.3[a]	43.0	36.0[b]	30.3	29.1[c]	23.4	<0.0001
Confectioneries	38.1	17.6	35.2[a]	17.9	38.0[b]	16.8	44.9[c]	21.0	<0.0001
Fats and oils	13.6	6.7	11.9[a]	6.4	13.7[b]	6.4	16.3[c]	8.1	<0.0001
Fish and shellfish	30.2	17.7	27.5[a]	17.5	30.4[b]	17.0	34.1[c]	22.8	<0.0001
Meats	33.7	16.9	29.2[a]	14.9	34.2[b]	16.6	39.2[c]	21.1	<0.0001
Dairy products	83.9	71.4	79.9	76.5	85.1	71.0	82.5	62.2	0.20
Vegetables	127.4	81.0	126.4	98.9	126.7	75.0	134.8	87.6	0.22
Fruits	50.0	51.9	47.6[a]	53.8	48.8[a]	49.6	65.6[b]	63.9	<0.0001
Soft drinks	33.4	53.1	24.4[a]	40.1	33.7[b]	54.4	50.2[c]	62.4	<0.0001

[a,b,c] Mean values within a row with different superscript letters are significantly different, $p<0.05$ (post hoc Bonferroni test; when the overall p from ANOVA was <0.05 the post hoc Bonferroni test was performed).

of confectioneries, fats and oils, fish and shellfish, meat, fruits, and soft drinks and the lowest intake of rice, bread, and noodles. No differences were observed among the categories of energy reporting status for dietary fiber, sodium, dairy products, or vegetables.

Table 2 shows non-dietary characteristics across categories of reporting status of energy intake. While the proportion of overweight or obese subjects was small (6.2 and 1.3%, respectively), many subjects perceived their own weight as too heavy or somewhat heavy (17.4 and 57.1%, respectively), suggesting excessive weight concerns in spite of actual leanness. Weight status, self-perceived weight status, whether currently trying to lose weight, physical activity, and residential status was associated with energy reporting status. Under-reporters of energy intake had the highest proportion of overweight and obese subjects, subjects who perceived their own weight as too heavy or too light, subjects currently trying to lose weight, subjects with an active lifestyle,

and subjects living alone. Over-reporters had the highest proportion of underweight subjects, subjects with a sedentary lifestyle, and subjects living with family.

ORs and 95% CIs for the risk of being an under-reporter compared to an acceptable reporter of energy intake are shown in Table 3. Results for crude and multi-variate-adjusted models were generally similar. In multivariate analysis, overweight and obese, perceiving their own weight as too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city were associated with a higher risk of being an under-reporter of energy intake. Currently trying to lose weight was associated with a higher risk of being an under-reporter in the crude model, but the association disappeared after consideration of other factors.

Table 4 shows ORs and 95% CIs for the risk of being an over-reporter compared to an acceptable reporter of energy intake. Results for crude and multivariate-adjusted models were generally similar again. On multi-

Table 2. Non-dietary characteristics across categories of reporting status of energy intake.

	All (n=3,956)		Under-reporters (n=729; 18.4%)		Acceptable reporters (n=2,893; 73.1%)		Over-reporters (n=334; 8.4%)		p^1
	n	%	n	%	n	%	n	%	
Weight status									<0.0001
Underweight (BMI: <18.5 kg/m²)	576	14.6	83	11.4	427	14.8	66	19.8	
Normal (BMI: ≥18.5 to <25 kg/m²)	3,080	77.9	545	74.8	2,287	79.1	248	74.3	
Overweight (BMI: ≥25 to <30 kg/m²)	247	6.2	77	10.6	151	5.2	19	5.7	
Obese (BMI: ≥30 kg/m²)	53	1.3	24	3.3	28	1.0	1	0.3	
Self-perceived weight status									<0.0001
Too heavy	690	17.4	200	27.4	430	14.9	60	18.0	
Somewhat heavy	2,260	57.1	386	53.0	1,702	58.8	172	51.5	
Just about right	830	21.0	113	15.5	637	22.0	80	24.0	
Somewhat light	151	3.8	22	3.0	111	3.8	18	5.4	
Too light	25	0.6	8	1.1	13	0.5	4	1.2	
Currently trying to lose weight									0.003
No	2,528	63.9	426	58.4	1,889	65.3	213	63.8	
Yes	1,428	36.1	303	41.6	1,004	34.7	121	36.2	
Dietary consciousness									0.42
Always	775	19.6	136	18.7	578	20.0	61	18.3	
Often	2,162	54.7	381	52.3	1,597	55.2	184	55.1	
Sometimes	571	14.4	113	15.5	410	14.2	48	14.4	
Seldom	390	9.9	84	11.5	269	9.3	37	11.1	
Never	58	1.5	15	2.1	39	1.4	4	1.2	
Physical activity									<0.0001
Sedentary	2,323	58.7	321	44.0	1,769	61.2	233	69.8	
Low active	1,317	33.3	305	41.8	927	32.0	85	25.5	
Active	242	6.1	76	10.4	150	5.2	16	4.8	
Very active	74	1.9	27	3.7	47	1.6	0	0	
Smoking status									0.30
Never	3,827	96.7	698	95.8	2,809	97.1	320	95.8	
Former	68	1.7	15	2.1	46	1.6	7	2.1	
Current	61	1.5	16	2.2	38	1.3	7	2.1	
Residential status									0.0002
Living with family	3,508	88.7	612	84.0	2,592	89.6	304	91.0	
Living alone	365	9.2	96	13.2	247	8.5	22	6.6	
Living with others	83	2.1	21	2.9	54	1.9	8	2.4	
Region									0.44
Hokkaido and Tohoku	388	9.8	69	9.5	293	10.1	26	7.8	
Kanto	1,358	34.3	230	31.6	1,003	34.7	125	37.4	
Hokuriku and Tokai	552	14.0	110	15.1	392	13.6	50	15.0	
Kinki	783	19.8	139	19.1	581	20.1	63	18.9	
Chugoku and Shikoku	427	10.8	93	12.8	302	10.4	32	9.6	
Kyushu	448	11.3	88	12.1	322	11.1	38	11.4	
Municipality level									0.047
Ward (i.e., metropolitan area)	784	19.8	122	16.7	598	20.7	64	19.2	
City	2,570	65.0	505	69.3	1,855	64.1	210	62.9	
Town and village	602	15.2	102	14.0	440	15.2	60	18.0	

