Different variations of tissue B-group vitamin concentrations in short- and long-term starved rats

Aya Moriya[1], Tsutomu Fukuwatari[1,2], Mitsue Sano[1,2] and Katsumi Shibata[1,2]*

[1]*Division of Life Style Studies, Section of Nutritional Sciences, Graduate School of Human Cultures, The University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522-8533, Japan*

[2]*Department of Food Science and Nutrition, Graduate School of Human Cultures, The University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522-8533, Japan*

Abstract

Prolonged starvation changes energy metabolism; therefore, the metabolic response to starvation is divided into three phases according to changes in glucose, lipid and protein utilisation. B-group vitamins are involved in energy metabolism via metabolism of carbohydrates, fatty acids and amino acids. To determine how changes in energy metabolism alter B-group vitamin concentrations during starvation, we measured the concentration of eight kinds of B-group vitamins daily in rat blood, urine and in nine tissues including cerebrum, heart, lung, stomach, kidney, liver, spleen, testis and skeletal muscle during 8 d of starvation. Vitamin B_1, vitamin B_6, pantothenic acid, folate and biotin concentrations in the blood reduced after 6 or 8 d of starvation, and other vitamins did not change. Urinary excretion was decreased during starvation for all B-group vitamins except pantothenic acid and biotin. Less variation in B-group vitamin concentrations was found in the cerebrum and spleen. Concentrations of vitamin B_1, vitamin B_6, nicotinamide and pantothenic acid increased in the liver. The skeletal muscle and stomach showed reduced concentrations of five vitamins including vitamin B_1, vitamin B_2, vitamin B_6, pantothenic acid and folate. Concentrations of two or three vitamins decreased in the kidney, testis and heart, and these changes showed different patterns in each tissue and for each vitamin. The concentration of pantothenic acid rapidly decreased in the heart, stomach, kidney and testis, whereas concentrations of nicotinamide were stable in all tissues except the liver. Different variations in B-group vitamin concentrations in the tissues of starved rats were found. The present findings will lead to a suitable supplementation of vitamins for the prevention of the re-feeding syndrome.

Key words: Starvation: Fasting: Energy metabolism

Starvation produces a series of metabolic changes that lead to a reduction in body weight, alterations in body composition and metabolic gene expression[1,2]. In mammals and birds, three distinct levels of energy depletion have been established[3–10]. The first phase (phase 1) is a rapid period of adaptation marked by an increase in mobilisation of fat stores and a lowering in protein utilisation. During the second phase (phase 2), which is a long period of thrift, most of the energy expenditure is derived from fats, and then fat stores are progressively exhausted, while body proteins are efficiently spared. The third phase (phase 3) is characterised by an increase in protein utilisation. In humans, the negative energy balance resulting from starvation can arise due to disease, eating or psychological disorders, or hunger strikes. Starvation and consequent re-feeding syndrome can lead to electrolyte disorders, especially

hypophosphataemia, along with neurological, pulmonary, cardiac, neuromuscular and haematological complications[11]. To avoid the re-feeding syndrome, an additional load of vitamins has been suggested to correct the vitamin deficiencies[11]. However, little is known about B-group vitamin status during starvation.

Several B-group vitamins take part in energy metabolism. For instance, vitamin B_2 functions as FAD and FMN in redox reactions including the electron transport chain and fatty acid oxidation. Nicotinamide is involved in more than 200 reactions, including the metabolism of carbohydrates, amino acids and fatty acids, and also in the electron transport chain. Vitamin B_1 catalyses carbohydrate metabolism including decarboxylation of α-ketoacids and *trans*-ketolation as a cofactor thiamin diphosphate; vitamin B_6 functions as pyridoxal 5′-phosphate in amino acid metabolism including aminotransferases,

decarboxylases, racemases and dehydratases as pyridoxal 5′-phosphate; and pantothenic acid is involved in fatty acid metabolism such as oxidation and synthesis. For these reasons, in the 'Dietary Reference Intakes for Japanese, 2010', dietary requirements for vitamin B_1, vitamin B_2 and niacin are expressed per 4186 kJ (1000 kcal), and the requirement for vitamin B_6 is expressed in terms of protein intake[12].

As mentioned earlier, prolonged starvation sifts the energy source from glucose to fats and then to protein, and B-group vitamins are involved in the metabolism of carbohydrates, fatty acids and amino acids. Thus, in the present study, we investigated how changes in energy metabolism altered B-group vitamin utilisation during starvation. We comprehensively determined eight kinds of B-group vitamin concentrations in rat blood, urine and tissues including the brain, heart, lung, stomach, kidney, liver, spleen, testis and skeletal muscle during 8 d of starvation.

Materials and methods

Diets

The composition of the purified diet is shown in Table 1. Vitamin-free milk casein, L-methionine and sucrose were purchased from Wako Pure Chemical Industries Limited (Osaka, Japan). Maize oil was purchased from Nisshin OilliO Group, Limited (Tokyo, Japan). Gelatinised maize starch, the mineral mixture (AIN-93G) and the vitamin mixture (AIN-93VX) were obtained from Oriental Yeast Company, Limited (Tokyo, Japan).

