

Effect of Fasting on the Urinary Excretion of Water-Soluble Vitamins in Humans and Rats

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Summary Recent studies showed that the urinary excretion of the water-soluble vitamins can be useful as a nutritional index. To determine how fasting affects urinary excretion of water-soluble vitamins, a human study and an animal experiment were conducted. In the human study, the 24-h urinary excretion of water-soluble vitamins in 12 healthy Japanese adults fasting for a day was measured. One-day fasting drastically decreased urinary thiamin content to 30%, and increased urinary riboflavin content by 3-fold. Other water-soluble vitamin contents did not show significant change by fasting. To further investigate the alterations of water-soluble vitamin status by starvation, rats were starved for 3 d, and water-soluble vitamin contents in the liver, blood and urine were measured during starvation. Urinary excretion of thiamin, riboflavin, vitamin B₆ metabolite 4-pyridoxic acid, nicotinamide metabolites and folate decreased during starvation, but that of vitamin B₁₂, pantothenic acid and biotin did not. As for blood vitamin levels, only blood vitamin B₁, plasma PLP and plasma folate levels decreased with starvation. All water-soluble vitamin contents in the liver decreased during starvation, whereas vitamin concentrations in the liver did not decrease. Starvation decreased only concentrations of vitamin B₁₂ and folate in the skeletal muscle. These results suggest that water-soluble vitamins were released from the liver, and supplied to the peripheral tissues to maintain vitamin nutrition. Our human study also suggested that the effect of fasting should be taken into consideration for subjects showing low urinary thiamin and high urinary riboflavin.

Key Words human, rats, fasting, urine, biomarker

A biological assay technique to assess water-soluble vitamins in healthy human subjects was reported by Melnick et al. in 1945 (1), and this technique became popular in the 1960 s and 1970 s. Urinary excretion of thiamin, riboflavin and niacin metabolites has been used for setting Dietary Reference Intakes (DRIs) of vitamin B₁, vitamin B₂ and niacin in the USA and Japan (2, 3). Recent validation studies have developed the urinary compounds as nutritional markers to estimate nutrient intakes. We have recently reported that each of the water-soluble vitamins or its metabolite levels in the 24-h urine are strongly correlated with its intake when young women consume the standard Japanese diet with several amounts of water-soluble vitamin (4). Thirty-day mean thiamin levels in 24-h urine are also highly correlated with mean intake of vitamin B₁ (5). These findings show that urinary excretion of water-soluble vitamins reflects their intakes when subjects constantly take certain amounts of water-soluble vitamins. It is well known that pharmacological doses of water-soluble vitamins acutely increase their excretion to urine, and that a chronic water-soluble vitamin restricted diet reduces its levels in urine and blood (6). Effects of acute deprivation of water-soluble vitamins on their urinary

excretion have also been reported, and urinary thiamin and pyridoxine decreased with fasting while urinary riboflavin increased in human subjects (7, 8). To use urinary water-soluble vitamins as nutritional markers effectively, factors affecting their excretion need to be clarified.

In the present study, we focused on clarifying how fasting affects the metabolism of water-soluble vitamins. We first conducted a human study to investigate the alterations of urinary excretion of water-soluble vitamins by fasting, and we measured the 24-h urinary excretion of water-soluble vitamins in Japanese subjects fasting for a day. We then performed an animal experiment to precisely investigate the flux of water-soluble vitamins by measuring of their levels in urine, blood and liver during starvation.

MATERIALS AND METHODS

Subjects. Healthy Japanese adults (3 male, mean ages 27.0 ± 6.1 y; 9 female, mean ages 24.7 ± 6.9 y) participated in the study. The mean height, body weight and body mass index (mean \pm SD) of the male subjects were 173 ± 6 cm, 77.4 ± 21.5 kg and 25.9 ± 6.7 , respectively, and those of the female subjects were 158 ± 4 cm, 51.2 ± 8.5 kg and 20.5 ± 2.9 , respectively. None of the subjects took regular medication or dietary supple-

