Effects of four-week feed restriction on toxicological parameters in beagle dogs

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Abstract: This study was conducted to examine any changes caused by feed restriction in dogs to contribute to safety evaluation in toxicity studies. Two male 7-month-old beagle dogs/group were fed 300 (control), 150 (50% of control), or 70 g/animal of diet daily (23% of control) for 4 weeks. Effects of feed restriction, except for clinical signs, were noted depending on the feed dosage in almost all examinations. The principal outcomes were: decreased body weight and water consumption, ECG changes (decreased heart rate and prolonged QTc), and hematopoietic and lymphopoietic suppression (decreased reticulocyte ratio or white blood cell count in hematology, decreased nucleated cell count in bone marrow, decreased erythroid parameters in myelography, and hypocellularity of bone marrow and thymic atrophy in histopathology). In addition, some changes were noted in urinalysis (decreased urine volume and sodium and potassium excretion), blood chemistry (decreased ALP and inorganic phosphorus and increased creatinine), organ weights, and gastric histopathology. These results provide important reference data for distinguishing the primary effects of test compounds from secondary effects of decreased food consumption in toxicity studies in beagle dogs.

Key words: beagle dogs, bone marrow, electrocardiography, feed restriction, toxicological parameter

Introduction

Changes in the toxicological parameters of feed-restricted animals have been reported in rats [13, 18, 20]. Levin et al. [13] examined overall toxicological parameters with feed restriction for 2 weeks at 75, 50 or 25% of the control diet and found a myelosuppression in bone marrow in all the feed-restricted groups, indicating that the primary response to the feed restriction was hematopoietic suppression in rats. Other examinations focusing on hematopoiesis in feed-restricted rodents showed a decreased blood cell count and hypocellularity in bone marrow [3–9, 15, 17, 19]. These rodent examinations suggested, as described by a number of review articles...
[15, 21, 22], the importance of hematopoietic evaluation in the malnourished condition. In contrast to rodents, however, few malnourished examinations have been reported in dogs, despite this being the most commonly used non-rodent species in toxicity studies. For dogs, Hill et al. [11] and Lawlar et al. [12] used the grayhound or Labrador retriever, respectively, and they did not consider any toxicological aspects except for clinical pathology. Morita et al. [16] used the beagle which is a common breed in toxicity studies, and only conducted electrocardiography and hematology. Their data are considered insufficient as background data for toxicity studies in dogs.

Given this lack of data, we comprehensively evaluated the biological responses, with particular attention to hematopoiesis, in feed-restricted beagle dogs, including toxicological examination items for general signs, body weight, water consumption, electrocardiography, urinalysis, hematology, blood chemistry, bone marrow examination (including nucleated cell count and myelography), organ weight, and histopathology.

Materials and Methods

Study design and conditions

Animals: Six male beagle dogs purchased from Kita-yama Labes Co., Ltd (Iwakuni, Japan) were used. The dogs were 7 months old at the initiation of restricted feeding. This study was approved by the Institutional Animal Care and Use Committee and was performed in accordance with the animal welfare by laws of Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories. The animals were individually housed in stainless steel cages in a room controlled for temperature (20.4 to 23.9°C), humidity (37 to 80%), ventilation (15 times/h), and light (12 h/day: 07:00 to 19:00).

Feeding and water supply: NVE-10 (Nippon Pet Food, Co., Ltd., Tokyo, Japan) was provided to each animal daily between 14:30 and 16:00. On the days before blood sampling (for hematology and blood chemistry and blood glucose level measurement) and gross pathology, any remaining food was removed from each animal at approximately 17:00. Water was available ad libitum from an automatic water supply system or bottle for measurement of water consumption.

Grouping: The three groups established for feed restriction were 300 g/animal/day (standard feed dosage in toxicity studies), 150 g/animal/day (50% of control), and 70 g/animal/day (23% of control), with 2 animals allocated per group. Animals were identified by number, as follows: No. 1 and 2 for 300 g/animal/day (control group), No. 3 and 4 for 150 g/animal/day, and No. 5 and 6 for 70 g/animal/day.

Feed restriction period: A four-week period of feed restriction was determined, which is the standard dosing period for subacute toxicity studies.

Examination items

The examination days were defined as follows. The day of initiation of feed restriction was defined as day 1. The day before day 1 was defined as day –1.