Animals

Male rats of the Wistar strain, weighing 225–235 g, were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were individually housed in a temperature-controlled room ($22 \pm 2°C$ and 50–60 % humidity) with a 12 h light–12 h dark cycle and were allowed to acclimate to the environment for 7 d before starting the experiment. Body mass, food consumption and water intake were recorded daily (± 0.1 g). We also collected 24 h urine samples every day.

Experimental procedures

A total of twenty-five rats were randomly divided into five groups. After 1 week of acclimatisation, five rats were killed

Table 1. Composition of the diet

	(g/100 g)
Vitamin-free milk casein	20·0
L-Met	0·2
Gelatinised maize starch	46·9
Sucrose	23·4
Maize oil	5·0
Mineral mixture (AIN-93-G)	3·5
Vitamin mixture (AIN-93VX)*	1·0

* The composition of the vitamin mixture is described by Reeves et al.[13].

by decapitation as a control group (CONT, n 5). The other rats were deprived from food for 1 d (S1, n 5), 2 d (S2, n 5), 6 d (S6, n 5) or until they had been in phase 3 for 2 d; that is, they were starved for a total duration of 6–9 d (P3, n 5). The starving phase was determined by calculating the specific daily rate of body mass loss dM/Mdt (g/kg per d) for each animal (dM represents the loss of body mass during $dt = t_1 - t_0$ and M is the body mass of the rat at t_0[10,14]. Blood was taken from the tail vein at 09.00 hours every day, and 3-hydroxybutyric acid (3-HBA) concentration in the blood was measured with a 3-HBA Kit (Abbott Japan Company, Limited, Tokyo, Japan) to confirm the metabolic state of each animal because blood 3-HBA reflects fatty acid oxidation.

After the animals were killed, blood samples were collected into EDTA-2K tubes from the carotid artery and were centrifuged at 1700 $\boldsymbol{g}$ for 10 min at 4°C. Plasma glucose, TAG, urea N, aspartate aminotransferase and alanine aminotransferase were measured with FUJI DRI-CHEM (FUJIFILM Company, Tokyo, Japan).

The cerebrum, heart, lungs, stomach, kidneys, liver, spleen, testes and leg muscles were dissected and weighed (± 0.001 g). The stomach was cleared of its contents. All tissue samples were immediately homogenised in ultra-pure water at 1:10 (w/v) using a Teflon glass homogeniser and stored at $-20°C$ until needed. The present study was conducted according to the guidelines for the care and use of laboratory animals, and was approved by the Ethics Committee of the University of Shiga Prefecture (Shiga, Japan).

Analytical methods

Vitamin B_1. Thiamin in urine was measured directly. The vitamin B_1 content in the blood and tissue was determined as the sum of thiamin, thiamin monophosphate and thiamin diphosphate and was expressed as total thiamin. TCA (5 %) was added to whole blood and tissue homogenates, and the blood and homogenates were centrifuged for 5 min at 20 000 $\boldsymbol{g}$, and the supernatant of the mixture was used for measurement. Vitamin B_1 levels in the urine, blood and tissue were determined by the HPLC post-labelled fluorescence method[15].

Vitamin B_2. Riboflavin in urine was measured directly by HPLC[16]. Riboflavin, FMN and FAD in blood and tissue were converted to lumiflavin by photolysis. Briefly, the supernatant from a TCA-treated blood or tissue sample was added to an equal volume of 1 M-NaOH. The alkalised mixture was irradiated with a fluorescent lamp for 30 min, and acetic acid was added to the mixture. The neutralised mixture was filtered with a 0·45 μm microfilter and the filtrate was directly injected into the HPLC system for the measurement of lumiflavin[17]. The measured lumiflavin was expressed as total vitamin B_2.

Vitamin B_6. 4-Pyridoxic acid, a catabolite of vitamin B_6, in urine was measured directly by HPLC[18]. Serum pyridoxal and pyridoxal 5′-phosphate were determined by the HPLC method[19]. Vitamin B_6 vitamers, including phosphate esters in the tissue, were converted to free vitamin B_6 vitamers such as pyridoxal and pyridoxamine using an autoclave under acidic conditions. Briefly, the homogenate was added

to 0·06 M-HCl at 1:8 (v/v) and autoclaved at 121°C for 3 h, and the mixture was adjusted to pH 5·0 using 1 M-NaOH. These were measured as total vitamin B_6 by the microbioassay method using *Saccharomyces carlsbergensis* strain 4228 ATCC 9080[20].

Vitamin B_{12}. Urine, plasma and tissue homogenates were added to a 0·2 mM-acetate buffer (pH 4·8) with 0·0006 % potassium cyanide. These were put into a boiling water bath for 5 min to be converted to cyanocobalamin, and then 10 % metaphosphoric acid was added to be neutralised. Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmannii* ATCC 7830[21].

Niacin. Nicotinamide[22] and its catabolites, N^1-methylnicotinamide (MNA)[23], N^1-methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py)[22], in urine were measured directly by HPLC. For measuring the total nicotinamide content in blood and tissues, the whole blood and tissue homogenates were autoclaved at 121°C for 20 min to convert the coenzymes to nicotinamide. The resulting nicotinamide was then determined by the HPLC method[22,24].