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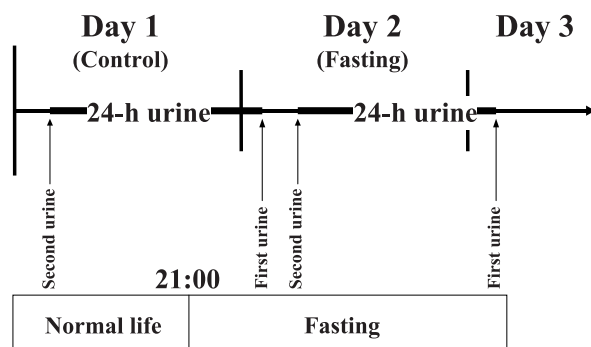


Fig. 1. Experimental design in human fasting study.

ments, or had habitual alcohol or cigarette consumption. Prior to the experiment, the subjects had physical checkups, and their hematological and blood biochemical values were within normal ranges. This study was reviewed and approved by the Ethical Committee of The University of Shiga Prefecture.

Human study. The experimental design is shown in Fig. 1. The subjects ate self-selected food until 21:00 on the first day, and then fasted until giving first urine sample on the third day. The subjects were permitted to drink water freely during the study. Twenty-four-hour urine samples of each day were collected from the second urinary excretion on the day to the first one on the next day. The urine samples collected on the first day were used as control samples, and those on the second day were used as fasting samples. The urine sample volumes were measured, and the samples were immediately treated as described under "Analyses" to avoid destruction of water-soluble vitamins, and then stored at -20°C until needed.

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

Twenty male Wistar rats aged 8 wk were obtained from CLEA Japan, Inc. (Tokyo, Japan) and immediately placed in individual CL-0301 rat metabolism cages obtained from CLEA Japan, Inc. The room temperature was maintained at around 22°C and about 60% humidity, and a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle was maintained. Rats were fed ad libitum a 20% casein 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 M mineral mixture and 1% AIN-93 vitamin mixture containing choline bitartrate for 7 d. The rats were starved for 3 d but were allowed to access water freely. The 24-h urine samples were collected every day at 09:00 during fasting in amber bottles containing 1 mL of 1 mol/L HCl, and were stored at -20°C until needed. Five rats each were killed at around 09:00 on days 0, 1, 2 and 3. Blood was collected, and the liver and thigh muscle were dissected to measure B-group vitamins.

Chemicals. Vitamin-free milk casein, sucrose, and L-methionine, thiamin hydrochloride, thiamin diphosphate (TDP) chloride, riboflavin, pyridoxine hydrochloride,

pyridoxal 5'-phosphate (PLP), nicotinamide (Nam), calcium pantothenate, pteroylmonoglutamic acid (folic acid), D(+)-biotin and L(+)-ascorbic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, the mineral mixture (AIN-93 M) (8) and the vitamin mixture (AIN-93-VX containing 25% choline bitartrate) (9) were obtained from Oriental Yeast Co., Ltd. (Tokyo, 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. N¹-Methylnicotinamide (MNA) chloride was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). N¹-Methyl-2-pyridone-5-carboxamide (2-Py) and N¹-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized (10, 11). All other chemicals used were of the highest purity available from commercial sources.

Determination of vitamins and their metabolites in urine, blood and liver.

Vitamin B₁: Vitamin B₁ contents in blood and liver were determined as sum of thiamin, TMP and TDP. Five percent trichloroacetic acid was added to whole blood, and the supernatant of the mixture was used for measurement. Liver was homogenized in a 5% trichloroacetic acid, and the supernatant of the homogenate was used for measurement. Urinary thiamin, blood vitamin B₁ and liver vitamin B₁ were determined by the HPLC-post labeled fluorescence method (12).

Vitamin B₂: Riboflavin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in blood and liver were converted to lumiflavin by photolysis, and lumiflavin was determined as total vitamin B₂ by the HPLC method (13). Urinary riboflavin was determined by the HPLC method (13).

Vitamin B₆: Vitamin B₆ vitamers including phosphate esters in the liver were converted to free vitamin B₆ vitamers such as pyridoxal and pyridoxine by autoclave under acidic conditions (4 h, at 121°C in 0.055 mol/L HCl), and measured as total vitamin B₆ by the microbioassay method using *Saccharomyces carlsbergensis* 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 B₁₂: Liver homogenate, plasma or urine were added to 0.2 mmol/L acetate buffer (pH 4.8), and the vitamin B₁₂ was converted to cyanocobalamin by immersion in a boiling water bath for 30 min with 0.0006% potassium cyanide at pH 4.5 (17). Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmanii*, ATCC 7830 (17).

