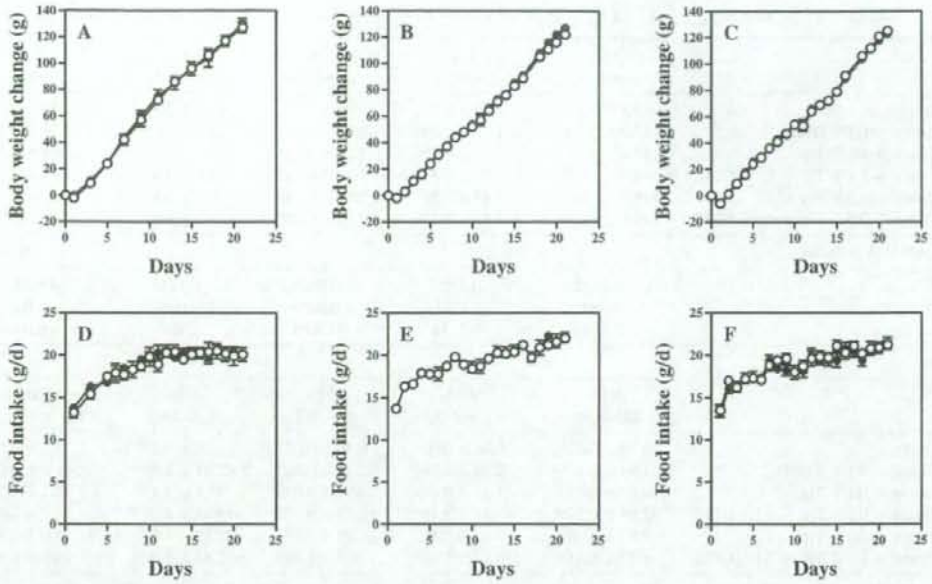

Fig. 1. Effect of Adding Free Trp to a 0.1% DEHP Diet on Body Weight and Food Intake.

Male rats of the Wistar strain (6 weeks old) were placed in individual metabolic cages. They were divided into six groups and then fed freely a 20% casein diet supplemented with 0% Trp (A and D), 0.1% Trp (B and E), or 0.5% Trp (C and F) with or without 0.1% DEHP for 21 d. ○, 0% DEHP diet; ●, 0.1% DEHP diet. Each point is the mean ± SEM (n = 5).

liver of each animal was removed, and a portion (approximately 0.2 g) was immediately treated as described in the literature.[18,19]

The contents of NAD ($NAD^+ + NADH$) and NADP ($NADP^+ + NADPH$) were measured by the colorimetric methods of Shibata and Murata[18] and Shibata and Tanaka[19] respectively. The urinary contents of Nam and of the catabolic metabolites MNA, 2-Py, and 4-Py were measured. The contents of Nam, 2-Py, and 4-Py in the urine were simultaneously measured by the HPLC method of Shibata *et al.*,[16] while the content of MNA in the urine was measured by the HPLC method of Shibata.[20] The contents of KA,[21] XA,[22] 3-HA,[22] AnA,[23] and QA[24] in the urine were measured by the HPLC method.

We have reported that growth retardation of young rats due to DEHP appeared at a level of 1.0% on a 20% casein diet.[7] Body weight gain on day 21 was 68.2 ± 2.5 g in the 40% casein diet group. It was 62.4 ± 2.7 g on a diet with 0.1% DEHP group, with no growth retardation, while growth retardation was observed at 0.5% on the 40% casein diet. Body weight gains at day 21 were 21.2 ± 3.9 g, 11.8 ± 3.5 g, and −9.4 ± 2.1 g on the diets with 0.5%, 1.0%, and 2.0% respectively.

The addition of free Trp to the DEHP diet did not affect body weight gain or food intake, as shown in Fig. 1. Liver weight was higher on each DEHP diet than on respective non-DEHP diet, as shown in Table 1. Liver weight was not affected by adding free Trp. NAD concentrations in the livers showed a tendency to increase with Trp intake. DEHP administration caused further increases in NAD concentration.

Table 1. Effect of Adding Free Trp to a DEHP Diet on Liver Weight and NAD Contents in Liver

	Liver weight (g/rat)	NAD (nmol/g of liver)
20% Casein	12.09 ± 0.36	830 ± 63[1]
20% Casein + 0.1% DEHP	17.61 ± 0.47*	1179 ± 86*,a
20% Casein + 0.1% Trp	12.31 ± 0.31	845 ± 51[1,2]
20% Casein + 0.1% Trp + 0.1% DEHP	17.54 ± 0.46*	1341 ± 95*,a,b
20% Casein + 0.5% Trp	12.61 ± 0.28	1066 ± 43[2]
20% Casein + 0.5% Trp + 0.1% DEHP	17.30 ± 0.71*	1458 ± 36*,b
2-way ANOVA *p*-values		
DEHP	<0.0001	<0.0001
Trp	0.9721	0.0024
DEHP × Trp	0.6560	0.5281

Each value is the mean ± SEM (n = 5); a different superscript letter means significant difference at $p < 0.05$, calculated by Bonferroni post test.
*; Significant difference between groups on the same Trp diets.
Numbers; Significant difference among groups on non-DEHP diets.
Letters; Significant difference among groups on 0.1% DEHP diets.

The metabolites of Trp to Nam were increased dose-dependent manner due to the addition of free Trp, as shown in Table 2. DEHP intake caused QA to increase extremely, as well as metabolites such as Nam, MNA, 2-Py, and 4-Py. Therefore, the conversion ratio of Trp to

Table 2. Effects of Adding Trp to a DEHP Diet on the Urinary Excretion of Metabolites Involved in Trp-Nam Pathway

	AnA (nmol/d)	KA (μmol/d)	XA (μmol/d)	3-HA (nmol/d)	QA (μmol/d)
20% Casein	33.1 ± 9.2^{1}	1.19 ± 0.20^{1}	1.05 ± 0.16^{1}	66.5 ± 3.8^{1}	0.54 ± 0.11
20% Casein + 0.1% DEHP	37.2 ± 5.2^{a}	1.37 ± 0.19^{a}	0.80 ± 0.04^{a}	78.0 ± 14.3^{a}	2.14 ± 0.19^{a}
20% Casein + 0.1% Trp	52.6 ± 5.8^{2}	2.39 ± 0.13^{2}	4.18 ± 0.30^{2}	46.8 ± 1.4^{1}	1.04 ± 0.11
20% Casein + 0.1% Trp + 0.1% DEHP	64.9 ± 1.6^{b}	2.80 ± 0.33^{b}	$2.10 \pm 0.31^{*,b}$	51.9 ± 4.9^{a}	7.90 ± 0.82^{a}
20% Casein + 0.5% Trp	118.9 ± 13.8^{3}	5.84 ± 0.59^{3}	9.67 ± 0.44^{3}	110.2 ± 9.6^{2}	2.74 ± 0.31
20% Casein + 0.5% Trp + 0.1% DEHP	140.1 ± 12.3^{c}	6.52 ± 0.17^{c}	$4.19 \pm 0.20^{*,c}$	114.2 ± 9.0^{b}	$54.66 \pm 8.51^{*,b}$
2-way ANOVA *p*-values					
DEHP	0.1022	0.1059	<0.0001	0.3237	<0.0001
Trp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DEHP × Trp	0.6440	0.7154	<0.0001	0.8895	<0.0001

	Nam (μmol/d)	MNA (μmol/d)	2-Py (μmol/d)	4-Py (μmol/d)	Conversion ratio of Trp to Nam (%)
20% Casein	0.14 ± 0.02^{1}	0.36 ± 0.02	0.36 ± 0.02	3.76 ± 0.29^{1}	2.3 ± 0.1
20% Casein + 0.1% DEHP	$1.08 \pm 0.11^{*,a}$	2.28 ± 0.18^{a}	2.35 ± 0.30^{a}	$12.81 \pm 1.40^{*,a}$	$11.7 \pm 0.6^{*,a}$
20% Casein + 0.1% Trp	$0.42 \pm 0.05^{1,2}$	1.45 ± 0.06	0.57 ± 0.09	9.08 ± 1.45^{2}	3.7 ± 0.5
20% Casein + 0.1% Trp + 0.1% DEHP	$2.66 \pm 0.33^{*,b}$	$45.14 \pm 3.18^{*,b}$	$10.23 \pm 0.75^{*,b}$	$19.13 \pm 1.29^{*,b}$	$22.7 \pm 1.0^{*,b}$
20% Casein + 0.5% Trp	0.83 ± 0.06^{2}	3.92 ± 0.32	1.99 ± 0.19	22.28 ± 1.62^{3}	4.0 ± 0.3
20% Casein + 0.5% Trp + 0.1% DEHP	$6.17 \pm 0.44^{*,c}$	$146.94 \pm 5.68^{*,c}$	$17.07 \pm 1.48^{*,c}$	17.83 ± 1.70^{b}	$24.3 \pm 0.9^{*,b}$
2-way ANOVA *p*-values					
DEHP	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Trp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DEHP × Trp	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Each value is the mean ± SEM (n = 5); a different superscript letter means significant difference at $p < 0.05$, calculated by Bonferroni post test.
*: Significant difference between the groups on the same Trp diets.
Numbers; Significant difference among the groups on non-DEHP diets.
Letters; Significant difference among the groups on 0.1% DEHP diets.