[1] Chi-square test.

variate analysis, a higher risk of being an over-reporter of energy intake was associated with sedentary lifestyle only. Underweight was associated with higher risk of being an over-reporter in crude model, but the association disappeared after consideration of other factors.

DISCUSSION

In this study in lean young Japanese women, misreporting, particularly under-reporting, of energy intake was common and differently distributed among populations. Under-reporting was associated with overweight or obesity, perceiving one's own weight as too heavy or

Table 3.   Risk of being an under-reporter of energy intake compared to being an acceptable reporter of energy intake.

	n of under-reporters/ acceptable reporters	Crude model[1]			Multivariate-adjusted model[2]		
		OR	95% CI	p	OR	95% CI	p
Weight status							
Underweight (BMI:<18.5 kg/m²)	83/427	0.82	0.63, 1.05	0.11	0.91	0.66, 1.25	0.55
Normal (BMI: 18.5 to<25 kg/m²)	545/2,287	1 (reference)			1 (reference)		
Overweight (BMI: 25 to<30 kg/m²)	77/151	2.14	1.60, 2.86	<0.0001	1.52	1.10, 2.12	0.01
Obese (BMI: 30 kg/m²)	24/28	3.60	2.07, 6.25	<0.0001	2.68	1.48, 4.86	0.001
Self-perceived weight status							
Too heavy	200/430	2.62	2.02, 3.40	<0.0001	2.03	1.47, 2.79	<0.0001
Somewhat heavy	386/1,702	1.28	1.02, 1.61	0.04	1.19	0.92, 1.53	0.19
Just about right	113/637	1 (reference)			1 (reference)		
Somewhat light	22/111	1.12	0.68, 1.84	0.66	1.17	0.69, 1.99	0.57
Too light	8/13	3.47	1.41, 8.56	0.007	4.06	1.57, 10.50	0.004
Currently trying to lose weight							
No	426/1,889	1 (reference)			1 (reference)		
Yes	303/1,004	1.34	1.13, 1.58	0.0006	1.11	0.93, 1.34	0.25
Dietary consciousness							
Always	136/578	1 (reference)			1 (reference)		
Often	381/1,597	1.01	0.82, 1.26	0.90	1.14	0.91, 1.44	0.26
Sometimes	113/410	1.17	0.89, 1.55	0.27	1.28	0.95, 1.72	0.11
Seldom	84/269	1.33	0.98, 1.81	0.07	1.54	1.11, 2.14	0.01
Never	15/39	1.64	0.88, 3.05	0.12	2.23	1.16, 4.28	0.02
Physical activity							
Sedentary	321/1,769	1 (reference)			1 (reference)		
Low active	305/927	1.81	1.52, 2.16	<0.0001	1.92	1.60, 2.31	<0.0001
Active	76/150	2.79	2.07, 3.77	<0.0001	3.28	2.40, 4.48	<0.0001
Very active	27/47	3.17	1.94, 5.16	<0.0001	3.90	2.36, 6.47	<0.0001
Smoking status							
Never	698/2,809	1 (reference)			1 (reference)		
Former	15/46	1.31	0.73, 2.36	0.37	1.08	0.58, 2.01	0.81
Current	16/38	1.70	0.94, 3.06	0.08	1.45	0.78, 2.70	0.24
Residential status							
Living with family	612/2,592	1 (reference)			1 (reference)		
Living alone	96/247	1.65	1.28, 2.12	0.0001	1.95	1.50, 2.55	<0.0001
Living with others	21/54	1.65	0.99, 2.75	0.06	1.79	1.05, 3.05	0.03
Region							
Hokkaido and Tohoku	69/293	1 (reference)			1 (reference)		
Kanto	230/1,003	0.97	0.72, 1.31	0.86	0.88	0.64, 1.21	0.43
Hokuriku and Tokai	110/392	1.19	0.85, 1.67	0.31	1.08	0.75, 1.56	0.68
Kinki	139/581	1.02	0.74, 1.40	0.92	0.89	0.64, 1.26	0.52
Chugoku and Shikoku	93/302	1.31	0.92, 1.86	0.13	1.05	0.72, 1.53	0.79
Kyushu	88/322	1.16	0.82, 1.65	0.41	1.15	0.79, 1.68	0.47
Municipality level							
Ward (i.e., metropolitan area)	122/598	0.75	0.60, 0.93	0.01	0.71	0.56, 0.90	0.005
City	505/1,855	1 (reference)			1 (reference)		
Town and village	102/440	0.85	0.67, 1.08	0.18	0.85	0.66, 1.09	0.20