Clinical signs: Animals were observed in the morning and afternoon for clinical signs including external appearance, behavior, respiration, position, urinary and fecal condition, response to stimulation, and mortality.

Body weight: Body weights were measured on days –21, –7, –1, 4, 7, 11, 14, 18, 21, 25, and 28.

Food consumption: The quantity supplied and remaining for each animal was weighed daily using an electronic balance, and daily food consumption calculated.

Water consumption: Water consumption was measured on days –18, –11, 6, 12, 19, and 26 using a graduated cylinder, and the difference between the volume of water supplied and remaining was calculated.

Electrocardiography: Electrocardiogram examinations were conducted on days –15, 1, 8, 14, 21, and 28. Data from standard leads (I, II, and III) were recorded continuously for 24 h, and analysis was performed at 10:00 and 18:00 on each examination day and at 10:00 on the following day using JET transmitters (JET-3ETA, Data Sciences International Inc., St. Paul, MN, USA), JET receivers (JET-RCV, Data Sciences International Inc.), and JET systems (Ponemah Physiology Platform Plus, version 4.9, Data Sciences International Inc.). Heart rate, PR interval, QRS duration, QT interval, and QTc (Matsunaga’s correction) from five consecutive waves of lead II at each time point as well as the mean values were calculated.

Urinalysis: Urinalysis was conducted on days –18, –11, 6, 12, 19, and 26. Six-hour urine (excreted from 9:00 to 15:00) and 18-h urine (excreted from 15:00 to 9:00 on the following day) were collected under ice-cooled conditions. The volume, pH, ketone bodies, bilirubin, occult blood, urobilinogen, protein, and glucose were measured or observed for 6- and 18-h urine, color and sediment were observed for 6-h urine, and specific
gravity, sodium, potassium, chloride, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (G-GTP), N-acetyl-beta-D-glucosaminidase (NAG), and osmolality were measured for 18-h urine.

Hematology: Blood was derived from the external jugular vein on days −17, −10, 7, 13, 20, and 27 to measure the erythrocyte count (RBC), leukocyte count (WBC), hematocrit value, hemoglobin concentration, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte ratio, eosinophil count, basophil count, monocyte count, lymphocyte count, neutrophil count, large unstained cell (LUC) count, prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen.

Blood chemistry: Blood was derived from the external jugular vein on days −17, −10, 7, 13, 20, and 27 and left at room temperature for 20 to 60 min. Serum was obtained by centrifugation at room temperature at 1,710 × g for 15 min to measure aspartate aminotransferase (ASA), alanine aminotransferase (ALAT), ALP, G-GTP, amylase, total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, total cholesterol, free cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, phospholipid, glucose, blood urea nitrogen (BUN), creatinine, inorganic phosphorus (IP), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), total bile acid, globulin, and albumin/globulin ratio (A/G). In addition to blood chemistry, circadian changes in blood glucose and total protein were measured on days −7, −4, 5, 11, 18, and 25 with the following procedure. Approximately 0.5 ml of blood was derived from the external jugular vein with a syringe containing heparin sodium at 10:00 and 18:00 on the day of examination and at 10:00 the following day. Blood was immediately cooled on ice and centrifuged at 4°C at 1,710 × g for 15 min. Plasma was collected at approximately 10:00 and 18:00 on the examination day and at 10:00 on the following day: total 3 points) to measure glucose and protein.

Gross pathology: The animals were weighed, anesthetized by an intravenous injection of sodium pentobarbital (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) solution (64.8 mg/ml, 0.5 ml/kg) into the cephalic vein of the forearm, and euthanized by exsanguination. External appearance, and internal organs and tissues were examined macroscopically.

Bone marrow examination: Bone marrow fluid was collected from the sternum at gross pathology. The bone marrow nucleated cell count was measured, and myelography were conducted.

- Bone marrow nucleated cell count: Bone marrow fluid was diluted and stained with Türk solution. The number of nucleated bone marrow cells was counted using an automatic F-820 cell counter (Sysmex Corporation, Kobe, Japan).
- Myelography: Bone marrow smears were prepared and stained with May-Grünwald and Giemsa stains. Myelograms of the smears were examined microscopically.