Nam was higher in each of DEHP groups than in the respective non-DEHP groups.

Shibata and Iwai have reported in 1980 that phthalic acid, an analog of QA, is a strong competitive inhibitor of quinolinate phosphoribosyltransferase (QPRT).[25] Hence, we investigated to determine whether the administration of phthalic acid esters, widely used as plasticizers, would cause adverse effects on rats when they were fed a niacin-deficient diet. Shibata *et al.* found in 1982 that growth promoting activity due to appropriate phthalic acid esters occurred when rats were fed a niacin-free, Trp-limiting diet.[26] We were surprised at that phenomenon, and so we started to sort out the contradiction. We found and have reported that mono(ethylhexyl)phthalate (MEHP), a metabolite of DEHP, inhibits the enzyme activity of ACMSD, and increases the formation of QA, causing it to increase further metabolites such as Nam.[7–12] Phthalic acid does not accumulate in the liver, so the reaction of QPRT might not be inhibited.

Phthalic acid esters are known to have adverse effects on mammals.[4] We have reported that the addition of a large amount of DEHP caused growth restriction.[7] Under such conditions, the conversion ratio of Trp to Nam increased extremely up to 30%, which mean that Nam formation in the body was about 35 mg/kg body weight of rat.[7] Nam administration of 40 mg/kg of body weight of rat caused significant growth retardation.[14] Hence, we present the hypothesis that part of the growth retardation induced by phthalic acid esters is due to the production of excess Nam. In the present experiment, we investigated whether the administration of a high-protein diet that contained high Trp to rats could cause a strengthening of growth retardation by DEHP. Growth retardation was observed at a level of 0.5% DEHP in the 40% casein diets, while we have reported that in the 20% casein diets, the retardation appeared at a level of 1.0% DEHP.[7] We found that feeding the high protein diet strengthened growth retardation due to DEHP. We investigated to determine whether the strengthened growth retardation due to feeding the high protein diet is attributed to high Trp intake. The addition of Trp only to the 20% casein diet containing 0.1% DEHP did not cause apparent growth retardation. A concentration of 0.1% DEHP was chosen because we anticipated that growth retardation would be strengthened by the addition of Trp, but no retardation of the body weight gain or food intake in young rats was observed (Fig. 1), although the conversion ratio was over 20% in the group consuming a 0.1% free Trp added diet containing 0.1% DEHP (Table 2), which indicates 60 mg of Nam formation per kilogram of body weight. Furthermore,

in the group consuming a 0.5% free Trp added diet containing 0.1% DEHP, Nam formation in the body was about 150 mg per kilogram body weight. Nevertheless, the body weight gain and food intake were almost the same as in the group on the 20% casein diet (control). These results indicate that our hypothesis that part of DEHP-growth retardation is due to an excess of Nam, is not plausible. In addition, the present data indicate that the growth retardation level in young rats exogenously taken excess Nam is lower than in rats endogenously formed excess Nam. This phenomenon implies that the peak concentration of Nam is the most important point in the toxicity of Nam, that is, as to whether Nam causes growth retardation. Even if the endogenous formation of Nam in 1 d are almost the same as the administered amount of exogenous Nam in 1 d, the peak Nam concentration in the body should not be much lower in endogenous Nam than in exogenous Nam, because endogenous Nam is continuously metabolized, or synthesized and catabolized, while the exogenous Nam causes an abrupt increase in the concentration of Nam in the body.

Acknowledgment

This report is a part of a continuing study by Katsumi Shibata on alleviation of the effects of putative endocrine disruptor phthalic acid esters. It was supported by a University of Shiga Prefecture grant for keynote research.

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Age-Related Alterations of B-Group Vitamin Contents in Urine, Blood and Liver from Rats

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Summary To investigate how aging alters B-group vitamin metabolism, rats were fed with niacin-free 20% casein diet from 3 to 80 wk old, and the urinary excretions of the B group vitamins were periodically measured. The blood and liver B-group vitamin levels in 80-wk-old rats were also compared with those in 8-wk-old rats. The urinary excretion of thiamin, riboflavin, vitamin B_6 metabolite 4-pyridoxic acid, pantothenic acid, folic acid and biotin were not altered during 540 d. The urinary vitamin B_{12} increased by 8-fold at 29 wk old, and further increased at 80 wk old. Conversion of nicotinamide from tryptophan gradually decreased to 60% from 29 to 48 wk old. Plasma PLP, vitamin B_{12} and folate levels in 80-wk-old rats were lower than those in 8-wk-old rats, consistent with lower liver vitamin B_6 and folate levels in aged rats. Plasma and liver biotin levels in aged rats were higher than those in young rats. Other B-group vitamins such as vitamin B_1, vitamin B_2, niacin and pantothenic acid levels in blood and liver from aged rats were same as those from young rats. Alteration of vitamin B_6 metabolism in particular is similar to the observations in eldery humans reported previously. Our findings suggest that aged rats can be useful models to investigate aging-related B-group vitamin metabolism.

Key Words aging, vitamin B_2, vitamin B_6, folate, biomarker

The precise cascade of pathological age-related events is still not clearly understood, but enhanced production of free radicals and its deleterious effects on macro-biomolecules such as proteins and DNA (*1*, *2*) as well as polyunsaturated fatty acids (*3*) are well known during aging. Aging is a major risk factor for various chronic disorders including cancer. It is well known that taking in nutrients suitable for life stages is important to prevent the aging process and chronic disorders. Food, namely nutrient intake, is a key factor affecting aging and the incidence of many chronic disorders (*4*–*6*). Decreased food intake and a sedentary lifestyle reduce energy expenditure and may alter their metabolism in elderly adults, and alteration of metabolism may cause alteration of nutrient requirement. However, there is not sufficient information on how aging alters vitamin metabolism (*7*, *8*). To investigate age-related alterations of B-group vitamins, rats were kept for 540 d from 3 to 80 wk old, and the urinary excretions of the B group vitamins were periodically measured. The blood and liver B-group vitamin levels in 80-wk-old rats were also compared with those in 8-wk-old rats.

MATERIALS AND METHODS

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, the mineral mixture (AIN-93M) (*9*), the niacin free-vitamin mixture (AIN-93-VX containing 25% choline bitartrate) (*9*) and nicotinamide adenine dinucleotide (NAD) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Thiamin hydrochloride, thiamin diphosphate (TDP) chloride, riboflavin, pyridoxine hydrochloride, pyridoxal 5′-phospahte (PLP), nicotinamide, calcium pantothenate, pteroylmonoglutamic acid (folic acid) and D(+)-biotin were purchased from Wako Pure Chemical Industries (Osaka, Japan). Thiamin monophosphate (TMP) chloride dihydrate and lumiflavin were purchased from Sigma-Aldrich Japan (Tokyo, Japan). 4-Pyridoxic acid (4-PIC) was made by ICN Pharmaceuticals (Costa Mesa, California, USA) and obtained through Wako Pure Chemical Industries. N^1-Methylnicotinamide (MNA) chloride was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized (*10*, *11*). All other chemicals used were of the highest purity available from commercial sources.

Animals and diets. The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Male Wistar rats aged at 3 wk old were obtained from CLEA Japan, Inc. (Tokyo, Japan) and immediately placed in individual wire-bottomed cages (260×180×380 mm, wide×height×depth). Rats fed ad libitum a niacin free-20% casein diet (Table 1) for 35 or 540 d.

The room temperature was maintained at around 22°C and about 60% humidity, and a 12-h light

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Table 1. Composition of the diet.

