A Suitable Diet for Recovery from Starvation Is a High-Fat Diet, but Not a High-Protein Diet, in Rats

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Summary The present study aims to determine the most suitable dietary balance of energy-producing nutrients for recovery from starvation. Rats were fed their standard high-carbohydrate diet (HCD, carbohydrate energy : protein energy : fat energy = 71 : 18 : 11) for 7 d and then deprived of food for 3 d (short-term starvation) or 8 d (long-term starvation). The starved rats were then fed the HCD, a high-protein diet (HPD, 31 : 57 : 12), or a high-fat diet (HFD, 34 : 14 : 52) for 8 d. Rats had ad libitum access to drinking water throughout the experimental period, including the starvation period. The reference group was allowed free access to the HCD throughout the experimental period. Characteristically, increased drinking, increased urea nitrogen in the plasma and urine, and hypertrophy of the kidneys, were observed in the HPD group. Furthermore, the recovery of plasma glucose level was insufficient in this group. Therefore, administration of a HPD was contraindicated in recovery from starvation. The recovery of body weight after starvation was excellent in the HFD group. No effect on the metabolism of B-group vitamins involved in energy metabolism was found with the administration of any diet. The effects of HCD and HFD administration on recovery from starvation were investigated in further detail. No adverse effects were observed on the tissue to body weight mass ratios or biochemical parameters in blood in the HFD group. From the above findings, it is hypothesized that a HFD is most suitable for quickly reversing the influence of starvation.

Key Words refeeding, high-protein diet, high-fat diet, high-carbohydrate diet, starvation

Energy homeostasis is one of the most important functions of our body. Negative energy balance from restricted feeding or total starvation can arise as a result of diseases, eating or psychological disorders, or hunger strikes. This induces severe weight loss. Refeeding syndrome is dependent on conditions such as degree of negative energy balance and the diet used for refeeding (1, 2). Thus, the goal of this study was to establish the optimal method for recovery from a period of negative energy balance.

Food intake regulation and energy balance are achieved through the complex coordination of peripheral signals and central regulatory circuitry (3, 4). These processes determine the initiation, termination, size, composition, and frequency of meals, and the long-term regulation of food intake in relation to body energy requirements (5). Notably, refeeding after starvation is followed by multiple adaptations in the liver and adipose tissue. In rats, meal feeding compared with nibbling causes similar metabolic changes as refeeding (6).

Rats select a diet containing 30–35% protein energy, 45–50% fat energy, and 15–20% carbohydrate energy (7), but the preference for these ratios is altered by starvation, food restriction, pregnancy, lactation, and an increase in energy consumption (8–10). Several studies have investigated the factors associated with self-selection of macronutrients and food intake patterns in rats after starvation (10–13). It is reported that starved-refed rats select a fat-rich diet because of its high energy content (13). Other studies have investigated the intake pattern of macronutrients, including kinds of fat, in starved rats. However, to the best of our knowledge, the influence of nutritional components on the recovery of starved rats, e.g., whether a high-fat diet has negative consequences such as obesity, has not been determined. Therefore, we decided to evaluate the benefits of specific macronutrients for the refeeding of starved rats.

In previous studies from our group, rats fed a 60% casein diet showed the same growth pattern as those on a standard diet (20% casein) (14), and rats receiving a 30% fat diet consumed the same number of calories as those receiving the standard diet (5% fat) (15). Therefore, we fed diets with these macronutrient compositions (which had no influence on non-starved rats) to starved rats.