[1] Each of the variables listed was entered into the model separately.
[2] All the variables listed were entered into the model simultaneously.

light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a ward (metropolitan area)); while over-reporting was associated with sedentary lifestyle. The most impressive finding was the association of perceived weight status with energy under-reporting, independent of actual weight status. To our knowledge, this is the first study to examine characteristics associated with under- and over-reporting of energy intake in young Japanese women, with consideration of individual physical activity level.

In this study of young Japanese women, about one-

Table 4. Risk of being an over-reporter of energy intake compared to being an acceptable reporter of energy intake.

	n of over-reporters/ acceptable reporters	Crude model[1]			Multivariate-adjusted model[2]		
		OR	95% CI	p	OR	95% CI	p
Weight status							
Underweight (BMI: <18.5 kg/m^2)	66/427	1.43	1.07, 1.91	0.02	1.33	0.92, 1.90	0.13
Normal (BMI: ≥18.5 to<25 kg/m^2)	248/2,287	1 (reference)			1 (reference)		
Overweight (BMI: ≥25 to<30 kg/m^2)	19/151	1.16	0.71, 1.90	0.56	0.93	0.54, 1.59	0.79
Obese (BMI: ≥30 kg/m^2)	1/28	0.33	0.05, 2.43	0.28	0.20	0.03, 1.53	0.12
Self-perceived weight status							
Too heavy	60/430	1.11	0.78, 1.59	0.56	1.21	0.79, 1.86	0.38
Somewhat heavy	172/1,702	0.81	0.61, 1.07	0.13	0.85	0.62, 1.17	0.32
Just about right	80/637	1 (reference)			1 (reference)		
Somewhat light	18/111	1.29	0.75, 2.24	0.36	1.17	0.66, 2.09	0.58
Too light	4/13	2.45	0.78, 7.70	0.12	2.22	0.69, 7.18	0.18
Currently trying to lose weight							
No	213/1,889	1 (reference)			1 (reference)		
Yes	121/1,004	1.07	0.84, 1.35	0.58	1.20	0.92, 1.55	0.17
Dietary consciousness							
Always	61/578	1 (reference)			1 (reference)		
Often	184/1,597	1.09	0.81, 1.48	0.57	1.08	0.79, 1.48	0.63
Sometimes	48/410	1.11	0.75, 1.65	0.61	1.13	0.75, 1.70	0.57
Seldom	37/269	1.30	0.85, 2.01	0.23	1.27	0.81, 1.99	0.30
Never	4/39	0.97	0.34, 2.81	0.96	0.84	0.29, 2.47	0.75
Physical activity							
Sedentary	233/1,769	1 (reference)			1 (reference)		
Low active	85/927	0.70	0.54, 0.90	0.007	0.68	0.53, 0.89	0.005
Active	16/150	0.81	0.48, 1.38	0.44	0.78	0.45, 1.33	0.36
Very active	0/47	—	—	—	—	—	—
Smoking status							
Never	320/2,809	1 (reference)			1 (reference)		
Former	7/46	1.34	0.60, 2.98	0.48	1.19	0.53, 2.71	0.67
Current	7/38	1.62	0.72, 3.65	0.25	1.60	0.69, 3.68	0.27
Residential status							
Living with family	304/2,592	1 (reference)			1 (reference)		
Living alone	22/247	0.76	0.48, 1.19	0.23	0.76	0.48, 1.20	0.24
Living with others	8/54	1.26	0.60, 2.68	0.54	1.25	0.58, 2.68	0.57
Region							
Hokkaido and Tohoku	26/293	1 (reference)			1 (reference)		
Kanto	125/1,003	1.40	0.90, 2.18	0.13	1.43	0.91, 2.25	0.12
Hokuriku and Tokai	50/392	1.44	0.87, 2.36	0.15	1.38	0.82, 2.32	0.23
Kinki	63/581	1.22	0.76, 1.97	0.41	1.24	0.76, 2.02	0.40
Chugoku and Shikoku	32/302	1.19	0.69, 2.05	0.52	1.23	0.70, 2.15	0.48
Kyushu	38/322	1.33	0.79, 2.24	0.29	1.31	0.76, 2.25	0.34
Municipality level							
Ward (i.e., metropolitan area)	64/598	0.95	0.70, 1.27	0.71	1.04	0.76, 1.42	0.83
City	210/1,855	1 (reference)			1 (reference)		
Town and village	60/440	1.21	0.89, 1.63	0.23	1.19	0.87, 1.63	0.27