Pantothenic acid. Pantothenic acid in urine was determined by HPLC[25]. To digest the bound pantothenic acid including coenzyme A and phosphopantetheine in tissue and plasma to free form, the homogenate or blood was incubated at 37°C for 24 h. Pantothenic acid in the plasma and tissue was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014[26].

Folate. Folate in urine and plasma was directly determined by the microbioassay method using *Lactobacillus casei* ATCC 27 773[27]. Folate in tissues was digested to monoglutamate forms by treatment with protease and conjugase. Briefly, 1 M-KH_2PO_4–K_2HPO_4 buffer (pH 6·1) was added to the tissue homogenate at 1:9 (v/v), and the homogenate was autoclaved at 121°C for 5 min. Proteinase MS (Kaken Pharmaceutical Company, Limited, Tokyo, Japan) was added to the homogenate at a final concentration of 2·5 mg/ml and then incubated at 37°C for 3 h. The reaction mixture was added

to the conjugase solution (extract from porcine kidney acetone powder, Sigma, Porcine, Type II) at 30:1 (v/v) and incubated at 37°C for 12 h. After centrifugation at 10 000 **g** for 10 min, the supernatant was used for determination by the microbioassay.

Biotin. Bound biotin in tissues was converted to the free form using autoclave under acidic conditions. Briefly, 1·5 M-H_2SO_4 was added to the homogenate at 1:1 (v/v), and the homogenate was autoclaved for 1 h at 121°C. The suspension was centrifuged at 10 000 **g** for 10 min at 4°C, and the supernatant was used to measure biotin. Biotin in urine and plasma was measured directly. The biotin content in urine, plasma and tissue was determined by the microbioassay method using *L. plantarum* ATCC 8014[28].

Statistical analysis

Values are expressed as means with their standard errors. P3 rats (starved for 6–9 d) were expressed at 8 d on the graph for convenience. To test the significance of the differences in mean values among all groups, one-way ANOVA with Tukey's *post hoc* test was employed. Repeated ANOVA with Bonferroni's *post hoc* test was used to analyse urinary excretion of B-group vitamins in P3 rats, and individual data points were compared with their data at day 0. All differences at $P < 0.05$ were considered to be statistically significant. Prism software (version 5; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

Results

Changes in body mass during starvation

Changes in body mass during starvation are shown in Table 2. Starvation for the first 24 h produced a weight loss of 7 %. From the second day to the last day of starvation, the rats lost 5 % weight for each 24 h (data not shown). The specific daily rate of body mass loss (dM/dMt) *v.* time in starved rats is presented in Fig. 1. The pattern of dM/dMt showed a

Table 2. Body mass and organ mass in the control and starved rats

(Mean values with their standard errors, *n* 5)

	CONT*		S1		S2		S6		P3†	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body mass (g)	252·9	3·3	253·1	2·8	246·4	6·2	252·2	3·1	249·0	3·2
Final body mass (g)	252·9[a]	3·3	235·1[a,b]	2·3	219·3[b]	5·6	182·7[c]	2·5	166·6[c]	5·4
Organ mass (g, wet wt)										
Cerebrum	1·29	0·02	1·30	0·01	1·28	0·01	1·27	0·02	1·23	0·03
Heart	0·84[a]	0·04	0·87[a]	0·03	0·81[a]	0·04	0·66[b]	0·02	0·58[b]	0·02
Lungs	1·28	0·08	1·19	0·10	1·09	0·06	1·09	0·13	0·95	0·06
Stomach	1·16	0·02	1·15	0·04	1·14	0·04	1·20	0·05	1·19	0·08
Kidneys	1·94[a]	0·05	1·89[a,b]	0.6	1·69[b,c]	0·03	1·53[c,d]	0·02	1·43[d]	0·04
Spleen	0·75[a]	0·04	0·67[a]	0·03	0·50[a]	0·05	0·30[b]	0·01	0·25[c]	0·04
Testes	2·75[a,b]	0·07	2·66[b,c]	0·05	2·66[b,c]	0·04	2·47[b,c]	0·03	2·45[c]	0·06
Liver	11·18[a]	0·23	7·25[b]	0·22	6·07[b]	0·19	4·69[c]	0·12	3·83[c]	0·42

CONT, non-starved control rats; S1, 1-day starved rats; S2, 2-day starved rats; S6, 6-day starved rats; P3, starved to phase 3 rats.
a,b,c Mean values within a row with unlike superscript letters were significantly different determined by one-way ANOVA with Tukey's multiple comparison tests ($P < 0.05$).
* Since the control rats were killed at the beginning of the experiment, the initial body weight was same as the final body weight.
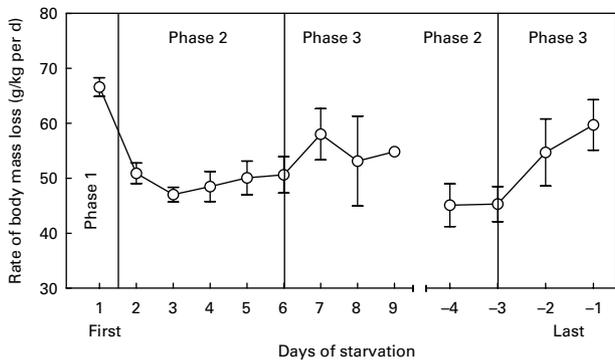† Phase 3 is determined by the rapid increase in dM/dMt (refer to Fig. 1).