MATERIALS AND METHODS

Diets. Three kinds of diets were prepared: a standard rat diet (namely a high-carbohydrate diet [HCD]), a
Table 1. Composition of the diets.

|                        | High-carbohydrate diet (HCD, ordinary diet) | High-protein diet (HPD) | High-fat diet (HFD) |
|------------------------|---------------------------------------------|-------------------------|---------------------|
| Energy value (kcal/100 g diet) | 398                                         | 381                     | 523                 |
| Energy % of carbohydrate (%) | 70.6                                        | 31.4                    | 34.6                |
| Energy % of protein (%)    | 18.1                                        | 56.8                    | 13.8                |
| Energy % of fat (%)       | 11.3                                        | 11.8                    | 51.6                |
| Vitamin-free milk casein  | 20.0                                        | 60.0                    | 20.0                |
| l-Methionine             | 0.2                                         | 0.6                     | 0.2                 |
| Gelatinized cornstarch    | 46.9                                        | 19.9                    | 30.2                |
| Sucrose                  | 23.4                                        | 10.0                    | 15.1                |
| Corn oil                 | 5.0                                         | 5.0                     | 30.0                |
| Mineral mixture (AIN-93-G) | 3.5                                         | 3.5                     | 3.5                 |
| Vitamin mixture (AIN-93)  | 1.0                                         | 1.0                     | 1.0                 |

Fig. 1. Effects of refeeding on body mass (A), water intake (B), food intake (C), and energy intake (D) in 3 d starved rats (Experiment 1). The rats were fed a standard high-carbohydrate diet (HCD) for 7 d then starved for 3 d. The starved rats were divided into three groups; the first group was fed a HCD (○), the second a high-protein diet (HPD, ■), and the third a high-fat diet (HFD, △) for 8 d. The reference group (●) was fed the HCD during the whole experiment. Each value is the mean ± SE of four rats. A different superscript letter on the last day of the experiment indicates a significant difference, p<0.05, as determined by one-way ANOVA followed by Tukey’s multiple comparison test.
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The compositions of these diets are shown in Table 1. Vitamin-free milk casein, l-methionine, and sucrose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Nisshin OilliO Group, Ltd. (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (AIN-93G) and a vitamin mixture (AIN-93) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). All rats were given ad libitum access to tap water.

**Chemicals.** Thiamin hydrochloride (C$_{12}$H$_{17}$ClN$_4$O$_2$S·HCl, molecular weight [MW] 533.27), riboflavin (C$_{17}$H$_{20}$N$_4$O$_6$, MW = 537.37), pyridoxal phosphate monohydrate (C$_{8}$H$_{10}$NO$_6$P·H$_2$O, MW = 265.16), and niacinamide (C$_{6}$H$_{6}$N$_2$O, MW = 122.13) were purchased from Wako Pure Chemical Industries. 4-Pyridoxic acid (4-PIC) (C$_8$H$_9$NO$_4$, MW = 183.16) was manufactured by ICN Pharmaceuticals (Costa Mesa, CA), was obtained from Wako Pure Chemical Industries. N$^1$-Methylnicotinamide (MNA) chloride (C$_7$H$_9$N$_2$O·HCl, MW = 159.61) was purchased from Tokyo Chemical Industry (Tokyo, Japan). N$^1$-Methyl-2-pyridone-5-carboxamide (2-Py) (C$_7$H$_8$N$_2$O$_2$, MW = 152.15) and N$^1$-methyl-4-pyridone-3-carboxamide (4-Py) (C$_7$H$_8$N$_2$O$_2$, MW = 152.15) were synthesized as described (17, 18). All other chemicals were of the highest purity available from commercial sources.

**Experimental procedures.** Male Wistar rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). Rats were individually housed in metabolic cages (CL-0355, CLEA Japan) in a temperature-controlled room (22±2°C, 50–60% humidity) with a 12-h/12-h light/dark cycle. Body mass, food, and water consumption were recorded daily (±0.1 g). This study was conducted according to the guidelines for the care and use of laboratory animals and approved by the Ethics Committee of the University of Shiga Prefecture (approval number 21-6).