[1] Each of the variables listed was entered into the model separately.
[2] All the variables listed were entered into the model simultaneously.

fourth of the participants were classified as either under- or over-reporters of energy intake (18.4 and 8.4%, respectively). In Western countries, the percentage of under-reporters ranged from 3 to 54% and that of over-reporters from 0.1 to 22% (2, 3, 6, 7, 10–16). In a Japanese study using total energy expenditure measured by doubly labeled water (n=140), 44% of subjects were defined as under-reporters and 20% as over-reporters (4). Other studies in Japan using the ratio of reported energy intake to estimated basal metabolic rate without consideration of individual physical activity reported that the prevalence of under-reporters was 20–37% while that of over-reporters was 2–10% (17, 18). Although comparisons of the prevalence of misre-

porting of energy intake between studies are hampered by differences in the criteria used to classify under- and over-reporting, dietary assessment instruments, and population characteristics, these findings suggest that not only under- but also over-reporting of energy intake is likely in many dietary surveys in both Western and Japanese populations.

In this lean Japanese population, we found that over-weight and obese subjects were more likely to under-report energy intake. This finding is consistent with numerous previous findings in Western countries (1–3, 6, 7, 10–16) and Japan (4, 17, 18). Further, subjects who perceived their own weight as too heavy were pre-dominant, and were more likely to under-report energy intake, independent of their actual weight status. More-over, under-reporting was also independently associated with perceiving one's weight as too light. This may be due to the excessive weight concerns and strong desire for thinness commonly observed in young Japanese women, irrespective of actual weight status (19, 20). A similar independent influence of both actual weight status and perceived weight consciousness on under-reporting has been observed in other obese populations (10, 14).

In this study, higher physical activity was associated with under-reporting of energy intake. This appears reasonable, given that active subjects with greater energy requirements can fall into the category of under-reporting (39). A similar association was observed in Japanese adult men and women (4). Although several studies have suggested an association between smok-ing status and energy misreporting (1, 3, 7, 14, 16), we found no such association, possibly due to the small per-centage of former and current smokers in the present study. We found some influence of variables related to residence (residential status and municipality level) on energy under-reporting, which is in accordance with several previous studies (3, 14). In contrast to a previ-ous study (14), lower dietary consciousness was asso-ciated with energy under-reporting, which may reflect carelessness or poor memory of dietary habits, or fac-tors potentially associated with dietary reporting such as knowledge of food and diet and enthusiasm in dietary assessment (18).

While previous studies have suggested several lifestyle factors as a risk factor of energy over-reporting, includ-ing low BMI (3, 10, 12, 14), none of these factors, including weight status, was associated with the risk of an being over-reporter in this study of relatively lean young Japanese women (except for sedentary lifestyle). On this basis, over-reporting may be a random rather than a systematic phenomenon compared with under-reporting, in the present population at least.

Consistent with previous Western studies (1, 3, 7, 10, 12–14, 16), energy-adjusted nutrient and food intakes differed among under-, acceptable, and over-reporters of energy intake, although nutrient and food intake in Jap-anese subjects appears to provide no clue as to whether the diet of under- and over-reporters is healthier or unhealthier than that of acceptable reporters (9, 17).

This supports the hypothesis that the under- and over-reporting of foods is selective and that this selective mis-reporting affects the energy-adjusted nutrient and food intake in a biased way (5–9), which in turn affects the diet-disease relationships thereby obtained (1, 3, 7).

Several limitations of the present study deserve men-tion. First, the participants selected were female dietetic students, not a random sample of Japanese people. To minimize the influence of nutritional education, the present survey was conducted in most institutions within 2 wk after the course began. Nevertheless, the participants may have had healthier dietary habits and lifestyles than the general population, although with regard to the reported intake of energy, fat, and carbo-hydrate and BMI at least, mean and SD values in the present study were reasonably comparable to those of a representative sample of Japanese women aged 15–19 y (1,852 (SD: 480) kcal/d, 29.3% (SD: 6.8%) of energy, 55.5% (SD: 7.8%) of energy, and 20.7 (SD: 3.0) kg/m^2, respectively) (40). Our results might not therefore be extrapolatable to the general Japanese population.