Fig. 1. Rate of body mass loss (dM/dt) in starved rats. Values are means with their standard errors (n 1–20 per d in the left; n 5 per d in the right). dM/dt (dM represents the loss of body mass during d$t = t_1 - t_0$ and M is the body mass of rat at t_0) was calculated for each animal. Abscissa: left, days counted from the beginning of starvation in all rats; right, counted to the end of starvation in the P3 group.

sharp decrease during the first hours of starvation and a steady rate at days 2–6 of starvation. Since obvious rapid increase was not observed after day 7 of starvation, we showed that the last part of starvation was counted to the end of starvation in the P3 group. The dM/dMt in the P3 group clearly showed a rapid increase from 3 d before the end of starvation. These patterns were exactly consistent with previous reports[10,14]. Therefore, we defined the phase at first decrease as phase 1, that of steady rate as phase 2 and the third part of the curve as phase 3 according to previous reports[10,14]. The S6 group showed the steady rate of body mass loss and the low blood 3-HBA concentration, and these were characteristics of both phases 2 and 3. These results showed that the S1 group was representative of phase 1, the S2 was of phase 2, the S6 group was in the marginal range between phases 2 and 3, and the P3 group was in phase 3.

Changes in mass of individual organs during starvation

Table 2 shows the changes in the mass of individual organs during starvation. The cerebrum, lung and stomach mass was not affected by starvation. The liver weight was gradually reduced by starvation, and that in the P3 group was 30 % of the control group. From 2 d of starvation, kidney mass

decreased. Heart and spleen mass decreased from 6 d. Testes mass decreased in P3 rats. Prolonged starvation reduced the spleen and liver weight the most.

Blood/plasma parameters

Table 3 shows the blood parameters. Blood 3-HBA increased more in S1 and S2 rats than in control rats. In contrast, the urea concentration in plasma was significantly higher in S6 and P3 rats, whereas there was a non-significant increase in the S1 and S2 rats. Plasma glucose level was 60 % significantly lower in the S1, S2 and S6 rats than in the control rats. Interestingly, plasma glucose returned to the basal level in the P3 rats. Plasma TAG was dramatically decreased after 1 d of starvation and then continued to decrease gradually throughout the remainder of the starvation period. Plasma aspartate aminotransferase was not affected by starvation. Plasma alanine aminotransferase began to increase after 6 d of starvation.

Effect of starvation on vitamin status

Table 4 shows B-group vitamin content in tissue, blood and urine in the control rats. We determined the B-group vitamin contents in nine tissues including the cerebrum, heart, lung, stomach, kidney, spleen, testis, skeletal muscle and liver, and five tissues were selected as representative variations in Fig. 2.

Cerebrum (Fig. 2(A)) and spleen. With the exception of biotin, all vitamin concentrations were unchanged by starvation. Biotin concentration was initially elevated to 150 % in the S1 rats, and then returned to basal level. B-group vitamin concentrations in the spleen showed a similar pattern that starvation did not affect their concentrations except for vitamin B_2. Vitamin B_2 concentration in the testis was elevated to 130 % after 6 d of starvation.

Heart (Fig. 2(B)). Vitamin B_1 and folate concentrations significantly decreased to approximately 60 % after 6 d of starvation. Pantothenic acid concentration was significantly lower in the S2 and S6 rats than in the control rats. Biotin and vitamin B_6 concentrations significantly increased to 160 and 250 % in the S1 and S6 rats, respectively. The other B-group vitamin concentrations were unchanged.

Table 3. Blood parameters in the control and starved rats

(Mean values with their standard errors, n 5)

	CONT		S1		S2		S6		P3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3-HBA (mmol/l)*	0·1[a]	0·0	1·8[a]	0·1	2·5[b]	0·2	0·7[a]	0·1	0·3[a]	0·1
Glucose (mmol/l)	6·36[a]	0·28	3·86[b]	0·32	3·84[b]	0·29	3·71[b]	0·37	6·44[a]	0·60
TAG (mmol/l)	3·01[a]	0·45	0·82[b]	0·08	0·81[b]	0·07	0·54[b]	0·10	0·46[b]	0·05
Urea (mmol/l)	7·45[a]	0·27	6·21[a]	0·42	6·21[a]	0·30	8·23[b]	2·36	13·08[b]	0·68
AST (U/l)	263	21	245	10	242	21	240	10	252	8
ALT (U/l)	39·8[a]	3·8	29·4[a]	1·9	31·8[a]	1·8	65·5[b]	10·0	73·3[b]	5·9

CONT, non-starved control rats; S1, 1-day starved rats; S2, 2-day starved rats; S6, 6-day starved rats; P3, starved to phase 3 rats; 3-HBA, 3-hydroxybutyrate; AST aspartate aminotransferase; ALT, alanine aminotransferase.