	%
Milk casein (vitamin-free)	20.0
L-Methionine	0.2
Gelatinized cornstarch	46.8
Sucrose	23.5
Corn oil	5.0
Mineral mixture (AIN-93)	3.5
Nicotinic acid free-vitamin mixture (AIN-93-VX)	1.0

(06:00–18:00)/12-h dark (18:00–06:00) cycle was maintained. Body weight and food intake were measured every 2 or 3 d at around 09:00. Urine samples (24-h; 09:00–09:00) were collected in amber bottles containing 1 mL of 1 mol/L HCl at 14, 23, 32, 39, 51, 57, 63, 70, and 80 wk during the experiment, and were stored at −20°C until needed. Five rats were killed at 8 wk old, and the other was at 80 wk old by decapitation at around 09:00. Blood was collected, and the liver was dissected to measure B-group vitamin.

Determination of vitamins and their metabolites in urine, blood and liver. For vitamin measurements, known concentration reconstituted samples were prepared as quality control (QC), and QC samples were analyzed for validation in all analysis. Stability of samples was also confirmed, and no degradation was observed for any vitamin measurement.

Vitamin B_1: Vitamin B_1 contents in blood and liver were determined as the sum of thiamin, TMP and TDP. Five percent trichloroacetic acid was added to whole blood, and supernatant of the mixture was used for measurement. Liver was homogenized in a 5% trichloroacetic acid, and supernatant of the homogenate was used for measurement. Urinary thiamin, blood vitamin B_1 and liver vitamin B_1 were determined by the HPLC-post labeled fluorescence method (*12*).

Vitamin B_2: Riboflavin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in blood and liver were converted to lumiflavin by photolysis, and lumiflavin was determined as vitamin B_2 by the HPLC method (*13*). Urinary riboflavin was determined by the HPLC method (*13*).

Vitamin B_6: Vitamin B_6 vitamers including phosphate esters in the liver were converted to free vitamin B_6 vitamers such as pyridoxal and pyridoxine by autoclave under acidic condition, and measured as total vitamin B_6 by the microbioassay method using *Saccharomyces carlsbergenisis* strain 4228 ATCC 9080 (*14*). Plasma PLP was determined by the HPLC method (*15*). Urinary 4-PIC was determined by the HPLC method (*16*).

Vitamin B_{12}: Liver homogenate, plasma or urine were added to 0.2 mmol/L acetate buffer (pH 4.8), and the vitamin B_{12} was converted to cyanocobalamin by boiling with 0.0006% potassium cyanide at acidic pH (*17*). Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmanii*, ATCC 7830 (*17*).

Niacin: NAD and NADP in the liver homogenate were converted to nicotinamide by autoclave, and nicotinamide was determined by the HPLC method (*11*). The contents of NAD (NAD^++NADH) in whole blood were measured by the colorimetric method (*18*). Urinary 2-Py, 4-Py and MNA, nicotinamide metabolites, were determined by the HPLC method (*11*).

Pantothenic acid: To digest bound pantothenic acid such as CoA and pantetheine in liver and blood to free form, liver homogenate or blood was incubated with 0.5 U alkaline phosphatase and 2% pigeon liver amidase in 250 μL of 20 mmol/L phosphate buffer (pH 7.0) at 37°C for 2 h. Pantothenic acid in urine, liver and blood were determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (*19*).

Folic acid: Folate in liver was digested to pteroylmonoglutamic acid by conjugase and protease (*20*). Plasma and urinary folate, and pteroylmonoglutamic acid digested from liver were determined by the microbioassay method using *Lactobacillus casei* ATCC 2733 (*20*).

Biotin: Bound biotin in liver was converted to free form by autoclave under acidic conditions, and biotin in urine, plasma and liver were determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (*21*).

Statistical analysis. For the statistical evaluation, the significance of the differences in the mean values between young and aged rats was tested by using Student's, two-tailed *t*-test and non-parametric Mann-Whitney U test. The differences of $p<0.05$ were considered to be statistically significant. Instat software (version 2.00; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

RESULTS

Body weight and food intake

The body weight increased linearly until approximately 10 wk old, and gradually increased to 80 wk old (Fig. 1A). This growth curve was the same as the data obtained from CLEA Japan, Inc. (*22*). The daily food intake was increased until approximately 10 wk old, and then kept constant around 20 g/d (Fig. 1B).

Comparison of blood B-group vitamin concentrations between young and aged rats

Table 2 shows comparison of B-group vitamin concentrations in whole blood or plasma between 8-wk-old young and 80-wk-old aged rats. Blood vitamin B_1, blood vitamin B_2, blood NAD and blood pantothenic acid concentrations in aged rats were the same as those in young rats. Plasma PLP, plasma vitamin B_{12} and plasma folate concentrations in aged rats were significantly lower than those in young rats, while plasma biotin in aged rats was significantly higher than that in young animals.

Comparison of liver B-group vitamin levels between young and aged rats

Table 3 shows comparison of liver B-group vitamin levels between 8-wk-old young and 80-wk-old aged rats. Vitamin B_1, vitamin B_2, nicotinamide and pantothenic acid levels in aged rats were the same as those

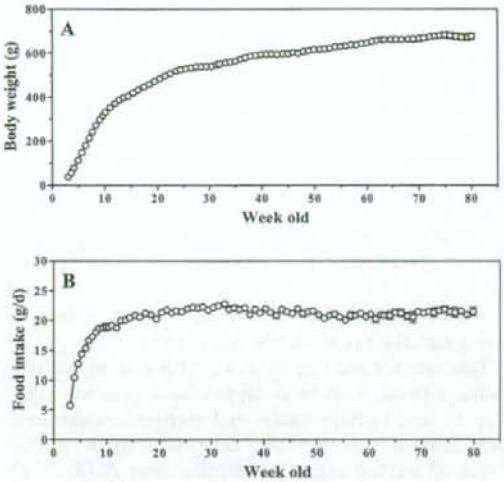

Fig. 1. Body weight (A) and daily food intake (B) in male rats of the Wistar strain fed with a 20% casein diet. Twenty male rats of the 3-wk-old Wistar strain were fed a niacin free-20% casein diet (Table 1) for 540 d. Values are expressed as means±SE for 20 rats.

Table 2. Comparison of the blood or plasma B-group vitamin levels between young and aged rats.

	Young rats[1] (8 wk old)	Aged rats[2] (80 wk old)
Blood vitamin B_1 (pmol/mL)	346±19	378±13
Blood vitamin B_2 (pmol/mL)	169±14	151±18
Plasma PLP (nmol/mL)	2.10±0.13	0.24±0.02*
Plasma vitamin B_{12} (pmol/mL)	7.18±0.09	1.13±0.06*
Blood NAD (NAD^++NADH) (nmol/mL)	79.4±3.8	78.4±2.9
Blood pantothenic acid (nmol/mL)	2.96±0.27	3.43±0.12
Plasma folate (pmol/mL)	277±11	52.4±2.9*
Plasma biotin (pmol/mL)	27.8±1.9	64.8±4.4*

[1]Values are expressed as mean±SE for 5 rats.
[2]Values are expressed as means±SE for 18–20 rats.
*Significant difference at $p<0.05$ between young and aged rats, as determined by non-parametric Mann-Whitney *U* test.

in young rats. Vitamin B_6 and folate levels in aged rats were significantly lower than those in young rats, while biotin in aged rats was significantly higher than that in young rats.

Age-associated alteration of urinary excretion of B-group vitamins

Urinary excretion of thiamin, riboflavin, vitamin B_6 metabolite 4-PIC, pantothenic acid and folic acid were not altered from 14 to 80 wk old (Fig. 2A, B, C, E and F). The urinary recovery rate of thiamin, riboflavin, 4-PIC, pantothenic acid and folic acid were approximately 20, 30, 40, 60 and 10%, respectively. The urinary excretion of cyanocobalamin increased from 23±2 to 178±9 pmol/d between 23 and 32 wk old, and then increased from 217±20 to 518±40 pmol/d between 70 and 80 wk old (Fig. 2D). The urinary recovery rate of cyanocobalamin was approximately 10% at 23 wk old, 40% at 51 wk old and 130% at 80 wk old. The urinary excretion of biotin increased from 1.9±0.1 to 4.9±0.2 nmol/d between 23 and 32 wk old, and kept constant after 32 wk old (Fig. 2G). The urinary recovery rate of biotin was approximately 10% at 23 wk old and 30% at older than 32 wk old.

Table 3. Comparison of the liver B-group vitamin contents between young and aged rats.