Organ weight and histopathology: Measurement of organ weight and histopathologic examination were performed for the heart, thymus, spleen, lungs, submandibular glands, liver, pancreas, kidneys, pituitary, thyroid glands, adrenals, testes, prostate, and brain. In addition to these organs, histopathology was performed for the aorta, sternum, splanchnic bone marrow, femur, femoral bone marrow, submandibular lymph nodes, mesenteric lymph nodes, Peyer’s patches (ileum), trachea, bronchi, tongue, sublingual glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, gallbladder, urinary bladder, parathyroid glands, epididymides, optic nerves, spinal cord, sciatic nerves, eyeballs, lacrimal glands, femoral skeletal muscle, skin, and mammary glands. The eyeballs and optic nerves were fixed in a mixture of 3% glutaraldehyde and 2.5% formalin, and the testes were fixed in Bouin’s solution. Other organs were fixed in 10% neutral buffered formalin. The sternum and femur were decalcified with Kalkitox (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical analysis: Statistical analysis was not conducted.

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Results

Clinical signs

No abnormal clinical signs were observed in any animals.

Body weight

The time courses of body weight are shown in Fig. 1. The body weights in the 150 g/animal and 70 g/animal groups decreased gradually during the 4-week feed restriction period. The degrees of change depended on the feed dosage; that is, 150 g/animal and 70 g/animal groups showed decreases of approximately 14 and 25%, respec-
Food consumption

All animals consumed the entire amount of provided food during the examination period.

Water consumption

The time courses of water consumption are shown in Fig. 2. Water consumption decreased depending on the feed dosage in all animals in the 150 and 70 g/day groups. The amount of water consumption in the 150 and 70 g/day groups were approximately half and one-fourth of those on day -11, respectively, on all examination days during the feed restriction period.

Electrocardiography

The time courses of the mean values for electrocardiographic parameters in each group are shown in Fig. 3, and the individual values for the parameters at all examination points are listed in Supplementary Table 1. Decreased heart rate and prolonged PR interval were noted depending on the feed dosage in the 150 and 70 g/day groups. Since tendencies towards prolongation of QTc were observed in the 150 and 70 g/day groups (Fig. 3D), individual values were evaluated in detail using criteria based on the variation during the acclimation period (Supplementary Table 1). Although prolongation beyond the criteria based on the variation during the acclimation period was noted transiently in only one animal in the 150 g/day group, both animals in the 70 g/day group exhibited a tendency toward increased levels at some examination points. The time courses of animals with noticeable tendencies in each group are shown in Fig. 3E. Tendencies towards prolongation of the QRS duration were also noted in the 150 g/day group; however, the degree of changes was very slight. The above changes tended to continue during the feed restriction period.

Urinalysis

A notable change depending on the feed dosage was observed in urine volume. The time courses of the mean value for daily total urine volume in each group are shown in Fig. 4A. The urine volume in the feed-restricted groups was consistently lower than that in the 300 mg/day group. The ratios to that on day -18 ranged from 20 to 60% in both groups during the feed restriction period. Excretion of sodium and potassium in urine changed in a manner similar to the urine volume (Figs. 4B and 4C). No notable change was observed in other parameters in any groups. The individual values of parameters at all examination points are listed in Supplementary Table 2, except for parameters in which no changes were noted at any examination points.

Hematology

The mean values of parameters with notable changes caused by feed restriction are shown in Table 1. Decreases in reticulocyte ratio and WBC, including the neutrophil, eosinophil, basophil, monocyte, and lymphocyte count, were noted depending on the feed dosage from day 7 up to the end of the feed restriction period.

Fig. 1. Time course of body weight for each individual.

Fig. 2. Time course of water consumption for each individual.
The time course of the changes in reticulocyte ratio and WBC are shown in Fig. 5. The individual values of parameters at all examination points are listed in Supplementary Table 3, except for parameters in which no changes were noted at any examination points.

**Blood chemistry**

Both feed-restricted groups exhibited a decrease or tendency to decrease in serum ALP, amylase, phospholipid, inorganic phosphorus, glucose, and calcium and an increase or tendency to increase in ALAT, creatinine, G-GTP, chloride, BUN, and total bile acid. Only the 70 g/day group exhibited an increase in bilirubin. These data are shown in Table 2. Almost all these parameters changed only at day 27 or at random days during the feed restriction period (Supplementary Table 4); however, creatinine constantly increased, and ALP and inorganic phosphorus constantly decreased during the feed restriction period (Fig. 6), although the magnitudes of change were not necessarily large. Regarding examina-
No apparent circadian changes were noted in any groups, and no abnormalities were observed in any animals (supplementary Table 5). The individual values of parameters at all examination points are listed in supplementary Table 4, except for parameters in which no changes were noted at any examination points.