[a,b] Mean values within a row with unlike superscript letters were significantly different determined by one-way ANOVA with Tukey's multiple comparison tests ($P < 0.05$).

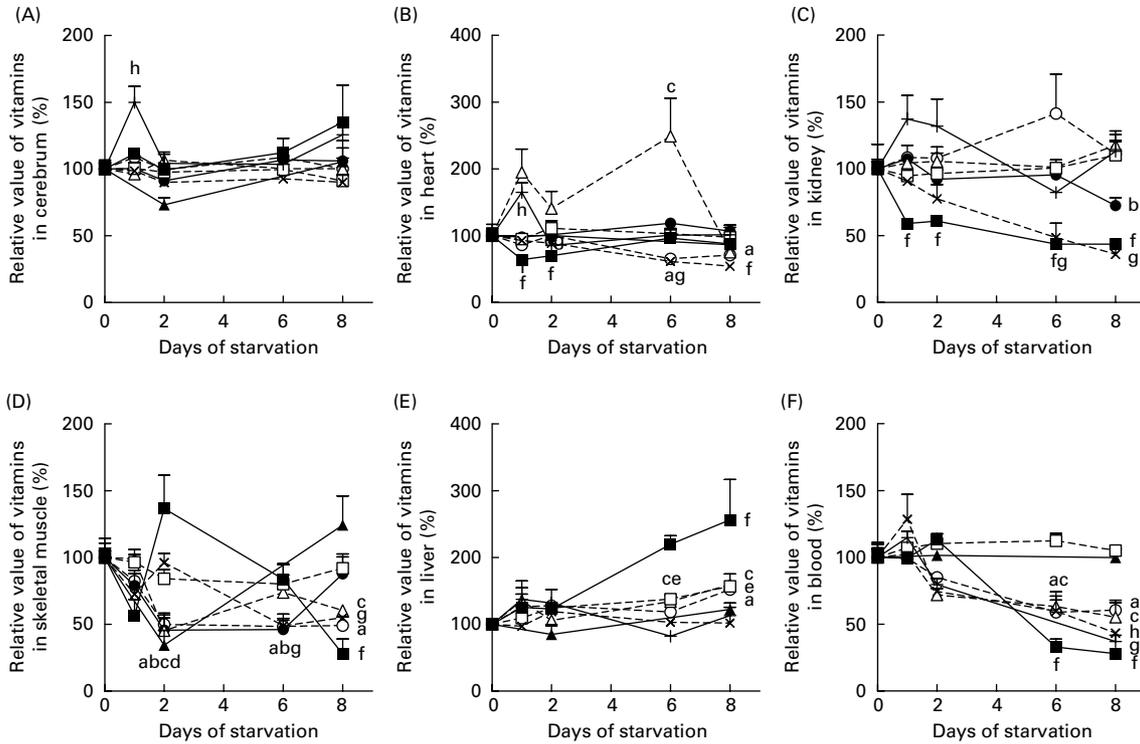* 3-HBA was measured in whole blood, and the others in serum.

Fig. 2. Relative value of B-group vitamin concentrations in (A) cerebrum, (B) stomach, (C) kidney, (D) skeletal muscle, (E) liver and (F) blood of rats during starvation. * Sum of serum pyridoxal and pyridoxal-5′-phosphate is expressed as serum vitamin B_6. Values are reported as means with their standard errors, *n* 5 per d. Values of control rats are expressed as 100 %. P3 is expressed at 8 d of starvation. Means with unlike letters were significantly different from day 0 in [a]vitamin B_1 (○), [b]vitamin B_2 (●), [c]vitamin B_6* (△), [d]vitamin B_{12} (▲), [e]nicotinamide (□), [f]pantothenic acid (■), [g]folate (×) and [h]biotin (+; $P < 0.05$).

Kidney (Fig. 2(C)) and testis. Pantothenic acid concentration dramatically decreased to 50 % of control during starvation. Folate concentration significantly decreased to 40 % in the S6 and P3 rats. Vitamin B_2 concentration significantly decreased to 70 % in the P3 rats. The reduction in pantothenic acid concentration from day 1 of starvation was also observed in the stomach and testis, and their maximal reduction was 60 %. In the testis, vitamin B_2 concentration also significantly reduced to 50 % during starvation, and other vitamin concentrations were not changed.

Skeletal muscle (Fig. 2(D)) and stomach. Concentrations of vitamin B_1, vitamin B_2 and vitamin B_6 significantly decreased to 50 % from 2 d of starvation, but only vitamin B_2 concentration returned to control levels in the P3 rats. Vitamin B_{12} concentration significantly decreased to 40 % only in the S2 rats. Pantothenic acid concentration significantly decreased in the P3 rats. Folate concentration significantly decreased to 50 % in the S6 and P3 rats. Pantothenic acid concentration was same as control until 6 d of starvation, and then dramatically decreased to 30 % in the P3 rats. Similar pattern was observed in the stomach that starvation reduced several B-group vitamin concentrations. In brief, pantothenic acid and biotin concentrations reduced to 30 and 60 % from day 1 of starvation, respectively. Vitamin B_6 concentration significantly decreased to 50 % from day 2, and vitamin B_2 did to 40 % from day 6 of starvation.