Experiment 1 (Short-term starvation): Male Wistar rats (8 wk of age, weighing 250–260 g, n = 16) were initially fed the HCD diet for 7 d and then randomly divided into four groups (each group, n = 4). The first group was used as a reference group for Experiment 1 and fed the HCD during the whole experiment. Each value is the mean±SE of four rats. A different superscript letter on the last day of the experiment indicates a significant difference, p<0.05, as determined by one-way ANOVA followed by Tukey’s multiple comparison test.

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*Fig. 2. Effects of refeeding on body mass (A), water intake (B), food intake (C), and energy intake (D) in 8 d starved rats (Experiment 2). The rats were fed a standard high carbohydrate diet (HCD) for 7 d then starved for 8 d. The starved rats were divided into three groups; the first group was fed a HCD (○), the second a high-protein diet (HPD, ■), and the third a high-fat diet (HFD, △) for 8 d. The reference group (●) was fed the HCD during the whole experiment. Each value is the mean±SE of four rats. A different superscript letter on the last day of the experiment indicates a significant difference, p<0.05, as determined by one-way ANOVA followed by Tukey’s multiple comparison test.*
Table 2. Effects of dietary macronutrient balance on organ mass in rats when refeeding after 3 d (Experiment 1) or 8 d (Experiment 2) of starvation.

|        | Reference-1, 11 d feeding with HCD (Experiment 1) | Short-term starvation (Experiment 1) (3 d starved→8 d fed) | Reference-2, 16 d feeding with HCD (Experiment 2) | Long-term starvation (Experiment 2) (8 d starved→8 d fed) |
|--------|--------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------|----------------------------------------------------------|
|        | HCD                                             | HPD                                                      | HFD                                             | HCD                                                      |
|        | (8 d starved→8 d fed)                           | (3 d starved→8 d fed)                                     | (8 d starved→8 d fed)                           | (8 d starved→8 d fed)                                     |
| Cerebrum | 0.396±0.030                                     | 0.417±0.010                                             | 0.383±0.011                                    | 0.424±0.018                                              |
| Heart   | 0.311±0.002                                     | 0.322±0.005                                            | 0.302±0.012                                    | 0.305±0.005                                              |
| Lung    | 0.398±0.018                                     | 0.394±0.012                                             | 0.464±0.035                                    | 0.423±0.014                                              |
| Kidneys | 0.644±0.022                                     | 0.690±0.024                                            | 0.876±0.016                                    | 0.643±0.013                                              |
| Liver   | 3.81±0.017                                      | 3.73±0.06                                              | 4.32±0.21                                    | 3.44±0.02                                               |
| Spleen  | 0.224±0.009                                     | 0.234±0.007                                            | 0.235±0.005                                    | 0.230±0.007                                              |
| Testes  | 0.939±0.011                                     | 1.00±0.02                                              | 0.974±0.026                                    | 1.03±0.02                                               |

Each value is expressed as g/100 g body weight and the mean±SE of four rats. A different superscript letter in the same row within the same experiment indicates a significant difference; * p<0.05, as determined by one-way ANOVA followed by Tukey’s multiple comparison test; ** p<0.05 as determined by Student’s t-test compared with the respective reference value. HCD, high-carbohydrate diet; HPD, high-protein diet; HFD, high-fat diet.

Table 3. Effects of dietary macronutrient balance on plasma variables and urine urea nitrogen in rats when refeeding after 3 d (Experiment 1) or 8 d (Experiment 2) of starvation.