**Bone marrow examination**

Notable results from the bone marrow examination are shown in Table 3. The nucleated cell count decreased in the feed-restricted groups depending on the feed dosage. The feed restricted-groups also exhibited decreased ratio of erythroblasts at the proerythroblast, basophilic erythroblast, polychromatic erythroblast, and mitotic erythroblast stages of the erythroblastic series and of total erythropoietic cells (Ecs), and increased ratio of granulocytes at the myeloblast, eosinophilic metamyelocyte, segmented neutrophil, and eosinophil stages of granulocytic series and of total granulopoietic cells.
(GCs) and monocytes. These changes led to an increased ratio of total GCs to total ECs.

**Gross pathology**

The thymus was found to be small in 1 animal in the 150 g/day group and both animals in the 70 g/day group. One animal in 70 g/day also exhibited a white focus of the mucosa in the stomach.

**Organ weight**

All organ weight data are shown in Table 4. Organ weight changes were evaluated by comparing the feed-restricted group values with the control group values or control background data with 300 g/day feeding. All animals in the feed-restricted groups exhibited a decrease or tendency to decrease in the absolute and relative thymus weights and absolute liver and kidney weights and an increase in relative brain and lung weights. One animal in the 150 g/day group and both animals in the 70 g/day group exhibited decrease in absolute and/or relative heart weight. One animal in each of the 150 g/day and 70 g/day groups also exhibited a decrease in absolute spleen weight. Only the 70 g/day group exhibited an increase in relative pituitary weight and absolute and relative adrenal weight and a decrease in absolute and relative testis weights and absolute prostate weight.

**Histopathology**

No abnormal changes were noted in the 300 g/day group. In the 150 g/day group, hypocellularity and an increase in the mature granulocyte ratio in the sternal and femoral bone marrow (Figs. 7B and 7E), as well as atrophy of the thymus and testicular seminiferous tubules, were observed in 1 animal. In the 70 g/day group, an increase in the mature granulocyte ratio in the sternal and femoral bone marrow and atrophy of the thymus were observed in both animals. Hypocellularity in the sternal and femoral bone marrow (Figs. 7C and 7F) and atrophy of the seminiferous tubules were observed in 1 animal, and regeneration was observed in the mucosa in the stomach body corresponding to a gross lesion (white focus in the mucosa) in another animal (Fig. 8). In the two animals with hypocellularity in the bone marrow, no necrotic picture was observed in the bone marrow (Fig. 7). An atrophic change was noted in the seminiferous tubules in the 70 g/day group (Supplementary Fig. 1). Atrophic seminiferous tubules with a reduced number of germinal cells were randomly distributed in testicular cross sections. No degenerative or necrotic cells were observed in the atrophic tubules, and many other seminiferous tubules looked intact. The magnitude of the testicular change was the same as that in the 150 g/day group, and a similar change was occasionally noted in the control background data.

**Discussion**

We examined the toxicological parameters of beagle dogs with a 4 weeks of feed restriction at 150 or 70 g/animal/day versus a standard diet of 300 g/animal/day. All animals ate all the supplied feed, indicating that the experimental condition for evaluation of the feed-re-
restricted effects were established. Decreases in body weight and water consumption that corresponded to the levels of feed restriction were noted.

The most notable finding in the present study was the effect on the hematopoietic system. Changes in a number of parameters were noted in hematology and bone marrow examinations, especially in the erythroid ratio, which was decreased. The decreased erythroid ratio was reflected in histopathology as an increase in the mature granulocyte ratio in the sternal and femoral bone marrow. In addition, a decrease in the numbers of nucleated bone marrow cells was noted. One animal in each feed-restricted group exhibited hypocellularity of the bone marrow. With regard to effects on leukocytes, decreases in the numbers of neutrophils, eosinophils, basophils, and monocytes were noted in
these groups. These results suggest that feed restriction affected all types of hematopoietic cells, particularly erythroid cells. Effects were also observed in the lymphoid system accompanied by thymic atrophy and a decreased lymphocyte count. Myelosuppression and thymic atrophy have been reported in feed-restricted rats by Levin et al. [13]. Morita et al. [18] reported decreased leukocytic parameters in feed-restricted dogs. These findings indicate that the effects on the hematopoietic and lymphocytic systems are reproducible and common events across different species.