Liver (Fig. 2(E)). Vitamin B_6 concentration was significantly higher in the S1, S6 and P3 rats, and the relative value in the P3

rats was 160 % of the control animals. Nicotinamide concentration increased in the S2, S6 and P3 rats. Vitamin B_1 and pantothenic acid concentrations were higher in the P3 rats, and their values were 150 and 250 %, respectively. Other B-group vitamins concentrations were unchanged.

Blood (Fig. 2(F)). Whole blood vitamin B_1, serum vitamin B_6 and plasma pantothenic acid concentrations decreased in the S6 and P3 rats. Plasma folate and biotin concentrations decreased in the P3 rats. The relative values of vitamin B_1 and vitamin B_6 in the P3 rats were 60 % of control, those of folate and biotin were 50 %, and those of pantothenic acid were 30 %.

Urinary contents of B-group vitamins (Fig. 3). Vitamin B_1 excretion acutely decreased to 10 % after 1 d of starvation. Urinary excretion of riboflavin, pyridoxal metabolite 4-pyridoxic acid and vitamin B_{12} gradually decreased during 4 d of starvation. Subsequently these values were stable, at approximately 20, 20 and 50 % of each control value. Urinary folate was initially unchanged in the S1 rats and then decreased to 40 % of the baseline value. Urinary pantothenic acid was increased to 170 % in 3rd and 4th days of starvation, and then returned to the control level. Although biotin excretion increased to 460 % during the first 3 d of starvation, it subsequently returned to the basal level.

Urinary contents of nicotinamide and its catabolites (Fig. 4). Nicotinamide excretion increased after 1 d of starvation and then returned to the basal level. 2-Py and 4-Py decreased after an initial increase on day 1. In contrast, MNA excretion

British Journal of Nutrition

Table 4. Contents of B-group vitamins in each tissue, blood and urine of control rats
(Mean values with their standard errors, n 4–5)

	Vitamin B$_1$ (nmol/g tissue)		Vitamin B$_2$ (nmol/g tissue)		Vitamin B$_6$ (nmol/g tissue)		Vitamin B$_{12}$ (pmol/g tissue)		Nicotinamide (nmol/g tissue)		Pantothenic acid (nmol/g tissue)		Folate (nmol/g tissue)		Biotin (nmol/g tissue)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cerebrum	9·1	0·4	6·6	0·1	18·3	0·4	35	2	292	12	61	3	0·52	0·02	0·19	0·01
Heart	18·9	1·5	34·4	1·6	14·5	1·6	202	20	578	46	166	9	0·75	0·03	0·23	0·04
Lung	5·7	0·5	8·4	0·6	3·6	0·3	49	4	375	11	59	8	0·78	0·03	0·13	0·02
Stomach	11·7	1·7	11·5	0·3	3·4	0·6	77	12	221	22	51	5	1·14	0·02	0·27	0·01
Kidney	16·8	3·1	57·2	2·7	18·8	1·1	269	19	920	27	250	18	6·47	0·35	1·40	0·12
Spleen	7·0	0·6	6·4	0·4	1·4	0·2	74	8	645	26	33	4	1·17	0·04	0·05	0·01
Testis	25·2	6·4	9·6	0·8	13·9	0·4	61	7	186	7	101	8	0·07	0·00	0·08	0·01
Skeletal muscle	4·3	0·2	7·5	0·5	35·5	1·8	38	5	400	24	22	2	0·30	0·01	0·03	0·00
Liver	32·2	1·8	68·0	3·2	17·1	0·6	144	10	862	34	242	10	14·14	0·69	1·84	0·11
Blood	285 (pmol/ml WB)	15	208 (pmol/ml WB)	12	1·46 (pmol/ml serum)	0·16	4·97 (pmol/ml plasma)	0·13	121 (nmol/ml WB)	5	7·02 (nmol/ml plasma)	0·39	43·2 (pmol/ml plasma)	3·6	54·6 (pmol/ml plasma)	3·5
Urine	96 (nmol/d)	4	65 (nmol/d)	12	345 (nmol/d)	4	38 (pmol/d)	2	3·93 (μmol/d)	0·17	567 (nmol/d)	68	6·20 (nmol/d)	0·68	2·96 (pmol/d)	0·25

WB, whole blood.

increased during the starvation period. Urinary excretion of the sum of nicotinamide and its catabolites increased 1·4-fold after 1 d of starvation and then decreased by less than half of the food sufficient state.

Discussion

The effects of metabolic changes, which are designated as the changes in the main energy sources such as glucose, lipids and protein, during starvation on the tissue and urine vitamin concentrations are currently poorly understood[11]. Elucidation of the effects will lead to a suitable supplementation for preventing the refeeding syndrome. Therefore, we investigated the effects of short- and long-term starvation on the vitamin concentrations in organs, muscle, blood and urine in rats.