	Young rats[1] (8 wk old)	Aged rats[2] (80 wk old)
Vitamin B_1 (nmol/g)	36.9±2.3	24.4±0.9
Vitamin B_1 (nmol/g)	74.3±3.8	64.8±2.2
Vitamin B_6 (nmol/g)	48.9±1.4	19.9±1.2*
Vitamin B_{12} (pmol/g)	91.5±9.7	not measured
Nicotinamide (nmol/g)	932±28	1,160±70
Pantothenic acid (nmol/g)	551±37	455±13
Folate (nmol/g)	26.3±1.3	13.6±0.6*
Biotin (nmol/g)	1.26±0.10	1.86±0.13*

[1]Values are expressed as mean±SE for 5 rats.
[2]Values are expressed as means±SE for 18–20 rats.
*Significant difference at $p<0.05$ between young and aged rats, as determined by non-parametric Mann-Whitney *U* test.

Age-associated alteration of the conversion ratio of tryptophan-nicotinamide

Since nicotinamide and its coenzyme forms were sufficiently supplied only from tryptophan via the tryptophan-nicotinamide conversion pathway in rats fed with a niacin-free 20% casein diet (23), and tryptophan-nicotinamide metabolism can be assessed in the rats fed with a niacin-free 20% casein diet. The urinary excretion of nicotinamide metabolites gradually decreased to 60% from 32 to 51 wk old (Fig. 3A). The conversion ratio of tryptophan to nicotinamide also gradually decreased from 1.5 to 0.8% (Fig. 3B).

DISCUSSION

To investigate the alterations of B-group vitamin metabolism during aging, 3 wk old rats were kept for 540 d until 80 wk old. Urinary excretion of B-group vitamins was periodically measured from 14 to 80 wk old, and blood and liver B-group vitamin levels in aged rats were compared with those in young rats. As for vitamin B_6 and folic acid, the blood and liver contents in aged rats were much lower than those in young rats, while aging did not affect their urinary excretion. Plasma vitamin B_{12} in aged rats was also lower than that in young rats. Plasma and liver biotin levels in aged rats were higher than those in young rats, and urinary biotin also increased in aged rats. As for vitamin B_1, vitamin B_2 and pantothenic acid, aging did not affect their blood and liver contents or urinary excretion. These findings show that aging affects the metabolism

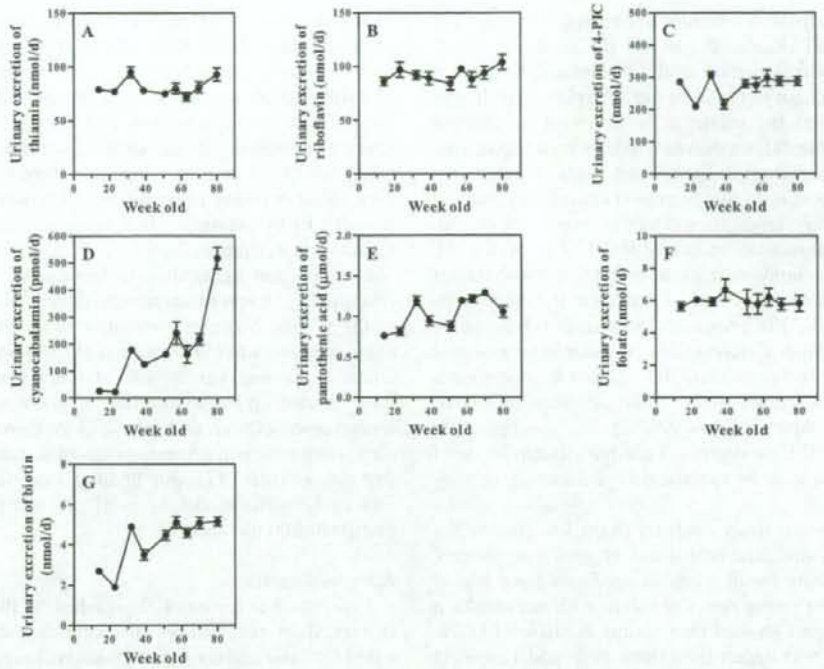

Fig. 2. Age-associated alteration of the urinary excretion of thiamin (A), riboflavin (B), 4-pyridoxic acid (4-PIC) (C), cyanocobalamin (D), pantothenic acid (E), folate (F) and biotin (G). Values are expressed as means±SE for 20 rats.

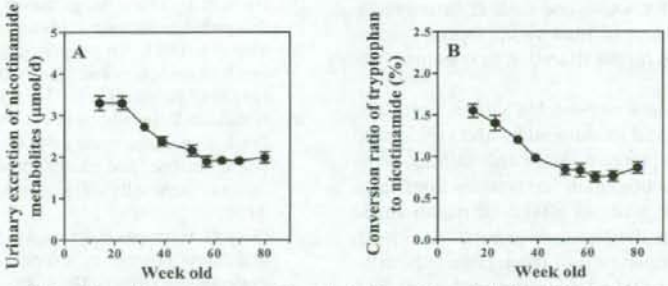

Fig. 3. Age-associated alteration of the urinary excretion of nicotinamide metabolites. Values are expressed as means±SE for 20 rats.

of some B-group vitamins, such as vitamin B_6, vitamin B_{12}, folic acid and biotin.

Plasma PLP concentration in elderly adults aged 65–75 y was lower than that in young adults aged 25–35 y, while dietary vitamin B_6 intake and urinary 4-PIC were the same among these groups (*24*), consistent with the present results for age-related alteration of vitamin B_6 metabolism obtained from the animal experiment. The vitamin B_6 requirements of the elderly over 60 y were determined as 1.9–2.0 mg/d from their plasma PLP and urinary 4-PIC (*25*), and these values are higher than those reported for younger adults (*26*). An oral vitamin B_6 loading study shows that vitamin B_6 absorption and ability to synthesize PLP are not different between elderly and young adult (*24*). Since urinary 4-PIC did not change in elderly adults (*24*) or aged rats, one of the possibilities to explain age-related PLP decrease may the regulation of 4-PIC production in the liver. Further study will elucidate the mechanism of age-related vitamin B_6 metabolism.

The framingham cohort study showed that prevalence of low serum cobalamin concentration in elderly people was 2.3 times higher than that in control young subjects (*27*). Vitamin B_{12} is different from other B-group vitamin because of its mechanism of absorption. Free vitamin B_{12} binds with haptocorrin and gastric intrinsic factor (IF) in the stomach and the duodenum, respectively, and this IF-vitamin B_{12} complex attaches

to the intestinal IF-vitamin B_{12} receptor in the ileal mucosa, and vitamin B_{12} enters the enterocyte (*28*). The high prevalence of mild cobalamin deficiency in healthy older subjects can be partly explained by inadequate vitamin B_{12} intake or severe atrophic gastritis (*29*). Since we did not measure indices for atrophic gastritis or IF excretion in the present study, whether gastric function in aged rats decreased vitamin B_{12} status is unclear. Interestingly, the urinary recovery rate of vitamin B_{12} dramatically increased to 130% at 80 wk old. Vitamin B_{12} binds to carrier protein transcobalamin (TC) in serum (*30*), and TC–vitamin B_{12} complex is reabsorbed in the proximal convoluted tubule via a receptor-mediated system (*28*). Megalin is an essential receptor for reabsorption of TC–vitamin B_{12} complex in the proximal tubule (*31*). Although little is known about how aging affects vitamin B_{12} absorption or megalin's function, decreased plasma vitamin B_{12} level in aged rats may be explained by enhancing urinary loss.

In the present study, contrary to the low vitamin B_6, vitamin B_{12} and folic acid status in aged rats, plasma, liver and urine biotin levels in aged rats were higher than those in young rats. Consistent with our results, a previous report showed that plasma biotin level in 24-mo old rats was higher than those in 3- and 12 mo old rats (*32*). This age-related alteration of biotin status is due to an increase of biotin transport in the intestine (*32*). An epidemiological study for elderly Japanese people showed that the mean serum biotin level in elderly people aged over 65 y was same as that in reference people, while prevalence of high serum biotin concentration in elderly was higher than that in reference subjects (*33*).

Niacin is a unique water-soluble vitamin provided from nicotinic acid and nicotinamide, and synthesized from amino acid tryptophan. Since the amount of de novo synthesized nicotinamide derivatives from tryptophan is almost the same as intakes of nicotinamide and nicotinic acid in the Japanese population (*34*), to determine the conversion ratio of tryptophan to nicotinamide is important to calculate niacin equivalent. The rats were given a niacin-free diet in the present experiment, because to assess the age-related alteration of tryptophan-nicotinamide metabolism was difficult in rats fed with a niacin-containing diet. Weaning rats need 1 mg (8 μmol) of niacin in 100 g of diet or 85 mg (420 μmol) of tryptophan in 100 g of niacin-free diet for maximum growth (*35, 36*). Since the 20% casein diet used in the present study contains 230 mg (1.1 mmol) of tryptophan in 100 g of diet, and young rats convert 2% of tryptophan into nicotinamide in molar basis (*37*), the 20% casein diet contains enough tryptophan to supply nicotinamide for maximum growth in rats. Therefore our findings in the rats fed with the niacin-free 20% casein diet are considered to be applied to rats fed with niacin-containing diet. In the present study, the conversion ratio of tryptophan to nicotinamide was 1.6% at 14 wk old, gradually decreased to 0.8% until 51 wk old, and then kept this level at over 51 wk old. Blood NAD and liver nicotinamide levels in aged rats were the same as those in young rats, and these results suggested that the low conversion ratio was still enough to maintain niacin status for the aged rats fed with 20% casein diets. Our reports showed that urinary excretion of nicotinamide metabolites and blood NAD level in elderly Japanese were not different from those in young Japanese (*38, 39*). However, there is no report to investigate the tryptophan-nicotinamide metabolism in elderly people.