At present, the only way to obtain unbiased infor-mation on energy requirements in free-living settings is to use doubly labeled water as a biomarker (1). This technique is expensive and impractical for applica-tion to large-scale epidemiologic studies, and alterna-tive procedures are accordingly used (3, 7–18). In the present study, we calculated estimated energy require-ments based on self-reported information on age, body height and weight, and physical activity with the use of an equation from the US Dietary Reference Intakes (32). Although the equation was developed based on a large number of highly accurate measurements of total energy expenditure by the doubly labeled water method, these were predominantly conducted in Caucasians (32), and might therefore be inappropriate for the pres-ent Japanese population. Moreover, this calculation used self-reported rather than measured body weight and height, although previous studies have generally shown that while weights are on average underestimated and heights are on average overestimated, the correlations between self-reported and measured values are markedly high (41, 42). Additionally, we are unable to determine whether the associations found between misreporting of energy intake and several characteristics are true, or were artifacts caused by the procedure used to identify misreporters or to calculate energy requirements.

Energy intake was assessed using a self-administered dietary assessment questionnaire (i.e., DHQ). Actual dietary habits were not observed and, as is often the case in such dietary questionnaires (6, 43–46), the validity of the DHQ in terms of energy intake appears somewhat insufficient against total energy expenditure as measured by doubly labeled water (4). Thus, the pres-ent findings might be specific to this dietary assessment questionnaire and should be interpreted in this context, albeit there is some evidence that people tend to report dietary intake similarly across dietary assessment meth-ods (1).

All the variables used in this study were based on

self-reporting, which might have been biased and hence influenced the results. For example, BMI calculated based on self-reported measures are generally underestimated, although the correlation between self-reported and measured BMI is markedly high (41, 42). It is thus likely that the percentages of overweight and obese subjects based on self-reported data in this study are underestimated, which might have influenced the results by attenuating or strengthening the association.

In conclusion, this study in lean young Japanese women showed that misreporting, particularly underreporting, of energy intake was common and differently distributed among populations. Under-reporting was associated with overweight or obesity, perception that one's weight was too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a ward (metropolitan area)); while over-reporting was associated with a sedentary lifestyle. The most impressive finding was the association of perceived weight status with energy under-reporting, independent of actual weight status. These results suggest that dietary data in young Japanese women should be treated and interpreted with marked caution. Further studies are needed to examine whether the associations observed in the present study are commonly observed across different dietary assessment methods and in other populations.

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Towards a better National Health and Nutrition Survey in Japan

In his Comment (Oct 1, p 1205),[1] Satoshi Sasaki doubts the value of the National Health and Nutrition Survey in Japan[2] (hereafter, the Survey), mentioning that "as long as the Survey continues to be done and reported in the current manner, it will not fulfil its potential as a valuable resource for health." He raises three points. First, the use of data from the Survey is limited; second, there are problems with methods and quality control; and third, access to Survey information is limited. We would like to address the first point, and offer proposals as to the other two points, in light of his comments.

Since 1948, the Survey has been carried out annually by the Ministry of Health, Labour and Welfare, together with the National Institute of Health and Nutrition and in collaboration with local or registered dietitians and randomly selected Japanese people (currently about 9000 individuals of 4000 households). The Survey is, a priori, meant to obtain a set of national statistics to get an overview of the present status of health and nutrition in Japan, and of long-term trends for launching governmental policy and initiatives. It is also concurrently serving to provide a wide range of basic information for setting dietary reference intakes for Japanese people;[3] an exercise and physical activity reference for health promotion;[4] regulatory measures for food additives and contamination with insecticides or pesticides, organic mercury, and radioactive substances; and reference values for consumption of energy and nutrients for the victims of the Great Eastern Japan Earthquake of March 11, 2011.

Sasaki's first comments do not seem reasonable because the Survey is a set of cross-sectional observations that show the status quo of health and nutrition in Japan as a whole, and has its own limits in showing how the traditional Japanese diet has contributed towards achieving the world's highest longevity. Furthermore, the Survey cannot be counted on to have a role in analytical epidemiological approaches, including case-control studies, cohort studies, or randomised controlled trials, to investigate the associations between individual health and disease and physical activity and nutrition, and the interactions between environmental factors and host genetic factors.

Second as Sasaki points out, the participation rate is rather low at about 60%, suggesting that there is non-response bias. Descriptions remain somewhat unclear about presently adopted semi-weighed food records, assessment of individual intake from household data, standardisation of consumption data, validity, and reproducibility. Thus, there might be issues of generalisability. We at the National Institute of Health and Nutrition have committed ourselves to managing data quality control and standardisation of the Survey methods, but we should keep on exerting every effort to improve the Survey. Since information on energy and nutrients is scarcely given for cooked dishes and prepared food, in particular, in the Standard Tables of Food Composition in Japan, the quality and quantity of table data should be improved with all due speed.