|        | Reference-1, 11 d feeding with HCD (Experiment 1) | Short-term starvation (Experiment 1) (3 d starved→8 d fed) | Reference-2, 16 d feeding with HCD (Experiment 2) | Long-term starvation (Experiment 2) (8 d starved→8 d fed) |
|--------|--------------------------------------------------|-----------------------------------------------------------|--------------------------------------------------|----------------------------------------------------------|
|        | HCD                                             | HPD                                                      | HFD                                             | HCD                                                      |
|        | (8 d starved→8 d fed)                           | (3 d starved→8 d fed)                                     | (8 d starved→8 d fed)                           | (8 d starved→8 d fed)                                     |
| Plasma |                                                  |                                                           |                                                 |                                                          |
| Glucose (mg/dL) | 73.5±2.3                                     | 71.8±6.0                                                  | 60.8±4.7                                        | 82.5±11.4                                                |
| Triglyceride (mg/dL) | 282±50                                    | 136±8*                                                   | 126±15*                                        | 126±17*                                                  |
| Creatinine (mg/dL)  | 0.275±0.025                                  | 0.225±0.025                                              | 0.225±0.025                                    | 0.250±0.029                                              |
| Urea nitrogen (mg/dL) | 21.8±1.1                                    | 18.7±1.6*                                                | 51.4±3.3*                                      | 19.9±0.9*                                                |
| AST (U/L)       | 205±15                                         | 220±11                                                   | 242±18                                        | 248±24                                                   |
| ALT (U/L)       | 34.3±1.9                                       | 26.3±1.4                                                  | 37.3±5.1                                      | 34.3±3.0                                                  |
| Urine |                                                  |                                                           |                                                 |                                                          |
| Urea nitrogen (g/d) | 0.413±0.058                                  | 0.294±0.026                                              | 0.417±0.010                                    | 0.424±0.018                                              |

Each value is the mean±SE of four rats; a different superscript letter in the same row within the same experiment indicates a significant difference; * p<0.05, as determined by one-way ANOVA followed by Tukey’s multiple comparison test; ** p<0.05 as determined by Student’s t-test compared with the respective reference value. HCD, high-carbohydrate diet; HPD, high-protein diet; HFD, high-fat diet; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
The fourth group was deprived of food for 3 d and then fed the HFD for 8 d.

Experiment 2 (Long-term starvation): Male Wistar rats (8 wk of age, weighing 250–260 g, n = 16) were initially fed the HCD diet for 7 d and then randomly divided into four groups (each group, n = 4). The first group was used as the reference group for Experiment 2 and fed the HCD ad libitum during the whole experimental period (23 d). The second group was deprived of food for 8 d and then fed the HCD for 8 d. The third group was deprived of food for 8 d and then fed the HPD for 8 d. The fourth group was deprived of food for 8 d and then fed the HFD for 8 d.

On the last day of the respective experiments, animals were euthanized by decapitation. Whole blood from the carotid artery was immediately collected into tubes containing EDTA-2K for plasma preparation. 5% trichloroacetic acid for vitamin B1 measurement, water for vitamin B2 preparation, and isonicotinamide for nicotinamide measurement. For plasma preparation, the whole blood in tubes containing EDTA-2K was centrifuged at 2,000 \( \times g \) for 10 min at room temperature and the resulting supernatants were used as the plasma samples. Whole blood samples treated with trichloroacetic acid, water, or isonicotinamide, and plasma samples, were stored at −20°C until analysis.

Twenty-four-hour urine samples were periodically collected and stored at −20°C until analysis. Urinary excretion of urea nitrogen, and vitamins (vitamin B1 (19), vitamin B2 (20), 4-PIC (21), and nicotinamide and its catabolites (MNA, 2-Py, 4-Py) (18, 22)) was measured in Experiments 1 and 2.

Plasma glucose, urea nitrogen, triglyceride, total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured with a FUJI DRI-CHEM analyzer (Fujifilm, Tokyo, Japan). Vitamin B1 (19), vitamin B2 (20), and nicotinamide (18) were measured in whole blood, and vitamin B6 (23) was measured in plasma in Experiments 1 and 2.

Rats were euthanized after the last urine samples had been collected. The tissues and organs listed in Table 2 were dissected and weighed. Vitamin B1 (19), vitamin B2 (20), vitamin B6 (23), and nicotinamide (18) were measured in liver in Experiments 1 and 2.