In conclusion, aging alters metabolism of vitamin B_6, vitamin B_{12}, tryptophan-nicotinamide, folic acid and biotin in rats. A strong association between homocysteine concentration and vitamin B_6, vitamin B_{12} and folate status was found (*40*), and many researchers have focused on the association of homocysteine and several diseases such as cardiovascular disease, depression, cognitive impairment, pregnancy complications and osteoporosis (*41*). Our findings suggest that aged rats can be useful models to investigate aging-related B-group vitamin metabolism.

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資　料

水溶性ビタミン混合剤投与中止1週間後の尿中水溶性ビタミン排泄量

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Urinary Excretion of Water-Soluble Vitamins One Week After Stopping Administration of the Water-soluble Vitamin Mixture

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The number of people taking large amounts of vitamin supplements is increasing in Japan. Taking large amounts of water-soluble vitamins is not considered risky because excess water-soluble vitamins are quickly excreted in the urine. In the present study, we investigated the clearance of water-soluble vitamins in Japanese. In the first week, six young female subjects ate the diet. In the second week, they ate the same diet with 6 times the recommended water-soluble vitamin dose based on the Dietary Reference Intake (DRI) for Japanese. In the third week, they ate the same diet without the vitamin mixture. The 24-h urine samples were collected every week. All water-soluble vitamins except vitamin B_{12} in 24-h urine samples were measured. The urinary excretion of nicotinamide metabolites, biotin, and ascorbic acid in the third week was same as that in the first week. Urinary thiamin in the third week was 4.4 times higher than in the first week, riboflavin was 3.4 times, vitamin B_6 metabolite 4-pyridoxic acid 2.0 times, pantothenic acid 2.4 times, and folic acid 3.1 times.

Key words: vitamin mixture, human, urine, excess, water-soluble vitamins

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緒　言

ある種の疾病がビタミン欠乏に起因することが20世紀初頭から半世紀をかけて明らかにされてきた[1]．20世紀の後半から現在にかけては，ビタミン欠乏症を予防するにはどの程度のビタミン摂取が必要であるかが明らかにされつつある[2]．このような中で，20世紀末には，ビタミンCに欠乏症である壊血病を予防する生理機能だけではなく，新たに抗酸化作用が見いだされ，この抗酸化作用を通じて疾病予防が期待できることが明らかにされたことにより[3)-6)]，あらためてビタミンの機能が注目されてきた．

このことがきっかけとなり，必要量を超えた各種ビタミンを摂取するヒトが増えてきた．水溶性ビタミンといっても，化学構造上の共通性はないため，異化代謝経路もさまざまであると推察される．また，大量に摂取した後のクリアランスの速度も異なることが予想されるが，未だ明確なデータはみあたらない．また，疫学調査において，ビタミン剤の摂取が無いときのビタミンの尿中排泄量を調べるうえで，ビタミン剤の補給を中止する期間を知る

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必要がある．本研究では，ヒトにおける各水溶性ビタミンのクリアランスを比較した．

実験方法

被験者

被験者は，あらかじめ実験内容の説明を受け，書類にて，実験への参加を希望した女子学生 6 名で，喫煙，飲酒の習慣がなく，朝食など規則正しい食習慣をもつ者である．被験者の身体的特徴を Table 1 に示した．本研究は，滋賀県立大学倫理審査委員会において承認を受け，ヘルシンキ宣言の精神に則って行われた．

食　事

2 種類の規定食を摂取させた．その栄養素成分は Table 2 と Table 3 に示した．ビタミン B_{12} を除く 8 種類の水溶性ビタミンは実測値である．他は五訂増補日本食品標準成分表を用いて計算した[7]．

実験計画

概略を Fig. 1 に示した．規定食摂取時の第 1 週の Day 4 の尿を採取・測定し，対照値とし，Data 1 とした．Day 5 ～ Day 7 は自由食としたが，日本人の食事摂取基準（2005 年版）に示された量の約 6 倍量の水溶性ビタミン混合（ビタミン B_{12} を除く）を投与した．第 2 週の Day 1 ～ Day 4 は規定食に加えて日本人の食事摂取基準（2005 年版）に示された量の約 6 倍量の水溶性ビタミン混合（Table 4）を投与した．第 2 週の Day 4 の尿を採取・測定し，Data 2 とした．Day 5 ～ Day 7 は自由食を摂取させた．但し，ビタミン混合の付加はしなかった．第 3 週は，クリアランスを知るために，第 1 週と同じ規定食を摂取させ，Day 4 の尿を採取・測定し，Data 3 とした．なお，付加量を食事摂取基準に示されたビタミン量を各々約 6 倍量とした理由は，明確に尿中排泄量の増大が認められ，かつ 1 週間適度の間投与し続けても過剰摂取による健康障害が現れないと思われた量と考えたからである．

蓄尿中の尿は氷中に保存し，24 時間尿の採取が終わると，直ちに容量を測定した．ビタミン B_1，ビタミン B_2，ビタミン B_6 異化代謝産物である 4-ピリドキシン酸，ニコチンアミド異化代謝産物である N^1-メチルニコチンアミド（MNA），N^1-メチル-2-ピリドン-5-カルボキサミド（2-Py），N^1-メチル-4-ピリドン-3-カルボキサミド（4-Py）の測定には，尿 9 mL に 1 mol/L HCl を 1 mL 添加し，-20℃で保存したサンプルを用いた．ビタミン C の測定には，尿 5 mL に 10％メタリン酸 5 mL を加え，-20℃で保存したサンプルを用いた．葉酸の測定には，尿 8.1 mL に 1 mol/L L-アスコルビン酸溶液 0.9 mL を添加し，-20℃で保存したサンプルを用いた．パントテン酸とビオチンの測定には，無処理尿を -20℃で保存したサンプルを用いた．

分析方法

ビタミン B_1

定量の標準品として使用したチアミン塩酸塩は和光純薬工業株式会社（大阪）から購入した．尿中のビタミン B_1 の定量方法は文献 8 に示した HPLC 法で行った．値はチアミンとして示した．

ビタミン B_2

定量の標準品として使用したリボフラビンは和光純薬工業株式会社（大阪）から購入した．尿中のビタミン B_2 の定量方法は，文献 9 に記載された HPLC 法に従って測定した．

ビタミン B_6 異化代謝産物 4-ピリドキシン酸

定量の標準品として使用した 4-ピリドキシン酸は Sigma Chemical Company（米国）から購入した．尿中の 4-ピリドキシン酸の定量方法は文献 10 に記載された HPLC 法を用いて測定した．

ニコチンアミド異化代謝産物 MNA，2-Py，4-Py

MNA 定量の標準品として使用した MNA 塩酸塩は東京化成工業株式会社（東京）から購入した．尿中の MNA の定量は，MNA を強アルカリ性下でアセトフェノンと縮合させることにより蛍光物質に変換した後測定する HPLC 法を用いた[11]．

2-Py 定量の標準品として使用した 2-Py は Pullman と Colowick[12] の方法にしたがって合成した．4-Py 定量の標準品として使用した 4-Py は Shibata ら[13] の方法にしたがって合成した．尿中の 2-Py および 4-Py は弱アルカリ性下で，

Table 1. Characteristics of the Subjects

Subjects	Age (Yr)	Height (cm)	Body weight (kg)	BMI
Female 1	21	161.0	50.0	19.3
Female 2	21	161.0	52.5	20.3
Female 3	21	162.0	46.0	17.5
Female 4	22	154.0	48.0	20.2
Female 5	21	160.5	53.0	20.6
Female 6	21	165.0	52.5	19.3
Mean	21.2	160.6	50.3	19.5
SD	0.4	3.6	2.9	1.1