Other changes noted in the feed-restricted groups were a decreased urinary volume and urinary potassium excretion, which were considered to be associated with decreased water consumption. Regarding cardiovascular events, feed-restricted animals exhibited a decreased heart rate. Some animals exhibited a prolongation of the PR interval and tendency towards prolongation of QRS that were associated with decreased heart rate. Bradycardia might be related to possible depression of cardiac function in response to malnutrition [1, 2]. Morita et al. [16] also reported a decreased heart rate in feed-restricted beagle dogs and considered the cause to be a decreased circulating blood volume. The decreased water consumption of the feed-restricted dogs in our examination is also consistent with the cause suggested by Morita et al. Regarding other cardiac parameters, slight prolongation of the mean QTc in the feed-restricted groups was noted during the feed restriction period. In individual analysis, 1 animal in each feed-restricted

Table 3. Individual and mean parameter values in bone marrow examination

| Parameters                          | 300 g/day | 150 g/day | 70 g/day |
|-------------------------------------|-----------|-----------|----------|
| No. 1 | No. 2 | Mean | No. 3 | No. 4 | Mean | No. 5 | No. 6 | Mean |
| Nucleated cell count (10⁴/mm³)     | 126       | 150       | 138      | 152   | ↓65  | 109   | ↓82   | ↓86  | 84   |
| Myelogram (%)                       | 0.4       | 0.4       | 0.4      | ↓0.2  | ↓0.0 | 0.1   | 0.4   | ↓0.0 | 0.2  |
| Proerythroblasts                    | 2.4       | 1.7       | 2.1      | 1.6   | ↓0.2 | 0.9   | 1.6   | ↓0.6 | 1.1  |
| Basophilic erythroblasts            | 38.6      | 38.9      | 38.8     | ↓26.3 | ↓14.6 | 20.5  | ↓13.7 | ↓13.0 | 13.4 |
| Polychromatic erythroblasts         | 0.8       | 1.0       | 0.9      | ↓0.0  | ↓0.4 | 0.2   | ↓0.0  | ↓0.6 | 0.3  |
| Mitotic erythroblasts               | 42.4      | 42.2      | 42.3     | ↓28.1 | ↓15.2 | 21.7  | ↓15.9 | ↓14.4 | 15.2 |
| Total erythropoietic cells (ECs)    | 0.0       | 0.0       | 0.0      | 0.0   | 0.0  | 0.0   | ↑0.2  | 0.0  | 0.1  |
| Myeloblasts                         | 0.8       | 0.4       | 0.6      | ↑1.3  | ↑3.6  | 2.5   | ↑1.2  | 0.6  | 0.9  |
| Basophilic metamyelocytes           | 7.2       | 3.5       | 5.4      | ↑10.3 | ↑10.2 | 10.3  | ↑15.3 | ↑12.4 | 13.9 |
| Basophilic metamyelocytes           | 1.6       | 0.8       | 1.2      | ↑2.5  | ↑10.6 | 6.6   | ↑4.0  | ↑2.1  | 3.1  |
| Total granulopoietic cells (GCs)    | 44.4      | 49.4      | 46.9     | ↑62.9 | ↑70.9 | 66.9  | ↑70.3 | ↑57.4 | 63.9 |
| Monocytes                           | 0.6       | 0.2       | 0.4      | ↑0.9  | ↑0.8  | 0.9   | ↑1.0  | ↑1.2  | 1.1  |
| Total GCs/Total ECs                | 1.05      | 1.17      | 1.11     | ↑2.24 | ↑4.66 | 3.45  | ↑4.42 | ↑3.99 | 4.21 |

Symbols: ↑, increase; ↓, decrease (evaluated only for individual data).
group exhibited apparent QTc prolongation. Although the mechanism of QTc prolongation in the feed-restricted dogs was unclear, this finding suggested that animals with decreased food consumption might exhibit QTc prolongation. To our knowledge, this is the first report of QTc prolongation in feed-restricted dogs.