Niacin: Phosphate ester forms of niacin such as NAD and NADP in the liver homogenate were converted to Nam by autoclave (10 min, at 121°C in water), and total Nam was determined by the HPLC method (11). Urinary 2-Py, 4-Py and MNA, Nam metabolites, were determined by the HPLC method (11, 18).

Pantothenic acid: To digest pantothenate compounds

such as CoA and phosphopantetheine in liver and blood to free form, liver homogenate or blood was incubated in 250 μ L of 20 mmol/L phosphate buffer (pH 7.0) containing 0.5 U alkaline phosphatase (from calf intestine, SIGMA P7923, 2,000 U) and the pigeon liver amidase at 37°C for 2 h. The pigeon liver amidase was prepared as follows: A 0.5 g of the liver acetone powder from pigeon (SIGMA L8376) was suspended in 25 mL of 0.02 mol/L KHCO_3 , and gently mixed for 30 min at 4°C. The suspension was centrifuged at 10,000 $\times g$ for 10 min at 4°C. The resulting supernatant was dialyzed for 24 h against the 0.02 mol/L KHCO_3 (3×2 L) to remove pantothenic acid including the original pigeon liver acetone powder. The dialyzed solution was centrifuged at 10,000 $\times g$ for 10 min at 4°C and the supernatant was used as the pigeon liver amidase. The free pantothenic acid in urine and blood, and digested pantothenic acid compounds in the liver 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 (1 h, at 121°C in 1.25 mol/L H_2PO_4), and biotin in urine, plasma and liver were determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014 (21).

Vitamin C: Reduced and oxidized ascorbic acid, and 2,3-diketogluconic acid in the urine samples were determined by the HPLC method (22).

Statistical analysis. Each value is expressed as the mean \pm SD in the human study, and the mean \pm SE in the animal experiment. For the statistical evaluation, the significance of the differences in the mean values between control and fasting subjects in the human study was tested by using Student's two-tailed paired *t*-test. The statistical significance in the animal experiment was determined by ANOVA, this being followed by Tukey's multiple-comparison test. The differences of $p < 0.05$ were considered to be statistically significant. Graph Pad Prism4.0 (Graph Pad Software, San Diego, CA, USA) was used for all the analyses.

RESULTS

Human study

The subjects freely ate a self-selected diet on the first day, and fasted on the second day. Their initial body weight was 57.8 ± 16.7 kg, and final body weight was 56.9 ± 16.4 kg. Their average body weight decrease by fasting was 0.9 ± 0.3 kg.

Urinary excretion of water-soluble vitamins or their metabolites in the control and fasting periods is shown in Table 1. Urinary excretion of thiamin was reduced to 30% by 1-d fasting, and urinary riboflavin increased by 3.1-fold. Fasting moderately increased urinary excretion of vitamin B₁₂ to 1.7-fold. Fasting did not affect uri-

Table 1. Urinary excretion of water-soluble vitamins in humans before (control) and during fasting.

Water-soluble vitamins	Control	Fasting
Thiamin (nmol/d)	459 \pm 261	136 \pm 101**
Riboflavin (nmol/d)	344 \pm 242	885 \pm 793**
4-PIC (μ mol/d)	3.87 \pm 1.25	3.58 \pm 0.77
Vitamin B ₁₂ (pmol/d)	33.5 \pm 21.9	57.8 \pm 18.2**
Nam metabolites (μ mol/d)	87.3 \pm 29.3	105.5 \pm 26.0
PaA (μ mol/d)	16.6 \pm 3.1	15.7 \pm 4.3
Folate (nmol/d)	32.5 \pm 1.6	40.6 \pm 10.9
Biotin (nmol/d)	84.9 \pm 39.1	107.4 \pm 38.1
Ascorbic acid (μ mol/d)	51.6 \pm 20.8	44.3 \pm 26.9

Twelve human subjects fasted for a day, and 24-h urine samples before (Control) and during fasting (Fasting) were collected. Values are expressed as means \pm SD for 12 subjects.

** $p < 0.01$ versus control determined by paired Student's *t*-test.

4-PIC, 4-pyridoxic acid; Nam, nicotinamide; PaA, pantothenic acid.

nary excretion of vitamin B₆ metabolite 4-PIC, Nam metabolites, pantothenic acid, folate, biotin or ascorbic acid.