Vitamin concentrations in organs and muscle showed different patterns for each vitamin. For noticeable characteristics, biotin concentration, which means the value in terms of g tissue, was increased in most organs of the S1 rats. A part of the reason is a reduced organ mass at S1. It was unclear why the biotin concentrations in organs remained at the same level regardless of organ mass during starvation. Vitamin B$_1$ is the vitamin that has the most rapid turnover[30], but the levels in the kidney were maintained. This may point to the necessity of vitamin B$_1$ in kidneys of starving rats. In terms of the metabolic state, vitamin B$_1$ was expected to decrease in the early days of starvation, because glucid is the main energy source in this period[3]. However, vitamin B$_1$ concentrations in tissues and blood were stable in the S1 rats. This is due to the sharp decrease in liver weight and in the urinary excretion of vitamin B$_1$. Along with the shifts in the main energy source from glucid to fat, vitamin demands appear to change. Next to vitamin B$_1$, pantothenic acid requirement may be the highest because it is involved in the metabolism of fatty acids[31], and also, biotin requirement may be higher because the gluconeogenesis is more active at the deficient state of glucose[32]. However, the present results were contrary to our expectations. Pantothenic acid concentrations in the heart, stomach, kidneys and testes were decreased in the S1 rats, and a similar phenomenon was observed in biotin concentrations. The urinary excretion of pantothenic acid and biotin was significantly increased by starvation. A similar phenomenon was already reported by Fukuwatari et al.[30]. Shibata and co-workers reported[33–38] that the urinary excretion of water-soluble vitamins reflects recent intake of the vitamins over the last few days, and in addition, the decreased urinary excretion of vitamins means the elevated demand for vitamins, whereas the increased urinary excretion of vitamins means the reduced demand for vitamins when their intake of vitamins is almost the same[33]. The increased urinary excretion suggests that the requirement of pantothenic acid and biotin was reduced by starvation, that is, lower concentrations of pantothenic acid and biotin in the body might prefer to live for a long time during starvation. A possible inferable reason for the increase in urinary pantothenic acid and biotin might be a mechanism to prevent the stored fat in the body from overspending or to decrease the amount of acetyl-coenzyme A,
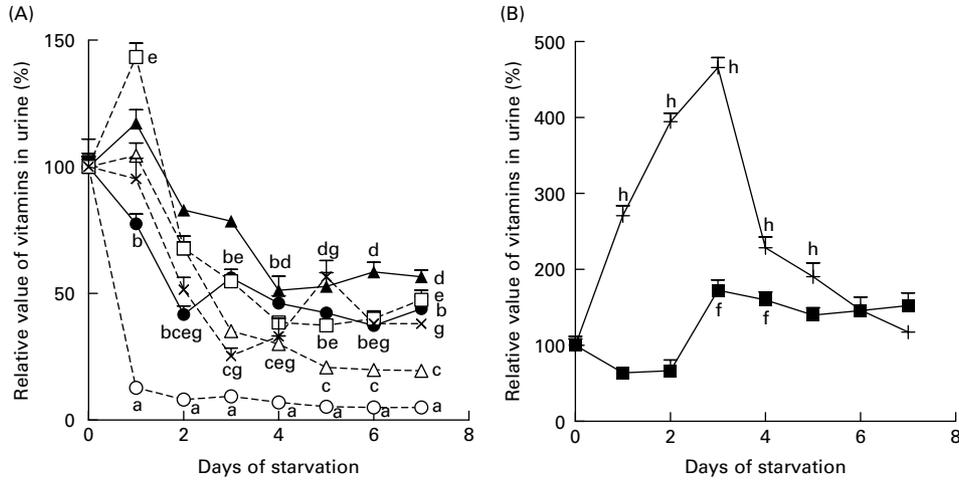
Fig. 3. Relative value of urinary B-group vitamin contents in P3 rats during starvation. Those of vitamin B$_1$, vitamin B$_2$, vitamin B$_6$, vitamin B$_{12}$, nicotinamide and folate are shown in (A), and pantothenic acid and biotin in (B). Thiamin is expressed as vitamin B$_1$, riboflavin as vitamin B$_2$, 4-pyridoxic acid as vitamin B$_6$, and sum of nicotinamide and its catabolites as nicotinamide. Values are reported as means with their standard errors, n 5 per d. Values of control rats are expressed as 100 %. P3 is expressed at 8 d of starvation. Means with unlike letters were significantly different from day 0 in [a]vitamin B$_1$ (○), [b]vitamin B$_2$ (●), [c]vitamin B$_6$* (△), [d]vitamin B$_{12}$ (▲), [e]nicotinamide (□), [f]pantothenic acid (■), [g]folate (×) and [h]biotin (+; $P<0.05$).

which modifies several functional proteins such as histone[39] and some enzymes[40], and in addition, to decrease holoenzymes of carboxylases[41,42]. Acetylation generally activated some enzymes in fatty acid oxidation[40]. The physiologically active form of biotin is covalently attached at the active site of a class of important metabolic enzymes in gluconeogenesis, lipogenesis and amino acid metabolism[41,42]. Accordingly, decreased acetylation and biotin-dependent enzymes lead to reduced fatty acid oxidation and to save fat in the body.