Experiment 3: Male Wistar rats (7 wk of age, weighing 220–230 g, n = 16) were initially fed the HCD diet
for 7 d and then deprived of food for 3 d. They were then randomly divided into two groups (each group, n=8). The first group was refed the HCD for 8 d and the second group was refed the HFD for 8 d.

Four rats per group were euthanized at each of the following timepoints: at the beginning of the experiment (pre-starvation), at day 3 of starvation (the end of starvation), at day 1 of refeeding, and at day 8 of refeeding (the last day of the experiment). The heart, kidneys, liver, epididymal and perirenal white adipose tissue, interscapular brown adipose tissue, inguinal subcutaneous fat, soleus muscle, and gastrocnemius muscle were dissected and weighed (±0.01 g).

**Statistical methods.** Values are expressed as the mean±SE for four rats. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparison tests (Figs. 1, 2, 7, 8, and 9, and Tables 2 and 3) and by Student’s t-tests (Figs. 3, 4, and 5, and Tables 2 and 3). Differences between groups with a p value of less than 0.05 were considered statistically significant. GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) was used for all statistical analyses.

**RESULTS**

**Changes in body mass, water intake, and food intake during starvation and refeeding (Experiments 1 and 2)**

Changes in body mass, water intake, and food intake during 3 d and 8 d of starvation and refeeding are shown in Figs. 1 and 2, respectively. A body weight loss of 7% was produced by starvation for the first 24 h in both experiments. From the second day to the last day of starvation, the rats lost 5% body weight every 24 h in both experiments. Refeeding with the HFD restored body weight more rapidly than refeeding with the HCD or HPD. The HPD group showed the slowest recovery of body weight (Figs. 1A and 2A).

Water intake decreased gradually during starvation and returned to the initial level after refeeding, except in the HPD group (Figs. 1B and 2B). Administration of the HPD induced polydipsia and polyuria (data not shown).

Food intakes after short-term (3 d) starvation were the same by weight as before starvation in all diet groups (Fig. 1C). Thus, the rats fed the HFD consumed approximately 30 kcal/d more than the other groups (Fig. 1D). Long-term (8 d) starvation groups showed...
a slight anorexia from day 1 to day 3 of refeeding. In contrast, after 4 d of refeeding, the rats demonstrated a trend toward excess eating (Fig. 2C). Thus, the energy intake of rats fed the HFD was approximately 30 kcal/d higher than that of the other two diet groups (Fig. 2D).

Changes in individual organ mass of starved-refed rats (Experiments 1 and 2)

Table 2 shows the changes in mass of individual organs in Experiments 1 and 2. Cerebrum, heart, lung, spleen, and testes mass were little affected by starvation in both experiments. Kidney mass in the HPD group was significantly higher than in the other groups in both experiments. In Experiment 2, liver mass in the HCD group was significantly higher than in the HFD group. In both experiments, liver mass in the HCD group was significantly higher than in the respective reference groups.

Blood parameters of starved-refed rats (Experiments 1 and 2)

Table 3 shows the blood parameters of starved-refed rats. After long-term starvation (Experiment 2), the concentration of plasma glucose was lower in the HPD group than in the other two diet groups. Notably, the concentrations of triglyceride and creatinine in the starved groups were not restored to the reference values in this experiment. Urea nitrogen concentration in the HPD group was significantly higher than in the other two diet groups in both experiments. AST and ALT levels were not significantly altered in either experiment.

Urea nitrogen in urine of starved-refed rats (Experiments 1 and 2)

Table 3 also shows the urea nitrogen levels in urine of starved-refed rats. Notably, the concentration of urea nitrogen in the HPD group increased by up to 3-fold in the short- and long-term starvation experiments.