Table 2. The Composition of the Diet 1

	Breakfast	Lunch	Dinner	Total
Energy (kcal)	402	689	617	1708
Protein (g)	19.5	23.8	25.2	68.5
Fat (g)	15.7	25.5	9.6	50.8
carbohydrates (g)	46.0	85.8	104.4	236.2
Fat-soluble vitamins				
Vitamin A (μg)	150	309	419	878
Vitamin D (μg)	1	0	2	3
Vitamin E (mg)	1.1	2.1	2.4	5.6
Vitamin K (μg)	8	204	98	310
Water-soluble vitamins[1]				
Vitamin B_1 (mg as thiamin)	0.35	0.17	0.07	0.59
Vitamin B_2 (mg as riboflavin)	0.47	0.20	0.25	0.92
Vitamin B_6 (mg as pyridoxine)	020	0.36	0.68	1.24
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.5	6.2	7.4
Niacin equivalent[2](mg)	7.0	8.4	14.9	30.3
Pantothenic acid (mg)	2.0	4.2	3.1	9.3
Folic acid (μg as pteroylmonoglutamic acid)	52	134	44	230
Biotin (μg)	21	20	26	67
Vitamin C (mg as L-ascorbic acid)	34	34	50	118
Minerals				
Na (mg)	794	1175	850	2819
K (mg)	592	601	625	1818
Ca (mg)	249	142	85	476
Mg (mg)	47	71	74	192
P (mg)	380	293	317	990
Fe (mg)	0.8	3.4	2.6	6.8
Zn (mg)	1.8	3.7	2.5	8.0
Cu (mg)	0.15	0.44	0.43	1.02

[1]Water-soluble vitamins except for vitamin B_{12} are measured. Other nutrients are calculated by using the Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition -2005-, Report of the Subdivision on Resources, The Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology, Japan.
[2]The niacin equivalent intake was calculates as follows: The average tryptophan content in food protein is 1.1 % and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

ジエチルエーテルで抽出後，HPLC で同時定量する方法を用いた[13]．

パントテン酸

定量の標準品として使用したパントテン酸カルシウムは和光純薬工業株式会社(大阪)から購入した．尿中のパントテン酸定量は，尿を直接試料検体として，*Lactobacillus plantarum* ATCC 8014 を用いる微生物学的定量方法で行った[14]．

葉　酸

定量の標準品として使用したプテロイルモノグルタミン酸は和光純薬工業株式会社(大阪)から購入した．尿中の葉酸定量は，尿を直接試料検体として，*Lactobacillus casei* ATCC 27773 を用いる微生物学的定量方法で行った[15]．

ビオチン

定量の標準品として使用した D(+)- ビオチンは和光純薬工業株式会社(大阪)から購入した．尿中のビオチン酸定量は，尿を直接試料検体として，*Lactobacillus plantarum* ATCC 8014 を用いる微生物学的定量方法で行った[16]．

ビタミン C

定量の標準品として使用した L-アスコルビン酸は和光

Table 3. The Composition of the Diet 2

	Breakfast	Lunch	Dinner	Total
Energy (kcal)	463	549	606	1618
Protein (g)	19.6	21.4	20.5	61.5
Fat (g)	22.3	12.8	10.0	45.1
carbohydrates (g)	46.1	85.6	105.5	237.2
Fat-soluble vitamins				
Vitamin A (μg)	294	144	444	882
Vitamin D (μg)	1	0	0	1
Vitamin E (mg)	2.7	0.6	2.9	6.2
Vitamin K (μg)	12	98	100	210
Water-soluble vitamins[1]				
Vitamin B_1 (mg as thiamin)	0.35	0.09	0.02	0.46
Vitamin B_2 (mg as riboflavin)	0.47	0.18	0.17	0.82
Vitamin B_6 (mg as pyridoxine)	0.20	0.35	0.31	0.86
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.3	10.3	11.3
Niacin equivalent[2](mg)	7.0	8.1	9.7	24.8
Pantothenic acid (mg)	2.0	3.7	3.6	9.3
Folic acid (μg as pteroylmonoglutamic acid)	52	125	105	282
Biotin (μg)	21	12	20	53
Vitamin C (mg as L-ascorbic acid)	34	25	53	112
Minerals				
Na (mg)	833	1237	1080	3150
K (mg)	594	851	615	2060
Ca (mg)	250	173	96	519
Mg (mg)	47	113	96	256
P (mg)	381	253	317	951
Fe (mg)	0.8	6.2	3.2	10.2
Zn (mg)	1.9	2.8	4.2	8.9
Cu (mg)	0.15	0.33	0.47	0.95

[1]Water-soluble vitamins except for vitamin B_{12} are measured. Other nutrients are calculated by using the Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition -2005-, Report of the Subdivision on Resources, The Council for Science and Technology, Ministry of Education, Culture, Sports, Science and Technology, Japan.

[2]The niacin equivalent intake was calculates as follows: The average tryptophan content in food protein is 1.1 % and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

純薬工業株式会社（大阪）から購入した．尿中のビタミン C 定量は Kishida ら[17]の方法に従った．

結　果

被験者に，第 1 週目は規定食のみを食べさせ，その時の尿に排泄されるビタミン量を対照値とした（Table 5-Data 1）．第 2 週目は食事摂取基準に示された推奨量あるいは目安量の約 6 倍量の水溶性ビタミン混合を規定食に付加した時の尿を集め分析を行った（Table 5-Data 2）．そして，クリアランスを調べるために，ビタミン混合の付加を中止した 1 週間後の尿を集め分析を行った（Table 5-Data 3）．

食事摂取基準に示された推奨量あるいは目安量の約 6 倍量の水溶性ビタミン混合剤を 1 週間投与し続けた時の尿中への水溶性ビタミンの排泄量（Data 2）は，ビタミン B_1 では対照値（Data 1）の約 20 倍であった．被験者のビタミン B_1 の第 1 週目の摂取量は 0.53 mg/ 日，第 2 週目の摂取量は 4.42 mg/ 日であったことから，8 倍の摂取量の増加に対し，尿中への排泄量は約 20 倍に増加したことになる．ビタミン B_2 では，摂取量が 0.87 mg/ 日から 6.61 mg/

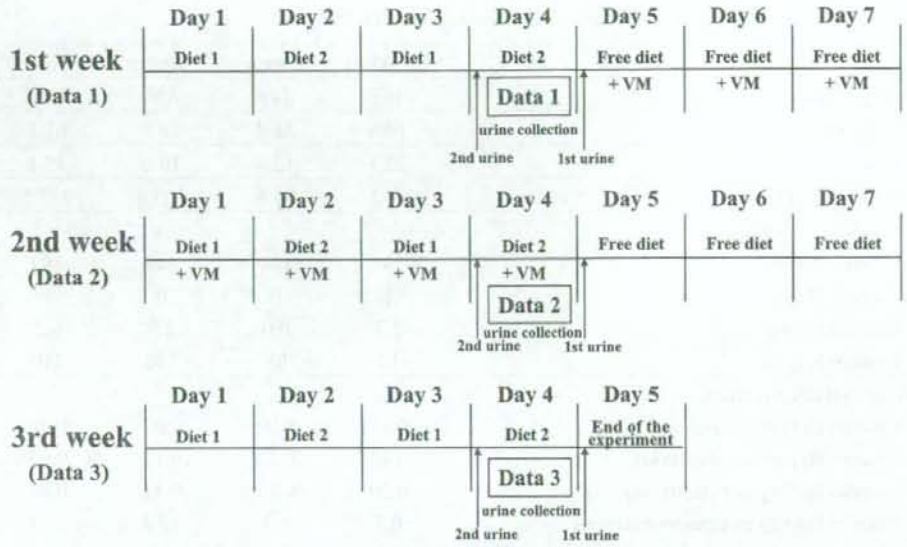

Fig. 1. The Scheme of the Experimental Design

The subjects were given the diet shown in Table 2 and 3 from day 1 to day 4 in each week. Six-fold of the water-soluble vitamin mixture (VM) based on Dietary Reference Intakes for Japanese, 2005[2], was given to the subjects from day 5 of week 1 to day 4 of week 2. The 24-h urine samples were collected from the second urinary excretion on day 4 to the first excretion on day 5 in each week. Composition of vitamin mixtures is shown in Table 4.

Table 4. The Composition of the Water-soluble Vitamin Mixture

Vitamins	Content in the mixture	Intake per day[a]
Thiamin	1.30 mg	3.9 mg
Riboflavin	1.91 mg	5.73 mg
Pyridoxine	2.20 mg	6.60 mg
Nicotinamide	22.5 mg	67.5 mg
Pantothenic acid	10.5 mg	31.5 mg
Pteroylmonoglutamic acid	0.45 mg	1.35 mg
D-Biotin	0.061 mg	0.183 mg
L-Ascorbic acid	200 mg	600 mg

[a] The subjects were taken the mixture at breakfast, lunch, and dinner, respectively.