Vitamin B$_6$ concentrations, expected to be the last vitamin decreased in tissues by starvation[3], decreased in the stomach, skeletal muscle and serum of the S2 rats. Vitamin B$_6$ in the
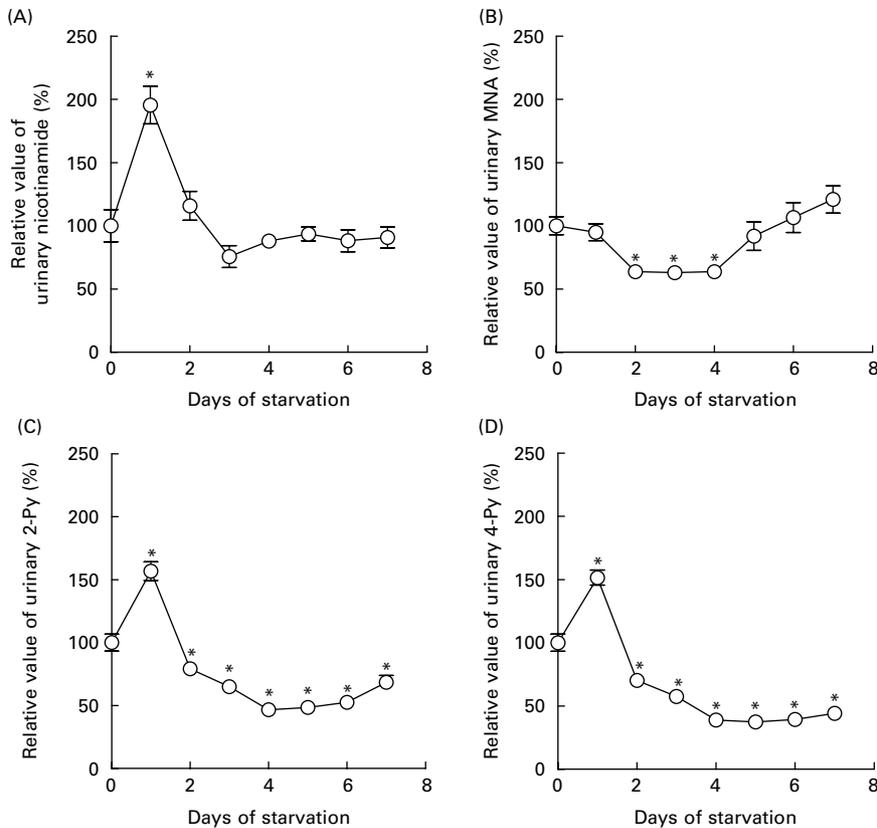

Fig. 4. Relative value of urinary nicotinamide (a) and its catabolites MNA (b), 2-Py (c) and 4-Py (d) contents in P3 rats during starvation. Values are reported as means with their standard errors, n 5 per d. *Mean values were significantly different from day 0 determined by one-way ANOVA with Tukey's multiple comparison tests ($P<0.05$). MNA, N^1-methylnicotinamide; 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide.

lung and serum decreased in the S6 rats. The differences in the pattern of vitamin B_6 decline may be due to the fat content of each tissue. Nicotinamide concentrations in tissues and blood were unchanged by starvation, despite this vitamin being involved in energy metabolism. Since nicotinamide is biosynthesised from tryptophan[43], nicotinamide concentrations in organs and blood were maintained. Urinary excretion of the sum of nicotinamide and its catabolites was high after 1 d of starvation and subsequently decreased. These results are in agreement with those reported by a previous study[30]. The proportion of nicotinamide, MNA, 2-Py and 4-Py in urine is controlled by enzymes involved in the metabolism of tryptophan to niacin. Starvation or food restriction induces a decline in MNA oxidase activity[44] and an elevation in nicotinamide methyltransferase[44]. This may explain why levels of 2-Py and 4-Py in urine decreased while those of MNA increased. MNA is an inhibitor of nicotinamide methyltransferase[45]. Therefore, an accumulation of MNA inhibits the activity of nicotinamide methyltransferase and leads to an increase in free form of nicotinamide, which inhibits the activities of histone deacetylase[46] and poly(ADP-ribose) synthetase[47]. This control might be suitable for living long during starvation.

To our knowledge, the present study presents the first data on vitamin status during the three phases of starvation. The changes in B-group vitamin concentrations in tissues and blood did not always correspond to metabolic states. The changes in vitamin content can be divided into three groups. First, vitamin B_1, vitamin B_2, nicotinamide and biotin levels declined gradually. Second, vitamin B_6 and vitamin B_{12} levels rapidly decreased after 1 d of starvation and then remained at a steady level. Finally, pantothenic acid and folate initially decreased in the S1 rats, then returned to near basal levels in the next day of starvation, then subsequently decreased again. This might mean that pantothenic acid and folate were mobilised to other tissues. We are unsure why such complicated changes occur. It is clear that further investigation, such as separate measurement of the free forms of the vitamins and of coenzymes, into the changes in the vitamin requirements of starving rats would be useful for the prevention of vitamin deficiency during starvation or for consequent refeeding.

Acknowledgements

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