Animal experiment

The rats were starved for 3 d, their body weight decreased day by day, and they lost approximately 35 g of 268 g in 3 d. Liver weight acutely decreased to 60% with 1-d starvation, and spleen weight was gradually decreased (Table 2). The weights of other tissues including the brain, heart, kidney, lung and testis did not change with 3-d starvation (data not shown).

The changes in water-soluble vitamin contents in urine samples during starvation are shown in Fig. 2. Urinary excretion of thiamin acutely decreased to 10% with 1-d starvation. Urinary excretion of riboflavin and vitamin B₆ metabolite 4-PIC gradually decreased during starvation, and their values were 30–50% of the food sufficient state after 3-d starvation. Urinary vitamin B₁₂ contents did not alter during starvation. Urinary excretion of Nam metabolites and folate increased by 1.3-fold with 1-d starvation, and then gradually decreased. Urinary pantothenic acid acutely decreased to 25% with 1-d starvation, and then returned to the initial level after 2-d starvation. Urinary excretion of biotin increased by 1.4-fold after 1- and 2-d starvation, and then returned to the initial level.

The changes in blood or plasma water-soluble vitamin levels during starvation are shown in Fig. 3. Starvation decreased blood vitamin B₁, and plasma PLP and folate levels to 80, 50 and 70%, respectively. Blood vitamin B₂ and Nam levels did not change significantly during starvation. Although 1-d starvation decreased plasma vitamin B₁₂ level to 90%, the levels were same as basal after 2- and 3-d starvation. One-day starvation increased plasma biotin level to 150%, and the levels were decreased to basal range after 2- and 3-d starvation. The blood pantothenic acid level was not detected

Table 2. Changes in tissue weights during starvation in rats.

	Day 0	Day 1	Day 2	Day 3
Liver (g)	12.06±0.30 ^a	7.60±0.12 ^b	6.47±0.07 ^c	5.93±0.06 ^c
Spleen (g)	0.72±0.03 ^a	0.68±0.02 ^a	0.56±0.02 ^b	0.51±0.02 ^b

Each value is expressed in g as the mean±SE or 5 rats. A different superscript letter means significant difference at $p<0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

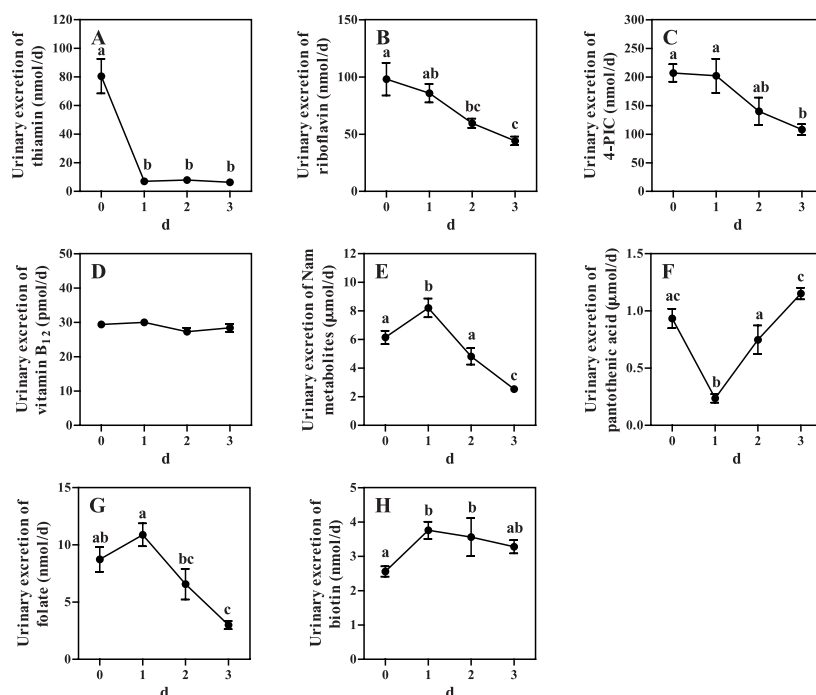


Fig. 2. Alteration of urinary excretion of thiamin (A), riboflavin (B), 4-pyridoxic acid (4-PIC) (C), vitamin B₁₂ (D), nicotinamide (Nam) metabolites (E), pantothenic acid (F), folate (G) and biotin (H) in rats during starvation. Five male Wistar rats aged 8 wk were starved for 3 d, and 24-h urine samples were collected every day during starvation. Values are expressed as means±SE for 5 rats. A different superscript letter means statistically significant difference at $p<0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

by the microbioassay.