Vitamin concentrations in urine, blood, and liver of starved-refed rats (Experiments 1 and 2)

Figure 3 shows the changes in vitamin B1 concentration in urine, whole blood, and liver of starved-refed rats in the short- and long-term starvation experiments. As shown in Fig. 3A, the urinary excretion of vitamin B1 steeply decreased to almost zero by day 1 of starvation and remained low during starvation. The recovery of urine vitamin B1 was quicker in the 3 d starvation experiment than in the 8 d starvation experiment (Fig. 3B and 3C). The urine vitamin B1 content in
the HFD group did not recover to the reference value in either experiment. The whole blood and liver concentrations of vitamin B₁ were completely recovered by day 8 of refeeding in all diet groups in both experiments (Fig. 3D and 3E).

Vitamin B₂. Figure 4 shows the changes in vitamin B₂ concentration in urine, whole blood, and liver of starved-refed rats. As shown in Fig. 4A, the urinary excretion of vitamin B₂ gradually decreased with starvation and remained low during starvation. The recovery of urine vitamin B₂ was almost the same in both experiments (Fig. 4B and 4C). The urine vitamin B₂ content recovered to the reference value at day 4 of refeeding. The whole blood and liver concentrations of vitamin B₂ were completely recovered by day 8 of refeeding in all diet groups in both experiments (Fig. 4D and 4E).

Vitamin B₆. Figure 5 shows the changes in vitamin B₆ concentration in urine, plasma, and liver of starved-refed rats. As shown in Fig. 5A, the urinary excretion of 4-PIC, a catabolite of vitamin B₆, increased at day 1 of starvation but gradually decreased after that. The recovery pattern of urine 4-PIC was almost the same in both experiments (Fig. 5B and 5C). Recovery was slower in the HPD group than in the other two diet groups. The plasma and liver concentrations of vitamin B₆ were completely recovered by day 8 of refeeding in all diet groups in both experiments (Fig. 5D and 5E).

Nicotinamide. Figure 6 shows the changes in nicotinamide concentration in urine, whole blood, and liver of starved-refed rats. As shown in Fig. 6A, the urinary excretion of the sum of nicotinamide and its catabolites (MNA, 2-Py, and 4-Py) increased at day 1 of starvation, then decreased at day 2 of starvation and remained constant after that. The recovery of urinary excretion of the sum of nicotinamide and its catabolites was slower in the 8 d starvation experiment than in the 3 d starvation experiment (Fig. 6B and 6C). Recovery was faster in the HPD group than in the other two diet groups in both experiments. The whole blood and liver concentrations of nicotinamide were completely recovered by day 8 of refeeding in all diet groups in both experiments (Fig. 6D and 6E).

Changes in body mass, food intake, and energy efficiency during starving and refeeding (Experiment 3)

Changes in energy intake, body mass, and energy efficiency (energy accumulated as body mass/energy consumed) during 3 d of starvation and 8 d of refeeding (HCD and HFD groups only) are shown in Fig. 7. Energy...
intake (kcal/d) in the HFD group was higher than in the HCD group (Fig. 7A) because daily food intakes (g/d) during the refeeding period were almost the same between the HCD and HFD groups. On day 1 of the refeeding period, body mass gain in the HFD group was higher than in the HCD group (Fig. 7B). On days 2 to 7 of the refeeding period, body mass gains in both groups were almost the same (approximately 10 g/d). Energy efficiency (Fig. 7C) was higher in the HCD group than in the HFD group on day 1 of the refeeding period. On days 2 to 7 of the refeeding period, energy efficiency was almost the same between the two groups.

Changes in the mass of individual organs, fat, and muscle of starved-refed rats (Experiment 3)

Figure 8 shows the changes in the mass of individual organs, fat, and muscle in terms of g/kg of body weight. Heart (Fig. 8A) and kidney (Fig. 8B) mass ratios were little affected by starvation and refeeding. Three days of starvation induced a decline in the mass ratios of liver (Fig. 8C), epididymal white adipose tissue (Fig. 8D), perirenal white adipose tissue (Fig. 8E), and interscapular brown adipose tissue (Fig. 8F) in both groups. In contrast, starvation induced an increase in the mass ratios of the soleus muscle (Fig. 8H) and gastrocnemius muscle (Fig. 8I) in both groups.

