Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.11, No.3, 123-130, 2024

The Japanese Society of Toxicology

Fundamental Toxicological Sciences

URL : http://www.fundtoxicolsci.org/index_e.html

Minireview

Understanding the effects of food restriction on toxicological parameters: A comparative analysis in rats, dogs, and monkeys

Nozomi Fujisawa

Translational Research Division, Chugai Pharmaceutical Co., Ltd.,
216 Totsuka-cho, Totsuka-ku, Yokohama, Kanagawa 244-8602, Japan

(Received May 29, 2024; Accepted June 3, 2024)

ABSTRACT — In non-clinical toxicity studies for drug development, reduced food intake in experimental animals can lead to fluctuations in various toxicological parameters, complicating the distinction between drug toxicity and secondary effects of reduced food intake. This review examines the parameters that change due to food restriction in rats, dogs, and monkeys, and discusses the presumed mechanisms behind each parameter change. The parameters include standard toxicological evaluation parameters, such as body weight, blood chemistry, hematology, urinalysis, bone marrow cell analysis, organ weight, and histopathology. This review also highlights the differences in parameter changes across animal species and food restriction conditions, providing crucial insights for improving the quality of non-clinical toxicity studies and enhancing human translatability. The review underscores the need for a comprehensive analysis of these parameters to understand animal nutritional status within toxicity studies. This information can improve the reliability of toxicity evaluations.

Key words: Food restriction, Toxicological parameters, Non-clinical toxicity studies, Rats, Dogs, Monkeys

INTRODUCTION

In non-clinical toxicity studies for drug development, it is common for animals to experience reduced food intake. This decrease can lead to fluctuations in various toxicological parameters, making it challenging to determine whether toxicity stems from the drug candidate or the secondary effects of reduced food intake. Therefore, understanding the impact of reduced food intake on toxicological parameters in non-clinical experimental animals is crucial. In humans, parameters such as protein, albumin, cholesterol, transthyretin, cholinesterase, transferrin, and lymphocytes are used to assess overall nutritional status. However, in non-clinical toxicity studies, it is rare to measure parameters like transthyretin, cholinesterase, and transferrin. Understanding the animal's nutritional state through a comprehensive analysis of toxicological parameters and evaluating toxicity in light of this information is challenging but crucial for assessing the inherent toxicity of a drug. This review outlines the toxicological parameters that change due to food restriction in rats, dogs, and monkeys in non-clinical toxicity studies. We also summarize the differences in parameter changes across animal species and food restriction conditions, and discuss the presumed mechanisms underlying each parameter change.

DISCUSSION

Numerous reports discuss the effects of food restriction on non-clinical experimental animals, with most studies mirroring the design of non-clinical toxicity studies

Correspondence: Nozomi Fujisawa (E-mail: fujisawa.nozomi53@chugai-pharm.co.jp)

(e.g., species, duration, examination method) and examining toxicological evaluation parameters. In each experimental animal, food restriction caused various changes in toxicological parameters (Table 1). The changes described in Table 1 are a rough summary of parameters clearly reported to have changed. Please note that parameters not listed in the table may not have changed, may have shown a trend of change but not significantly, or may not have been measured. Many reports have been made on rats under various food restriction conditions, with two reports on dogs and a single report on cynomolgus monkeys. Despite differences in animal species, strain, duration of food restriction, and level of food restriction, in most of the cases the parameters that changed were consistent, such as changes in weight loss, increased transaminase, decreased leukocytes, nutrients (e.g., glucose, total protein, albumin, and total cholesterol), bone marrow nucleated cell count, and thymus weight. However, especially in rats given the large number of reports, some parameters that increased or decreased after restricted feeding varied among studies (e.g., aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], glucose). These differences could be attributed to differences in experimental conditions such as age, level and duration of food restriction, nutritional constitution of food, strain, and feeding time. Possible mechanisms for these differences are discussed in the later part of this review.