The changes in liver water-soluble vitamin amounts during starvation are shown in Fig. 4. All water-soluble vitamin amounts in the liver decreased after 3-d starvation. One-day starvation decreased the vitamin B₂, vitamin B₆, vitamin B₁₂, Nam and folate amounts in the liver, and 3 d were needed to decrease the liver vitamin B₁, pantothenic acid and biotin amounts. The changes in liver water-soluble vitamin contents during starvation are shown in Fig. 5. Since the liver weight was extremely decreased on day 1 by starvation (Table 2), some vitamin contents in the liver decreased. Contents of vitamin B₁ and vitamin B₂ in the liver increased during 2-d starvation, and then decreased to the basal levels. Those of pantothenic acid and folate in the liver during 3-d starvation were higher than those values prior to starvation. Other water-soluble vitamin contents in the liver did not change during starvation.

A deficiency of vitamin B₁ appears as a disorder of skeletal muscles. The changes in water-soluble vitamin contents in the skeletal muscle during starvation are shown in Fig. 6. Three-day starvation did not alter vita-

min B₁, vitamin B₂, vitamin B₆, Nam, pantothenic acid or biotin contents in the skeletal muscle. One-day starvation decreased muscle vitamin B₁₂ content to 40%, and muscle folate content decreased to 60% on the third day.

DISCUSSION

To investigate how fasting affect the urinary excretion of water-soluble vitamins in humans, human subjects were fasted for a day, and urinary vitamin or its metabolite contents obtained during fasting were compared with those before fasting. One-day fasting drastically decreased urinary thiamin content, and increased urinary riboflavin content. These results are consistent with the previous reports (7, 8). Other water-soluble vitamin contents did not show dramatic change with fasting. The present findings showed that alterations of urinary excretion by fasting were observed only for vitamins B₁ and B₂.

To further investigate the alteration of water-soluble vitamin metabolism during starvation, rats were starved for 3 d, and water-soluble vitamin contents in

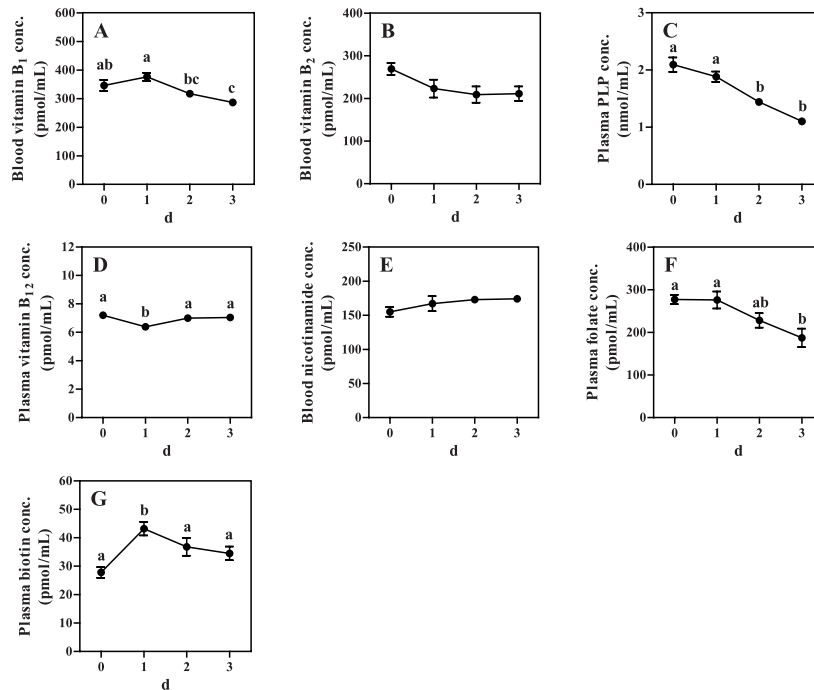


Fig. 3. Alteration of blood vitamin B₁ (A), blood vitamin B₂ (B), plasma PLP (C), plasma vitamin B₁₂ (D), blood nicotinamide (E), plasma folate (F) and plasma biotin (G) concentrations in rats during starvation. Values are expressed as means \pm SE for 5 rats. A different superscript letter means statistically significant difference at $p < 0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