On day 1 of refeeding, perirenal white adipose tissue
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(Fig. 8E) and subcutaneous fat inguinal brown adipose tissue (Fig. 8G) mass ratios further declined, while liver mass (Fig. 8C), epididymal white adipose tissue (Fig. 8D), and interscapular brown adipose tissue (Fig. 8F) mass ratios recovered to their respective reference values in both groups. The gastrocnemius muscle (Fig. 8I) mass ratio was still higher than the reference value at day 1 of refeeding in both groups.

By day 7 of refeeding after 3 d of starvation, the mass ratios of individual organs, fat, and muscle had recovered to their reference values in both groups. However, the mass ratios of epididymal white adipose tissue (Fig. 8D) in both groups, perirenal white adipose tissue (Fig. 8E) in the HFD group, and gastrocnemius muscle (Fig. 8I) in the HFD group, remained higher than their respective reference values.

Blood parameters of starved-refed rats (Experiment 3)

Blood parameters of starved-refed rats are shown in Fig. 9. Three days of starvation induced hypoglycemia (Fig. 9A) and a reduction in triglyceride (Fig. 9C) and total cholesterol (Fig. 9D) concentrations. The concentrations of plasma urea nitrogen (Fig. 9B), AST (Fig. 9E), and ALT (Fig. 9F) were not affected by short-term starvation. By day 1 of refeeding, starvation-induced hypoglycemia was recovered (Fig. 9A). Triglyceride concentrations in the HFD group were lower compared with the reference value; however, those in the HCD group were recovered by day 1 of refeeding (Fig. 9C). Cholesterol concentrations in both groups remained lower than the reference value (Fig. 9D). AST in the HCD group (Fig. 9E) and ALT in both groups (Fig. 9F) were significantly higher compared with the reference values. By day 7 of refeeding after 3 d of starvation, only triglyceride (Fig. 9C) and cholesterol (Fig. 9D) concentrations in the HFD group had not recovered to their respective reference values.
Refeeding syndrome is an important, yet commonly overlooked, condition affecting patients. More research is needed in this field as an evidence base is lacking. Refeeding syndrome is caused by rapid refeeding after a period of under-nutrition. In the present study, we compared the recovery effects of HCD, HPD, and HFD administration on body mass, water intake, food intake, biological parameters in blood, and vitamin levels in urine of starved rats. Drinking water was made freely available during the experimental period, including the starvation period. A reference group was allowed free access to the HCD throughout the experiment.

In general, the starvation state is particularly vulnerable to overfeeding because many metabolic processes and signal transduction systems are almost shut down. Characteristically, increased drinking, increased urea nitrogen in the plasma and urine, and kidney hypertrophy, were observed in the HPD group. Drinking water was made freely available during the experimental period, including the starvation period. A reference group was allowed free access to the HCD throughout the experiment.

In general, the starvation state is particularly vulnerable to overfeeding because many metabolic processes and signal transduction systems are almost shut down. Characteristically, increased drinking, increased urea nitrogen in the plasma and urine, and kidney hypertrophy, were observed in the HPD group. Drinking water was made freely available during the experimental period, including the starvation period. A reference group was allowed free access to the HCD throughout the experiment.