日へと8倍に増加すると，尿中排泄量は約20倍に増大した．ビタミンB_6では，摂取量が1.05 mg/日から7.66 mg/日へと7倍に増加すると，ビタミンB_6異化代謝産物である4-ピリドキシン酸の尿中排泄量は約8倍に増大した．ナイアシンでは，摂取量が27.6 mgNE/日から95.0 mgNE/日へと3.5倍に増加すると，総ニコチンアミド異化代謝産物(MNA+2-Py+4-Py)の尿中排泄量は約5倍に増大した．パントテン酸では，摂取量が9.3 mg/日から40.7 mg/日へと4倍に増加すると，尿中排泄量は約5倍に増大した．葉酸では，摂取量が0.26 mg/日から1.60 mg/日へと6倍に増加すると，尿中排泄量は約12倍に増大した．ビオチンでは，摂取量が60 μg/日から242 μg/日へと4倍に増加すると，尿中排泄量は約4倍に増大した．ビタミンCでは，摂取量が115 mg/日から715 mg/日へと6倍に増加すると，尿中排泄量は約10倍に増大した．

以上のように，ビタミンB_{12}を除く水溶性ビタミンの摂取量の増大により，尿中排泄量は摂取量と同等もしくはそれ以上の倍率で増大した．ビタミンB_{12}の主要な排泄経路は尿ではないため，今回は測定対象とはしなかった[18]．

メルクインデックスで調べたこれら水溶性ビタミンの尿中排泄量は単なる水への溶解度と対応しないが，参考として，水溶性ビタミンの水への溶解度を以下に示す[19]．チアミン塩酸塩は1 gが約1 mLの水に溶ける．リボフラビンは結晶形により異なるが，1 gが3000～15,000 mLの

Table 5. Urinary Excretion of Water-soluble Vitamins Before Administration (Data 1), During Administration (Data 2), and One Week After Stopping Administration of the Water-soluble Vitamin Mixture (Data. 3).

Vitamins	Data 1	Data 2	Data 3
Thiamin (nmol/day)	288 ± 74	6095 ± 1058	1259 ± 403
Riboflavin (nmol/day)	283 ± 73	5569 ± 1215	972 ± 609
4-Pyridoxic acid (μmol/day)	3.44 ± 0.41	27.96 ± 2.69	6.81 ± 2.60
Nicotinamide metabolites (μmol/day)	85.6 ± 10.8	560.4 ± 78.3	89.2 ± 22.7
Pantothenic acid (μmol/day)	14.6 ± 2.0	71.2 ± 11.0	34.9 ± 7.3
Folic acid (nmol/day)	21.9 ± 8.5	830.1 ± 235.8	68.6 ± 11.6
Biotin (nmol/day)	74.5 ± 12.0	315.0 ± 30.0	51.6 ± 15.0
Ascorbic acid (μmol/day)	294 ± 82	4514 ± 954	459 ± 408

Values are means ± SD for 6 subjects.

水に溶ける．ピリドキサミン塩酸塩は 1 g が約 4.5 mL の水に溶ける．ニコチンアミドは 1 g が約 1 mL の水に溶ける．パントテン酸カルシウムは 1 g が 2.8 mL の水に溶ける．プテロイルモノグルタミン酸は 1 g が約 625,000 mL の水に溶ける．ビオチンは 1 g が約 4,500 mL の水に溶ける．アスコルビン酸は 1 g が約 3 mL の水に溶ける．

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資 料

パンを主食とした食事中に含まれる水溶性ビタミンの遊離型ビタミンに対する相対利用率

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Relative Availability of Water-Soluble Vitamins in a White Bread Diet to Free Vitamins

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The relative availability of water-soluble vitamins to free vitamins in a white bread diet consumed by Japanese male and female subjects was determined. The subjects, 9 female Japanese college students in experiment 1, and 7 male Japanese and 5 female Japanese college students in experiment 2, consumed the test diet with or without a water-soluble vitamin mixture for five consecutive days, and the water-soluble vitamin levels in a 24-h urine sample were measured. The ratio of the urinary excretion rate for each water-soluble vitamin in the test diet to that in the water-soluble vitamin mixture was determined as the relative availability. The relative availability of each vitamin was as follows: B_1, 55%; B_2, 50%; vitamin B_6, 85%; niacin, 60%; pantothenic acid, 70%; folate, 50%; biotin, 85%; and C, 95%.

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1. 緒 言

現在の日本では，食パンは主要な糖質・たんぱく質供給源であると同時に，B群ビタミン，特にビタミンB_1，ビタミンB_2，ナイアシン，パントテン酸，葉酸の供給源ともなっている．五訂増補日本食品標準成分表[1]の値は，食品中に含まれる結合型のB群ビタミンを *in vitro* で化学的あるいは酵素的処理を行うことによりすべて遊離型にした後，測定した値である．したがって，消化率・吸収率および体内利用率を考慮した値ではなく，食品中に含まれる栄養素を資源的な見方から記載した数値である．一方，日本人の食事摂取基準（2005年版）において，ビタミンB_6，ビタミンB_{12}，葉酸の食事摂取基準は生体利用率を考慮して策定された[2]．「日本食品標準成分表」と「食事摂取基準」との間の整合性を保つことは，国民に質の高い健康・栄養をもたらすために必要不可欠なことである．

我々は，最近，一般的な食事を被験者に摂取させ，遊離型B群ビタミンを付加したときとしなかったときの尿中B群ビタミン排泄量を測定することにより，遊離型B群ビタミンに対する食事中のB群ビタミンの相対利用率を簡便に決定する方法を確立した[3]．数多くのメニューに対してこの方法を用い，データを蓄積していけば，日本人が摂取する食事中のビタミンの平均的な利用率を明らかにすることができる．本研究では，食パンを主食としたときの水溶性ビタミンの相対利用率を決定したので，資料として報告する．

2. 実験方法

(1) 被 験 者

被験者は，予め実験内容の説明を受け，書類にて，実験への参加を希望した者である．実験1の被験者は22～25歳の成人女性9名であり，年齢は22.7±1.2歳（平均値±SD），身長は157±4 cm，体重は49.4±5.6 kg，BMIは20.0±1.7であった．実験2の被験者は，

Table 1. The composition of test diets for female subjects in Experiment 1

	Breakfast	Lunch	Dinner	Snack	Total
Energy (kcal)	388	527	545	325	1,785
Protein (g)	9.7	19.5	27.0	8.6	64.8
Fat (g)	19.2	16.3	13.5	3.6	52.6
Carbohydrate (g)	44.9	74.3	83.3	67.3	269.8
Water-soluble vitamins*1					
Vitamin B_1 (mg as thiamin chloride)	0.28	0.19	0.47	0.17	1.11 (3.30 μmol)
Vitamin B_2 (mg as riboflavin)	0.09	0.14	0.33	0.04	0.60 (1.60 μmol)
Vitamin B_6 (mg as pyridoxine)	0.12	0.20	0.58	0.10	1.00 (5.92 μmol)
Vitamin B_{12} (μg as cyanocobalamin)	0.1	0.2	6.6	0	6.9 (5.09 nmol)
Niacin equivalent*2 (mg)	5.2	6.8	11.0	2.8	25.8 (210 μmol)
Pantothenic acid (mg)	0.9	2.1	4.8	1.8	9.6 (43.8 μmol)
Folic acid (μg as pteroylmonoglutamic acid)	11	35	99	16	161 (365 nmol)
Biotin (μg)	11.1	9.9	15.8	4.2	41.0 (168 nmol)
Vitamin C (mg as L-ascorbic acid)	16	7	45	36	104 (591 μmol)

*1 Water-soluble vitamins except for vitamin B_{12} were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. *2 The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

19～25歳の成人男性7名，21～22歳の成人女性5名であった．男性被験者の年齢は21.6±2.4歳，身長は172±6 cm，体重は64.5±7.1 kg，BMIは21.7±1.7であり，女性被験者の年齢は21.8±0.5歳，身長は159±4 cm，体重は52.5±9.4 kg，BMIは20.5±2.9であった．いずれも，喫煙，飲酒の習慣がなく，朝食など規則正しい食習慣をもつ者であった．被験者の本研究は，滋賀県立大学倫理審査委員会において承認を受け，ヘルシンキ宣言の精神に則って行われた．