A research group under the auspices of the Ministry of Health, Labour and Welfare suggested transfer of the Survey method from semi-weighed food records to 1-day (or multiple-day) 24-h dietary recall (with or without photos),[5] which is currently adopted worldwide, making international comparisons possible. This approach allows us to estimate individual consumption of energy, food, and nutrients; clarify the causative factors for health promotion and prevention of diseases; and elucidate the factors associated with life expectancy.

The third point relates to governmental statistics: that is, secondary (post-tabulated) data are provided to researchers. Thus, to obtain the Survey primary data, researchers must go through formalities and secure approval from the Ministry. Round table discussion on tabulation items to meet the current needs, open access to the Survey information, and provision of the primary data (or setting-up a data archive) should be made. The National Institute of Health and Nutrition proposes to launch a cohort study based on the Survey individual data to verify the associations between health and disease, physical activity and sports, and consumption of food and nutrients along with information on smoking, alcohol drinking, anthropometric measurements, and blood biomarkers.

Sasaki's comments serve to alert the Ministry and the Institute to modify the framework of the Survey, including replacement of the Survey methods, and to guarantee quality control, standardisation, and access to the Survey data.

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Note

The Urinary Excretory Ratio of Nicotinamide Catabolites Was Associated with the Conversion Ratio of Tryptophan to Nicotinamide in Growing Rats Fed a Niacin-Free 20% Casein Diet

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Weaning rats were fed a niacin-free 20% casein diet. Twenty-four-h-urine samples were collected, and nicotinamide and its catabolites were measured. A correlation was found between the urinary excretory ratio of nicotinamide catabolites (N^1-methyl-2-pyridone-5-carboxamide + N^1-methyl-4-pyridone-3-carboxamide)/ N^1-methylnicotinamide and the tryptophan-nicotinamide conversion ratio during growing period of the rats. This indicates the possibility that the conversion ratio can be deduced from the excretory ratio.

The vitamin Nam is biosynthesized from the essential amino acid Trp in mammalian liver, including the human liver.[1,2] The metabolism of nicotinic acid, Nam, and Trp in mammals is given in reference 3. It is said that the pathway Trp to Nam plays a critical role in preventing Nam deficiency pellagra in humans, because protein malnutrition frequently causes pellagra.[4] In order to calculate the conversion ratio of Trp to Nam, animals and humans must eat a special diet that configures a preformed niacin-free refined diet for several days.[5] This means that calculating the conversion ratio is very difficult.

Shibata[6] had found that the conversion ratio of Trp to Nam is affected by age, and the excretory ratio of (2-Py + 4-Py)/MNA is too, but the conversion ratio could not be calculated in the experiment[6] because the diet of rats contained a pre-formed niacin (niacin is a generic name for Nam and nicotinic acid).

We thought of the possibility that the excretory ratio of (2-Py + 4-Py)/MNA can be used as a surrogate biomarker of the conversion ratio of Trp to Nam during the growing period of rats. As a first step, we investigated the relationship between the excretory ratio and the conversion using 24-h urine samples. The urinary excretory ratio of Nam catabolites was associated with the conversion ratio of Trp to Nam in growing rats fed a niacin-free 20% casein diet. We report these results in detail here.

The care and treatment of the experimental animals confirmed to The University of Shiga Prefecture Guidelines for the Ethical Treatment of Laboratory Animals. The room temperature was maintained at about 22 °C and about 60% humidity and a 12 h/12 h light/dark cycle (06:00–18:00/18:00–06:00) was imposed.

Male 3-week-old Wistar rats purchased from CLEA Japan (Tokyo) were placed immediately in individual CL-301 metabolism cages purchased from CLEA Japan, and were fed freely with a conventional purified diet consisting of 20% vitamin-free milk casein, 0.2% L-methionine, 46.9% gelatinized cornstarch, 23.4% sucrose, 5% corn oil, 3.5% AIN-93-G mineral mixture,[7] and a 1% AIN-93 vitamin mixture[7] containing chorine bitartrate, but without niacin, for 30 d.

Twenty four-h urine samples were collected from 9:00 to next 9:00 for days 7, 16, 23, and 30 of the experiment in amber bottles containing 1 mL of 1 mol/L HCl, and were stored at −20 °C until needed. The urine contents of Nam, 2-Py, and 4-Py were measured simultaneously by the HPLC method of Shibata *et al.*[8] The urine content of MNA was also measured by this method.[9] The conversion ratio was calculated by comparing the Trp intake during urine collection with the sum of urinary excretion of Nam, MNA, 2-Py, and 4-Py.[10]

Pearson correlation coefficients were calculated to determine the association between the conversion ratio of Trp to Nam and the urinary excretory ratio of (2-Py + 4-Py)/MNA. The calculation was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

The weaning rats had free access to the niacin-free 20% casein diet for 30 d. The changes in food intake and in growth during the experiment were normal. Figure 1 shows the urinary excretion of Nam, MNA, 2-Py, and 4-Py. These compounds increased with age. The conversion ratio of Trp to Nam increased with age, as shown in Fig. 2A, and the excretory ratio of (2-Py + 4-Py)/MNA also increased with age as shown in Fig. 2B.