The recovery of weight after starvation was excellent in the HFD group. However, in general, the administration of a HFD has been associated with adverse effects such as an impairment of β-cell function (24) and an increase in fatty liver and adipose mass (25). Again, we want to stress that the HFD used for this study had no adverse effects on the general health of normal rats (15). Therefore, we investigated changes in individual organ mass and blood parameters at day 1 of refeeding after 3 d of starvation. Energy intake was higher in the HFD group than in the HCD group as food intakes by weight between the two groups were similar. Starvation leads to the exhaustion of energy-producing nutrients and thus an important component of recovery from starvation is the quick supply of energy-producing nutrients. However, starvation causes intestinal atrophy and decreases the specific activities of sucrase and maltase (26). This report suggests the superiority of a HFD to a HCD in the recovery from starvation. The HFD is a high-energy diet compared with the HCD, because it contains more energy per weight of food. The recovery of body weight was faster in the HFD group than in the HCD group at day 1 of refeeding after 3 d of starvation. The energy efficiency was higher in the HCD group than in the HFD group, which means that much more energy was needed to recover the lost body mass. This is because the starved rats could not eat the necessary amount of food because their digestive tissue was impaired as a result of starvation. The recovery of organ mass was almost the same between groups at day 1 of refeeding after 3 d of starvation and at day 8 of refeeding after 3 d of starva-
tion. Thus, HFD administration did not induce fat accumulation in the body. Therefore, we hypothesize that HFD administration is suitable for quickly reversing the effects of starvation. However, regarding the fact that the HFD is superior to the HCD, there is one caveat; i.e., the fat content of the HFD used in the present study was 30% of dietary weight (approximately 50% of energy), which did not induce adverse effects in non-starved healthy rats (15).

B-group vitamin supplementation, especially vitamin B₁, should be initiated with refeeding (1). Our previous paper revealed that the tissue concentrations of B-group vitamins including vitamin B₁, vitamin B₂, vitamin B₆, vitamin B₁₂, nicotinamide, pantothenic acid, folate, and biotin, were differently affected by starvation (27). In addition, we reported that the urinary excretion of B-group vitamins significantly decreased according to the length of starvation (27). In the present study, we investigated the recovery of B-group vitamins, including vitamin B₁, vitamin B₂, vitamin B₆, and niacin, using rats starved for 3 d (short-term starvation) or for 8 d (long-term starvation). Similar to our previous report (27), the urinary excretion of these vitamins decreased according to the period of starvation. When the starved rats were refed with a diet containing a normal level of vitamin mixture (1% AIN-93 vitamin mixture), the urinary vitamin levels gradually recovered. The recovery was faster in the 3 d starvation experiment than in the 8 d starvation experiment. The concentrations of vitamins in the blood and liver completely recovered to the reference values. An important discovery was that the vitamin concentrations in blood and liver were not affected by dietary amounts of energy-producing nutrients such as carbohydrate, protein, and fat. We previously reported that excess vitamin intake before starvation did not affect body mass, organ mass, or blood variables in starved rats (28). Nevertheless, we wish to emphasize the importance of vitamin B₁ during starvation. The urinary excretion of vitamin B₁ steeply decreases when food intake is restricted (27–30). This phenomenon suggests that the need for vitamin B₁ is much higher in starvation than in well-fed animals. Notably, tissue vitamin concentrations are maintained by changing the urinary excretion rate of vitamins in rats with restricted food intake (30). As coenzymes of B-group vitamins are involved in many metabolic processes such as glycolysis, β-oxidation, amino acid catabolism, the TCA cycle, and the electron transport system, a mechanism must exist to maintain the vitamin concentrations at constant levels. We hypothesize that this mechanism involves a decreased urinary excretion of B-group vitamins, supporting a strong requirement for dietary vitamins. Although we could not measure the metabolites of energy-producing nutrients during the starving and refeeding periods, undesirable metabolites such as 2-oxo acids (31, 32) might be excreted in urine.

In conclusion, to recover optimally from starvation, a quick supply of energy-producing nutrients is the most important consideration. A high-fat diet was found to be the best in this respect, although the fat content should only be 30% on a weight basis. B-group vitamins involved in energy metabolism were not associated with the recovery from starvation.

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Author contributions

A. M., T. F., and K. S. designed the study. A. M. conducted the experiments. A. M. and K. S. drafted the manuscript. A. M., T. F., and K. S. designed the study. A. M. conducted the experiments. A. M. and K. S. drafted the manuscript. A. M., T. F., and K. S. read and approved the final manuscript.

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