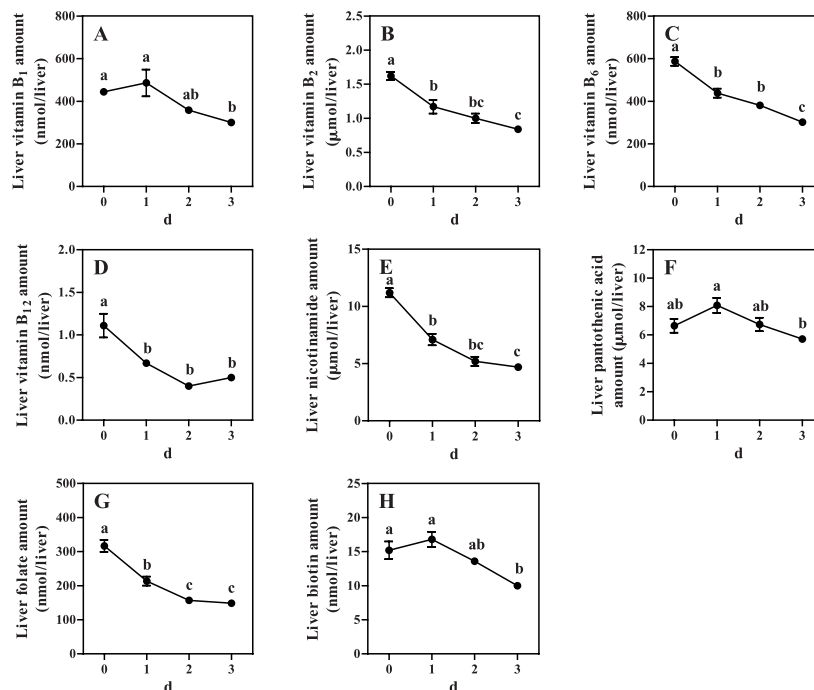


Fig. 4. Alteration of vitamin B₁ (A), vitamin B₂ (B), vitamin B₆ (C), vitamin B₁₂ (D), nicotinamide (E), pantothenic acid (F), folate (G) and biotin (H) amounts in rat liver during starvation. Values are expressed as means \pm SE for 5 rats. A different superscript letter means statistically significant difference at $p < 0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

the urine, blood, liver and skeletal muscle were measured. Liver water-soluble vitamin amounts gradually decreased during starvation, and the decrease corresponded to the liver weight during starvation, whereas most blood and muscle vitamin concentrations did not show dramatic change during starvation. A possible

explanation of the flux in water-soluble vitamins during the starvation is as follows: water-soluble vitamins are released from the liver into the blood, non-hepatic cells absorb the vitamins from blood, and extra vitamins are excreted into the urine. Several vitamin levels in urine and blood reflected their contents in the liver. However,