From a temporal perspective on parameter changes, while direct comparison is not possible due to the variety of reported food restriction conditions, a cross-sectional view of the studies did not indicate that longer food restrictions led to more parameter changes. It would be more appropriate to consider temporal changes within the same study. In dogs, time-dependent parameter changes have been reported (Morita *et al*., 2015), with decreases in leukocytes and increases in gamma-glutamyl transferase (GGT) being more pronounced at 8 weeks after food restriction than at 4 weeks. On the other hand, changes such as an increase in erythrocyte-related parameters and a decrease in reticulocytes were transient, changing at 4 weeks but recovering at 8 weeks. In monkeys, only data up to 2 weeks have been reported, and most parameter changes were more pronounced at the final time point of 2 weeks of food restriction (Fujisawa *et al*., 2024). Conversely, total ketone bodies were the highest on the fourth day of food restriction and decreased thereafter until 10 days later. Two rat studies reported temporal data within the same experiment (Hubert *et al*., 2000; Ogawa *et al*., 1985). No apparent trends were observed in the relationship between the number of altering parameters or the degree of variability and the duration of food restriction.

Focusing on reports with similar food restriction conditions (22-33% of normal food-supply for 2-4 weeks), Table 2 summarizes the toxicological parameter changes in different animal species (Asanuma *et al*., 2011; Fujisawa *et al*., 2024; Levin *et al*., 1993; Moriyama *et al*., 2008; Oishi *et al*., 1979; Takamatsu *et al*., 2015). Most changes were common across species, but there were sporadic parameters that only changed in rats or only in rats and dogs. While the detection sensitivity of parameter changes was high in rats due to the large number of reports, Moriyama *et al.* observed reddish urine and decreased activity/body temperature after a week of 25% food restriction in rats, suggesting that rats might be the most sensitive to food restriction (Moriyama *et al*., 2008). In addition, increases in erythrocyte-related parameters were commonly reported in rats, but not in dogs or monkeys. One of the reasons why rats are highly susceptible to food restrictions is their fast metabolism, due to their large body surface area.

Presumed mechanisms for each toxicological parameter change

It is easy to imagine that body weight decreases after food restriction and indeed, all of the studies in Table 1 exhibited this change. More precisely, in the 0%, 25%, and 55% restriction conditions, food intake per body weight was reported to converge over time to a similar level (Hubert *et al*., 2000). This report also found a close correlation between 2-year survival and body weight, suggesting a link between food restriction and decreased incidence of spontaneous degenerative diseases and tumors. In non-human primates, similar phenomena have been reported. Although the food restriction conditions were milder than those in the Table 1 studies, caloric restriction in rhesus monkeys reduced cancer incidence and increased survival rate (Mattison *et al*., 2017). The relationship between food restriction and health and longevity is a growing field of interest, and research on physiological, metabolic, and molecular changes due to food restriction is progressing (Green *et al*., 2022). On the other hand, Moriyama *et al*. showed a deterioration in general condition after a week of 75% food restriction in rats (Moriyama *et al*., 2008). Although no similar general condition changes were reported in other studies with similar conditions (75% food restriction), this suggested the possibility that excessive food restriction in rats leads to moribundity.

Substances included in the diet and absorbed into the bloodstream after feeding (e.g., nutrients) are natural-

Table 1. Changes in toxicological parameters after restricted feeding.