The slight increases in serum creatinine and bun levels in all and some feed-restricted animals, respectively, were considered to be due to a decreased glomerular filtration rate (GFR) associated with a decreased renal blood flow and circulating blood volume. Taking into consideration the small fluctuation ranges of the serum creatinine and BUN levels, the inhibitory effect on GFR caused by feed restriction was considered to be not so significant. While the serum inorganic phosphate (IP) concentration is expected to increase when GFR decreases, all feed-restricted animals exhibited a decrease in the serum IP concentration in our examination. The reduction in phosphorus intake associated with feed restriction is therefore considered to lead to a subsequent decrease in serum IP concentration. Another potential cause of the increase in BUN might be catabolism of protein, which is supported by animal No. 4 which showed a decrease in serum total protein and albumin levels with the largest increase in BUN, and in animal No.5 which showed an increase in serum ALAT levels, which is a parameter of protein catabolism.

Changes in organ weight were noted in the pituitary, adrenal, spleen, brain, heart, lung, liver, kidney, and prostate without accompanying histopathological changes, except for decreased weights of the thymus and testis, reflecting atrophic changes in histopathology. Trieb et al. [23] described that body weight ($x$) and absolute organ weight ($y$) have an equality of $y=ax^b$, where $a$ is the integration constant and $b$ is the ratio of the specific growth rates for the organ and species. When $b$ equals 1, this indicates an isometric increase with constant proportionality for body weight and organ weight. When $b$ is greater than 1, this indicates a positive allometric increase in organ weight compared with body weight.

### Table 4. Individual and mean organ weights in each animal

| Organ | (Upper value, Absolute weight; lower value, relative weight to body weight) |
|-------|--------------------------------------------------------------------------|
|       | 300 g/day | 150 g/day | 70 g/day |
|       | No. 1 | No. 2 | Mean | No. 3 | No. 4 | Mean | No. 5 | No. 6 | Mean |
| Pituitary (mg) | | | | |
| (mg/kg) | 62 | 73 | 68 | 64 | 58 | 61 | 64 | 61 | 63 |
| Thyroid gland (bilateral weight, g) | 0.98 | 0.96 | 0.97 | 1.02 | 0.67 | 0.85 | 0.70 | 0.90 | 0.80 |
| (g/kg) | 0.087 | 0.089 | 0.088 | 0.107 | 0.076 | 0.092 | 0.093 | 0.110 | 0.102 |
| Adrenal (bilateral weight, g) | 0.9 | 0.88 | 0.89 | 0.89 | 0.76 | 0.83 | 0.82 | 1.16 | 0.99 |
| (g/kg) | 0.080 | 0.081 | 0.081 | 0.094 | 0.086 | 0.090 | 0.109 | 0.141 | 0.125 |
| Testis (bilateral weight, g) | 15.0 | 18.4 | 16.7 | 14.1 | 14.9 | 14.5 | 11.2 | 19.6 | 10.4 |
| (g/kg) | 1.33 | 1.70 | 1.52 | 1.48 | 1.69 | 1.59 | 1.49 | 1.17 | 1.33 |
| Pancreas (g) | 21.8 | 30.4 | 26.1 | 20.4 | 21.5 | 21.0 | 16.7 | 17.4 | 17.1 |
| (g/kg) | 1.93 | 2.81 | 2.37 | 2.15 | 2.44 | 2.30 | 2.23 | 2.12 | 2.18 |
| Thymus (g) | 16.7 | 19.0 | 17.9 | 11.7 | 13.2 | 7.5 | 2.2 | 2.2 | 2.2 |
| (g/kg) | 1.48 | 1.76 | 1.62 | 1.23 | 0.36 | 0.80 | 10.29 | 10.27 | 0.28 |
| Submandibular gland (bilateral weight, g) | 9.6 | 10.2 | 9.9 | 9.7 | 9.1 | 9.4 | 8.9 | 7.6 | 8.3 |
| (g/kg) | 0.85 | 0.94 | 0.90 | 1.02 | 1.03 | 1.03 | 1.19 | 0.93 | 1.06 |
| Spleen (g) | 26.7 | 24.1 | 25.4 | 30.5 | 15.9 | 23.2 | 15.4 | 20.3 | 17.9 |
| (g/kg) | 2.36 | 2.23 | 2.30 | 3.21 | 1.81 | 2.51 | 2.05 | 2.48 | 2.27 |
| Brain (g) | 79.8 | 79.9 | 79.9 | 82.3 | 82.7 | 85.0 | 75.8 | 83.1 | 79.5 |
| (g/kg) | 7.06 | 7.4 | 7.23 | 8.67 | 9.94 | 9.31 | 10.11 | 10.13 | 10.12 |
| Heart (g) | 72.3 | 77.0 | 74.7 | 74.6 | 68.1 | 71.4 | 68.4 | 63.6 | 66.0 |
| (g/kg) | 6.40 | 7.13 | 6.77 | 7.85 | 7.74 | 7.80 | 7.92 | 7.76 | 8.44 |
| Lung (g) | 78.7 | 75.9 | 77.3 | 80.3 | 79.7 | 80.0 | 63.2 | 70.6 | 66.9 |
| (g/kg) | 7.96 | 7.03 | 7.00 | 8.45 | 9.06 | 8.76 | 7.84 | 8.61 | 8.52 |
| Liver (g) | 279.6 | 276.9 | 278.3 | 221.4 | 207.4 | 214.4 | 170.4 | 188.5 | 179.5 |
| (g/kg) | 24.74 | 25.64 | 25.19 | 23.31 | 23.57 | 23.44 | 22.72 | 22.99 | 22.86 |
| Kidney (bilateral weight, g) | 44.0 | 43.7 | 43.9 | 39.7 | 39.3 | 39.5 | 31.8 | 33.7 | 32.8 |
| (g/kg) | 3.89 | 4.05 | 3.97 | 4.18 | 4.47 | 4.33 | 4.24 | 4.11 | 4.18 |
| Prostate (g) | 3.5 | 2.6 | 3.1 | 3.1 | 2.7 | 2.9 | 2.6 | 1.8 | 2.2 |
| (g/kg) | 0.31 | 0.24 | 0.28 | 0.33 | 0.31 | 0.32 | 0.35 | 0.22 | 0.29 |