(2) 食　事

18～29歳の男性および女性の食事摂取基準に従い，男性に対してはエネルギー摂取量が約2,400 kcal，女性に対しては約1,800 kcalとなるような献立を作成し，被験者に摂取させた．献立は1日3回の主食をパンと，パン食に適した主菜と副菜とした．具体的な規定食の内容は，朝食は食パン，マーガリン，野菜ジュース，ミニトマト，ハム，ゆで卵，昼食は食パン，イチゴジャム，ツナ，おろしハンバーグ，キャベツ，夕食は食パン，マーガリン，ホタテのトマトスープ，ほうれん草炒め，温州ミカン，間食は食パン，ブルーベリージャム，グレープフルーツジュースとした．用いた規定食中の栄養素量をTable 1～3に示した．食品成分表に基づいて計算すると，実験1において，全食品に対するパンのエネルギー比率は53.3%，たんぱく質摂取量に対する比率は51.7%，脂質では30.2%，炭水化物では62.3%であった．パン由来の水溶性ビタミンについては，実測値に基づくと，ビタミンB_1では22.1%，ビタミンB_2では23.3%，ビタミンB_6では8.3%，ナイアシン当量では39.7%，パントテン酸では23.1%，葉酸では24.7%，ビオチンでは14.7%，ビタミンCが0%であった．実験2の男性に対しては，全食品に対するパンの比率は，エネルギーでは52.9

Table 2. The composition of test diets for male subjects in Experiment 2

	Breakfast	Lunch	Dinner	Snack	Total
Energy (kcal)	604	625	736	431	2,396
Protein (g)	25.2	28.3	35.8	13.0	102.2
Fat (g)	22.2	22.8	26.6	5.6	77.2
Carbohydrate (g)	74.9	74.1	91.8	84.6	325.5
Water-soluble vitamins*1					
Vitamin B_1 (mg as thiamin chloride)	0.32	0.17	0.40	0.17	1.35 (4.01 μmol)
Vitamin B_2 (mg as riboflavin)	0.28	0.32	0.52	0.06	1.17 (3.11 μmol)
Vitamin B_6 (mg as pyridoxine)	0.16	0.21	0.53	0.10	1.01 (5.98 μmol)
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.8	7.5	0	9.0 (6.64 nmol)
Niacin equivalent*2 (mg)	7.8	6.9	11.0	3.7	29.5 (240 μmol)
Pantothenic acid (mg)	2.3	2.8	4.8	1.7	11.7 (53.3 μmol)
Folic acid (μg as pteroylmonoglutamic acid)	36	54	139	30	249 (565 nmol)
Biotin (μg)	13.7	22.0	26.0	4.2	65.9 (270 nmol)
Vitamin C (mg as L-ascorbic acid)	52	23	40	46	162 (920 μmol)

*1 Water-soluble vitamins except for vitamin B_{12} were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. *2 The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

%，たんぱく質では43.7%，脂質では27.4%，炭水化物では68.9%，ビタミンB_1では24.2%，ビタミンB_2では15.9%，ビタミンB_6では11.0%，ナイアシン当量では46.3%，パントテン酸では25.3%，葉酸では21.3%，ビオチンでは12.2%，ビタミンCでは0%であった．実験2の女性に対しては，全食品に対するパンの比率は，エネルギーでは50.1%，たんぱく質では44.3%，脂質では26.6%，炭水化物では62.5%，ビタミンB_1では20.8%，ビタミンB_2では17.4%，ビタミンB_6では9.4%，ナイアシン当量では42.0%，パントテン酸では26.1%，葉酸では18.3%，ビオチンでは15.6%，ビタミンCでは0%であった

(3) 実験計画

実験計画の概略をFig. 1に示した．実験開始日をDay 1とした．1日のスケジュールは6時起床，6時30分～7時に朝食，13時～13時30分に昼食，18時～18時30分に夕食，23時に就寝とし，間食の摂取時間は自由にさせた．水はミネラルウォーターを自由摂取とした．Table 1に示した規定食をDay 1～Day 5およびDay 8～Day 12に摂取させた．Day 6とDay 7は被験者の負担軽減を考慮し，自由食とした．Day 6以降は，水溶性ビタミン混合を食後3回服用させた．1日当りの水溶性ビタミン混合の摂取量は，チアミン塩酸塩1.98 mg/日（7.12 μmol/日），リボフラビン3.45 mg/日（9.18 μmol/日），ピリドキシン2.94 mg/日（17.4 μmol/日），ニコチンアミド56.4 mg/日（459 μmol/日），パントテン酸19.0 mg/日（86.8 μmol/日），プテロイルモノグルタミン酸570 μg/日（1.29 μmol/日），ビオチン69 μg/日（283 nmol/日），アスコルビン酸90 mg/日（511 μmol/日）である．

Day 5の2回目の尿から翌日のDay 6の1回目までの尿を蓄尿し，これをDay 5の1日尿とした．Day

Table 3. The composition of test diets for female subjects in Experiment 2

	Breakfast	Lunch	Dinner	Snack	Total
Energy (kcal)	499	427	644	326	1,896
Protein (g)	21.5	16.8	28.0	9.3	75.6
Fat (g)	20.4	14.9	20.4	3.8	59.6
Carbohydrate (g)	56.3	55.2	91.7	65.9	269.1
Water-soluble vitamins*1					
Vitamin B_1 (mg as thiamin chloride)	0.38	0.14	0.48	0.18	1.18 (3.50 μmol)
Vitamin B_2 (mg as riboflavin)	0.26	0.13	0.36	0.05	0.80 (2.13 μmol)
Vitamin B_6 (mg as pyridoxine)	0.14	0.16	0.50	0.09	0.89 (5.27 μmol)
Vitamin B_{12} (μg as cyanocobalamin)	0.7	0.2	6.9	0	7.8 (5.76 nmol)
Niacin equivalent*2 (mg)	6.9	4.9	9.8	2.8	24.4 (198 μmol)
Pantothenic acid (mg)	2.1	1.4	3.6	1.4	8.5 (38.8 μmol)
Folic acid (μg as pteroylmonoglutamic acid)	25	40	131	21	217 (492 nmol)
Biotin (μg)	12.8	7.9	15.1	2.9	38.7 (159 nmol)
Vitamin C (mg as L-ascorbic acid)	52	23	40	46	162 (920 μmol)

*1 Water-soluble vitamins except for vitamin B_{12} were measured. Other nutrients were calculated by using the Standard Tables of Food Composition in Japan. *2 The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and the 1/60 (in weight basis) of tryptophan taken was converted into niacin in the body.

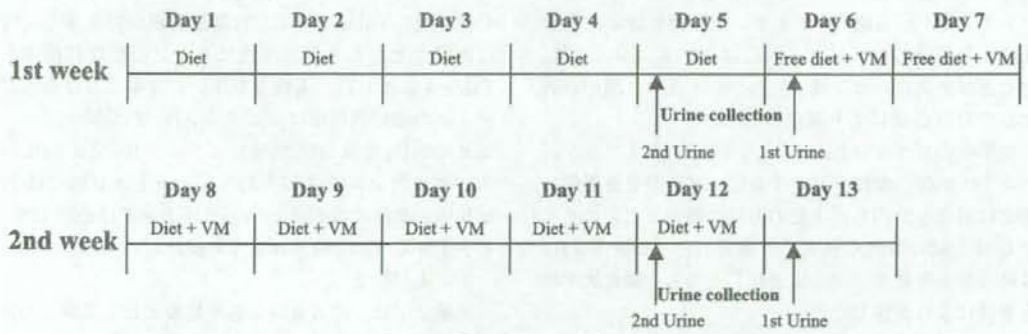

Fig. 1. Experimental design

The subjects were given the diet shown in Tables 1, 2 and 3 from Day 1 to Day 5 and from Day 8 to Day 12. The subjects freely took self-selected diet at Day 6 and Day 7. Water-soluble vitamin mixture (VM) containing 1.98 mg/d (7.12 μmol/d) of thiamin chloride, 3.45 mg/d (9.18 μmol/d) of riboflavin, 2.94 mg/d (17.4 μmol/d) of pyridoine, 56.4 mg/d (459 μmol/d) of nicotinamide, 19.0 mg/d (86.8 μmol/d) of pantothenic acid, 570 μg/d (1.29 μmol/d) of pteroylmonoglutamate, 69 μg/d (283 nmol/d) of biotin and 90 mg/d (511 μmol/d) of ascorbic acid was given to the subjects from Day 6 to Day 12. The 24-h urine samples were collected from the second urinary excretion on Day 5 and Day 12 to the first excretion on Day 6 and Day 13, respectively.