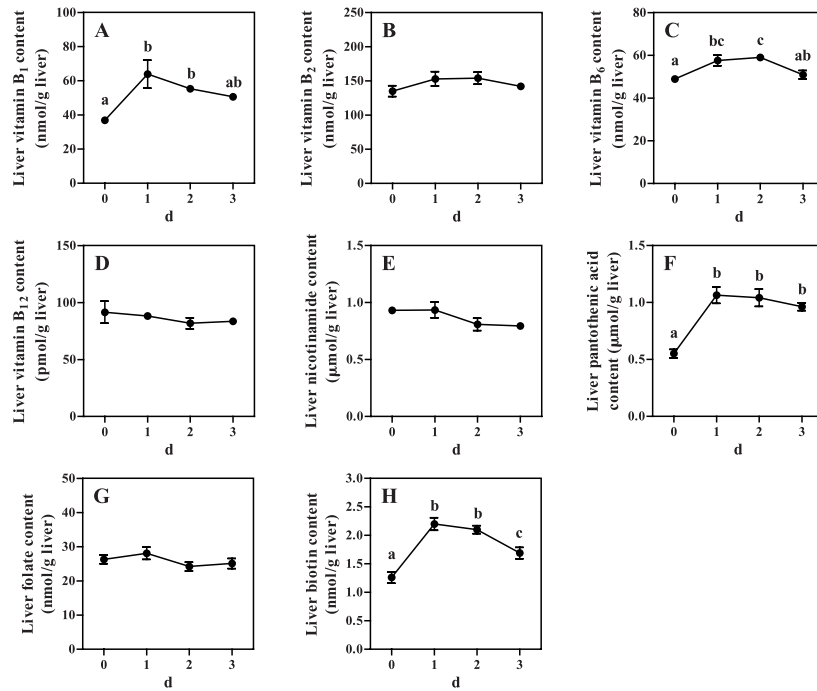


Fig. 5. Alteration of vitamin B₁ (A), vitamin B₂ (B), vitamin B₆ (C), vitamin B₁₂ (D), nicotinamide (E), pantothenic acid (F), folate (G) and biotin (H) contents in rat liver during starvation. Values are expressed as means±SE for 5 rats. A different superscript letter means statistically significant difference at $p < 0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

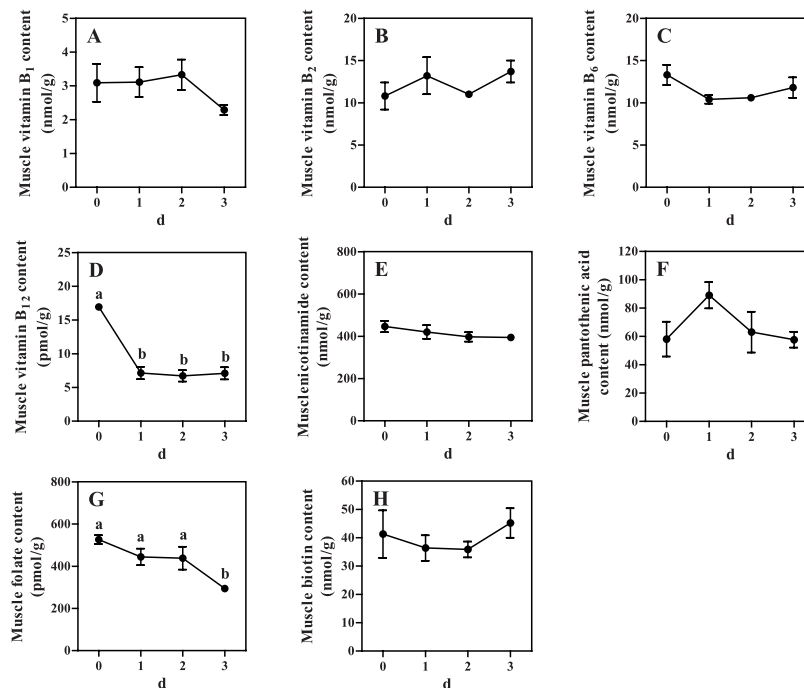


Fig. 6. Alteration of vitamin B₁ (A), vitamin B₂ (B), vitamin B₆ (C), vitamin B₁₂ (D), nicotinamide (E), pantothenic acid (F), folate (G) and biotin (H) contents in rat thigh muscle during starvation. Values are expressed as means±SE for 5 rats. A different superscript letter means statistically significant difference at $p < 0.05$ determined by one-way ANOVA followed by Tukey's multiple comparison test.

alteration of urinary excretion and blood concentration of some vitamins was not consistent with those in the liver. These results showed that urinary excretion and blood concentration of water-soluble vitamins do not always reflect their intake or nutritional status during fasting and starvation.

The changes of the urinary excretion of water-soluble vitamins by starvation can be classified into six types. The first is the vitamin B₁ type: urinary excretion of thiamin acutely decreased with 1-d starvation, and it became almost undetectable thereafter (Fig. 2A). The second is the vitamin B₂ and vitamin B₆ type: urinary

excretion gradually decreased after the 2nd day of starvation (Figs. 2B and 2C). The third is the niacin and folate type: urinary Nam metabolites and folate increased with 1-d starvation, and then gradually decreased (Fig. 2E and 2G). The fourth is the pantothenic acid type: the pantothenic acid excretion was significantly decreased on day 1 of starvation, and then returned to the basal level (Fig. 2F). The fifth is the vitamin B₁₂ type: the urinary vitamin B₁₂ did not alter during starvation (Fig. 2D). The sixth is the biotin type: the urinary excretion of biotin was significantly increased by starvation (Fig. 2H).