Symbols: ↑, increase; ↓, decrease; (↑), tended to increase; (↓), tended to decrease (evaluated only for individual data).
Negative allometry or slower organ growth results if $b$ is less than 1 [23]. The organs that decreased in weight in the present study had relatively large values for $b$ [14]. That is, the weights of the organs with a large rate of increase associated with body weight gain decreased along with body weight. These data indicate the importance of carefully assessing organ weight in toxicity studies referring to $b$ for weight change secondary to body weight change or as a direct effect of the test articles. In some organs, the weights of relative organs increased, indicating that the weight changes of these organs did not follow the rapid decrease in body weight.

In histopathology, atrophic changes in the bone marrow and thymus and regeneration of the gastric mucosa were noted. Regeneration of the gastric mucosa is considered to be a repair process for mucosal injury, which might be induced by stress through corticosteroid release [13]. Similar findings were also observed in rats by Levin et al. [13] and considered a common response in different species. Testicular changes, such as degeneration of

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**Fig. 7.** Histological section of the sternal bone marrow. Scale bars: A to C, 100 $\mu$m; D to F, 50 $\mu$m. A: 300 g/day. B: 150 g/day. C: 70 g/day. D: 300 g/day, magnified image. E: 150 g/day, magnified image. F: 70 g/day, magnified image. Hypocellularities are observed in B and C, and hypocellularity and an increase in the mature granulocyte ratio are observed in E and F.

**Fig. 8.** Histological section of the stomach at 70 g/day. Scale bars: A, 100 $\mu$m; B, 50 $\mu$m. A: A regenerative mucosa can be seen in the right half area of the picture. B: Regenerative mucosa, magnified image. The cytoplasm of the epithelium shows basophilia. Mitotic figures can be seen (arrows).
the seminiferous epithelium or reduced number of the spermatids, were reported as the effects of feed restriction in rats [13]. Atrophy of the seminiferous tubules was also observed in the present study; a similar change is occasionally noted as a spontaneous change [10] probably due to endocrinological imbalance. The precise mechanism of testicular atrophy caused by feed restriction is unclear.

In summary, the principal responses in feed-restricted dogs with decreased body weight and water consumption were suppressed hematopoiesis and lymphopoiesis, which are common in feed-restricted rats. The stomach mucosa and cardiovascular system were also targets: the dogs showed a decreased heart rate accompanied by prolongation of the PR interval and QTc. These results provide important information for assessing responses of beagle dogs with decreased food consumption in toxicity studies.

Acknowledgments

The authors thank the staff of Shin Nippon Biomedical Laboratories, Ltd., and Dr. Ohata, Dr. Ikeda, and Dr. Motegi of Astellas Pharm Inc. for their support regarding the initiation of this study.

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