Figure 3 shows the relation found between the conversion ratio of Trp to Nam and the urinary excretory ratio of Nam catabolites. The Pearson coefficient value

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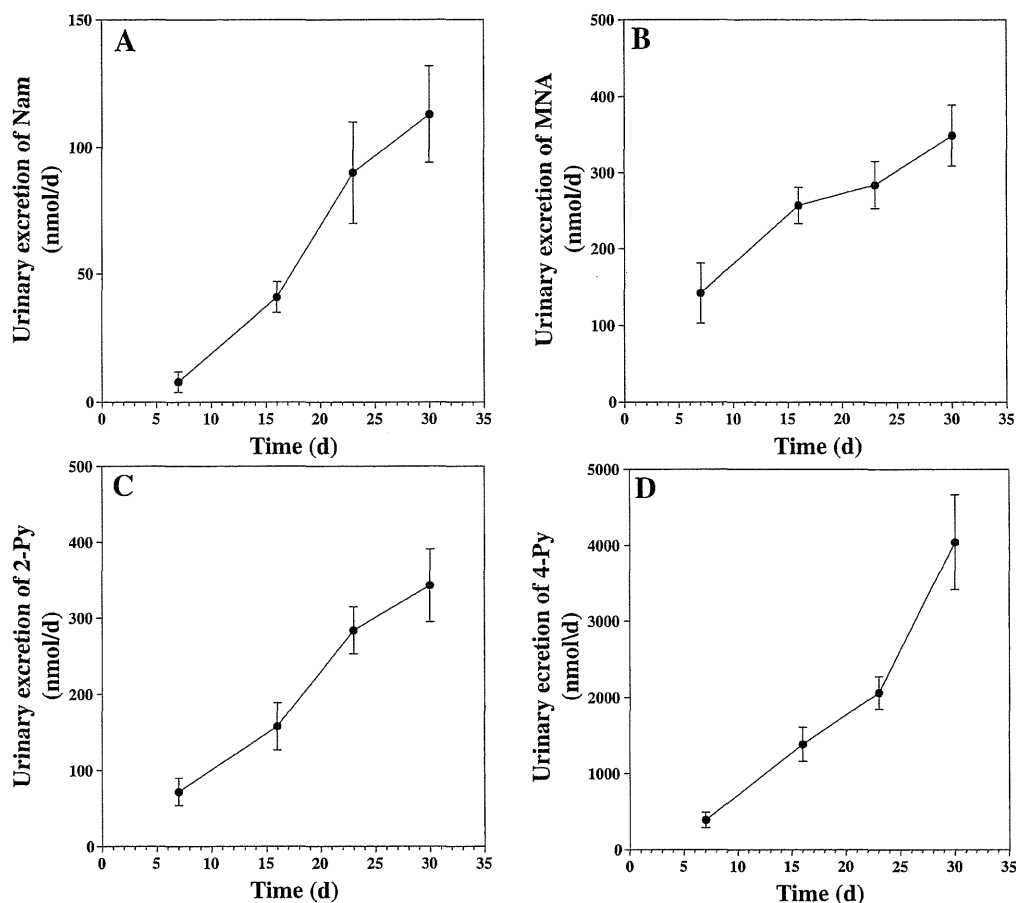
Fig. 1. Effects of Age on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C), and 4-Py (D).
Symbols mean represent ± SEM for six rats.
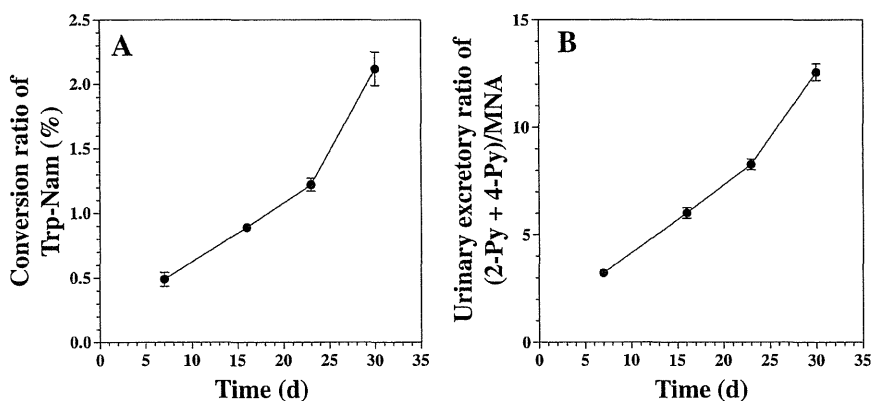
Fig. 2. Effects of Age on the Conversion Ratio of Trp to Nam (A) and the Urinary Excretory Ratio of (2-Py + 4-Py)/MNA (B).
Symbols mean represent ± SEM for six rats.

was 0.90, and p was 0.03. This correlation is significant. A very strong correlation was found between the urinary excretory ratio of Nam catabolites (2-Py + 4-Py)/MNA in the 24-h urine samples and the Trp-Nam conversion during the growing period of the rats.