It has been reported that even during World War II clinically recognizable vitamin deficiencies occurred only rarely among victims of starvation (23). The data obtained from the present animal experiment show that starvation for 3 d decreased liver vitamin contents but not cause acute vitamin deficiencies. Only 3 of 7 vitamin levels, namely vitamin B₁, PLP and folate, in blood, and 2 of 8 vitamin levels, namely vitamin B₁₂ and folate, in skeletal muscle decreased with starvation. Three-day starvation failed to decrease liver vitamin concentration. These results suggest that water-soluble vitamins were released from the liver, and supplied to the periphery. This homeostasis would be achieved by decreasing the body weight including the liver weight and by decreasing the urinary excretions.

An interesting phenomenon was observed in the urinary excretion of thiamin in humans and rats with fasting. Namely, the urinary excretion of thiamin abruptly decreased to almost zero level on day 1 of fasting in humans (Table 1) and rats (Fig. 2A). A similar phenomenon was reported by Stevenson (7) and Consolazio et al. (8), and they considered that repletion of body stores of vitamin B₁ was fairly rapid. Total thiamin content in adult human has been estimated to be approximately 30 mg (24). Vitamin B₁ requirement is closely related to energy consumption, particularly that derived from carbohydrate. Elson et al. (25) reported clinical signs of vitamin B₁ deficiency on intake of 0.35 mg/1,000 kcal. Based on information from Ferrebee et al. (26), one can calculate the quantity of thiamin that may be liberated from body tissue breakdown (0.5 mg/kg tissue). In the present fasting experiment for humans, the average weight loss was 0.9 kg/d, and 0.4–0.5 mg of thiamin was estimated to be liberated. As average energy consumption is approximately 2,000 kcal/d, usable vitamin B₁ might be 0.2 mg/1,000 kcal. This amount is lower than the value 0.35 mg/1,000 kcal (25). There is some possibility that the urinary excretion of vitamin B₁ was decreased to supplement this shortage.

The urinary excretion of riboflavin was increased by fasting in humans (Fig. 1B). The same phenomenon has also been reported as follows: acute starvation increased the excretion of vitamin B₂ approximately 5-fold when starved for 7 d under sedentary conditions (27), and an early marked increase of vitamin B₂ excretion was observed when obese subjects and young men were fasted (28, 29). Based on the data of Axelrod et al. (30), one can calculate the quantity of riboflavin that

may be liberated from the breakdown of body tissues as 2.9 mg/kg tissue and 17 mg/kg liver. These calculations suggest that very rapid depletion of vitamin B₂ in body store occurs compared with that of vitamin B₁, and that the greater part of vitamin B₂ is weakly associated with proteins and will be liberated under fasting conditions. Namely, any appreciable excess quantity of vitamin B₂ being excreted in the urine could be attributed to the breakdown of flavoproteins due to body weight loss. There are similar reports: Cayer and Cody (31) reported an increase of vitamin B₂ excretion in patients with peptic ulcers, and Pollack and Bookman (32) observed that large negative nitrogen balances precipitated by surgical procedures were associated with excretion of vitamin B₂ many times that of the quantity ingested. These reports suggest that vitamin B₂ requirement is related to the body protein status; thus the increased excretion of vitamin B₂ during fasting may be related to adequate reserves and protein catabolism. However, the same phenomenon was not observed in the animal experiment, and the urinary excretion of vitamin B₂ gradually decreased (Fig. 3B).

In conclusion, the present findings showed that urinary and blood water-soluble vitamin contents do not always reflect their intake or nutritional status during fasting and starvation. Our animal experiment suggested that water-soluble vitamins were released from the liver with its reduction, and supplied to the periphery to maintain vitamin nutrition. Recent findings show that urinary excretion of water-soluble vitamins reflect their intakes, and that those amounts can be useful nutritional markers (4–6). However, the present findings also suggest that the evaluator for vitamin status has to pay attention to a subject showing both low urinary thiamin and high urinary riboflavin.

Acknowledgments

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