Pellagra results from a diet deficient in Nam and/or Trp. This disease is considered a public health problem in many maize-consuming African and Asian countries, especially populations facing to emergency and conflict.[11–15]

Krehl et al.[16] found that Trp could completely counteract the growth retardation caused by corn grits

diet in rats. The conversion ratio of Trp to Nam is not constant: It is affected by age,[5] various nutritional factors,[10,17–25] hormones,[26–28] and chemicals.[29–31] Therefore, it is important in preventing a pellagra outbreak to know the conversion ratio of Trp to Nam under the conditions, but it is not possible to know this in case of emergency and conflict.

As for the biomarkers of pellagra, it is known that the blood NAD level does not reflect Nam nutritional status in pellagra patients,[32] and that the Nam itself does not appear in the urine even in healthy people.[3] On the contrary, urinary excretion of Nam catabolites such as

Fig. 3. Relation between the Conversion Ratio of Trp to Nam and the Urinary Excretory Ratio of Nam Catabolites.

Symbols mean represent ± SEM for six rats. The Pearson coefficient value was 0.90, and p was 0.03. The correlation is significant.

MNA, 2-Py, and 4-Py, and the excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples, are generally used as a laboratory test.[14]

Shibata and co-workers[10,17-22,33] found that the urinary excretory ratio of (2-Py + 4-Py)/MNA primarily reflected protein nutritional status, not Nam nutritional status, because the excretory ratio was decreased by the administration of an extremely large amount of Nam[34] and MNA[35] in rats. In addition, Shibata et al.[36] reported that the administration of 150 mg/d of Nam did not affect the excretory ratio in humans. Thus, increases in the excretory ratio do not bring improved Nam nutritional status. Shibata[33] proposed that the Nam catabolite excretory ratio reflects protein nutritional status.

Collection of a 24-h urine sample and feeding of a niacin-free refined diet are very hard to achieve in emergency and conflict situations. (2-Py + 4-Py)/MNA can be measured by using a spot urine sample instead of a 24-h urine sample. Therefore, it appears to be possible that the conversion ratio of Trp to Nam can be deduced by a spot urine sample instead of using a 24-h urine sample.

It is necessary to examine whether the same result obtains when weaning rats are fed a diet containing other proteins or different concentrations of dietary proteins. In addition, it is also necessary to examine diurnal variations in the urinary excretory ratio of (2-Py + 4-Py)/MNA, even though the collection of spot urine samples from rats is difficult.

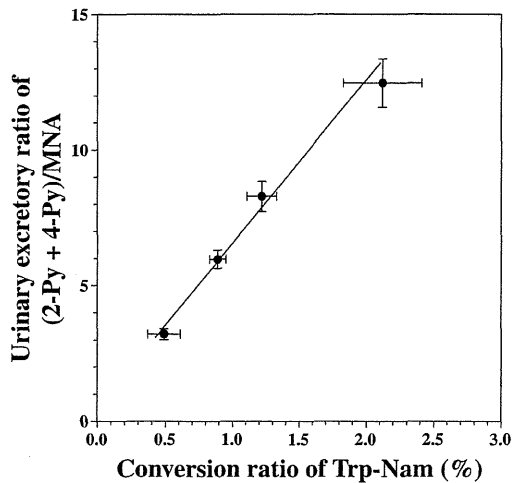In the future, we plan to study the relation between the conversion ratio of Trp to Nam in 24-h urine samples and the urinary excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples the growing period of humans.

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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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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

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

Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS\cdot HCl$; molecular weight 337·27), riboflavin ($C_{17}H_{20}N_4O_6$; 376·37), pyridoxine hydrochloride ($C_8H_{11}NO_3\cdot HCl$; 205·63), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$; 1355·40), nicotinamide ($C_6H_6N_2O$; 122·13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}\cdot Ca$; 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 ($C_7H_9N_2O\cdot HCl$; 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].

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 $\textbf{\textit{g}}$ 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$_2$PO$_4$–K$_2$HPO$_4$ 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 $\textbf{\textit{g}}$ 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$_2$PO$_4$–K$_2$HPO$_4$ 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 $\textbf{\textit{g}}$ 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$_2$PO$_4$–K$_2$HPO$_4$ 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 $\textbf{\textit{g}}$ for 10 min at 4°C. The supernatant was dialysed against a large amount of 50 mM-KH$_2$PO$_4$– K$_2$HPO$_4$ 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$_2$SO$_4$ 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 $\textbf{\textit{g}}$ 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

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.

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 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

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 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).

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.

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

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