Animal (sex, age*)	Duration of food restriction	Supplied food (% of control)	Toxicological parameters changes with food-restriction	References
SD rat (male and female, 7 weeks old)	3, 7 days	40-80%	Body weight and body weight gain Decreased Water consumption Decreased Hematology and blood chemistry Increased: RBC, HGB, HCT, MCH, MCHC, EOS, PT, APTT, AST, ALT, BUN, Na, Cl, GLDH Decreased: Reticulocyte, WBC, NEUT, LYMPH, MONO, FIB, ALP, CK, TC, TG, PL, Na, Ca, IP Urinalysis Increased: Na/K, Na, Cl Decreased: Volume, ALP, LDH, ALB, CRE, K, NAG Bone marrow cells Increased: M/E ratio Decreased: Nucleated cell, erythroid cell, lymphocyte Organ weight (absolute weight) Decreased: Thymus, heart, lung, liver, kidney, spleen, pituitary, thyroid, seminal vesicles, ovary Histopathological changes** Bone marrow, spleen, thymus, adipose tissue	(Umeya *et al*., 2024)
SD rat (male and female, 5-6 weeks old)	2 weeks	25-75%	Body weight and body weight gain Decreased Hematology and blood chemistry Increased: RBC, HGB, HCT, AST, ALT, ALP, BIL, GLUC, BUN, Na, Cl Decreased: MCV, MCH, MCHC, WBC, NEUT, LYMPH, MONO, PLT, TP, ALB, GLB, TC, K, Ca Organ weight (absolute weight) Decreased: Brain, thymus, heart, liver, kidney, adrenal, testis Histopathological changes** Bone marrow, thymus, testis, stomach	(Levin *et al*., 1993)
SD rat (male and female, 7 weeks old)	2 weeks	25-75%	General condition Reddish urine, decreased activity, cool to touch Body weight and body weight gain Decreased Hematology and blood chemistry Increased: RBC, HGB, HCT, MCHC, AST, ALT, BIL, A/G, BUN, Na, Cl Decreased: Reticulocyte, WBC, NEUT, LYMPH, MONO, EOS, BASO, PLT, GLUC, TP, ALB, GLB, TC, TG, K, Ca, IP Urinalysis Decreased: pH Organ weight (absolute weight) Decreased: Brain, thymus, heart, liver, kidney, spleen, pituitary, thyroid, prostate, testis, ovary Histopathological changes** Bone marrow, lymphoid tissues, salivary glands, pancreas, liver, kindney, stomach, adrenal, reproductive organs	(Moriyama *et al*., 2008)
SD rat (male, 6 weeks old)	2 weeks	33-67%	Body weight and body weight gain Decreased Water consumption Decreased Hematology and blood chemistry Increased: RBC, HGB, HCT, BUN, Fe Decreased: Reticulocyte, WBC, ALP, TIBC, UIBC Bone marrow cells Increased: M/E ratio Decreased: Nucleated cell, erythroid cell, lymphocyte, mitotic cell Histopathological changes** Bone marrow	(Asanuma *et al*., 2011)

Table 1. (Continued).

Animal (sex, age*)	Duration of food restriction	Supplied food (% of control)	Toxicological parameters changes with food-restriction	References
Wistar rat (male, 5 weeks old)	4 weeks	22-88% (5-15 g/day)	Body weight and body weight gain Decreased Water consumption Decreased Hematology and blood chemistry Increased: RBC, HGB, Na Decreased: MCV, WBC, ALT, ALP, GLUC, TP, TC, TG, BUN, IP Urinalysis Decreased: Volume Organ weight (absolute weight) Decreased: Thymus, heart, lung, liver, kidney, spleen, adrenal, prostate, seminal vesicle, testis	(Oishi *et al*., 1979)
Wistar rat (male and female, 5 weeks old)	3 months	33-67%	Body weight and body weight gain Decreased Hematology Increased: RBC, HGB, HCT Decreased: Reticulocyte, MCV, WBC Bone marrow cells Increased: M/E ratio, lymphocyte Decreased: Nucleated cell, erythroid cell Organ weight (absolute weight) Decreased: Brain, thymus, heart, lung, liver, kidney, spleen, adrenal, testis	(Ogawa *et al*., 1985)
SD rat (male and female, 6 weeks old)	13 weeks	55-85%	Body weight and body weight gain Decreased Hematology and blood chemistry Increased: ALP, Na Decreased: WBC, GLUC, TP, CRE, lipid peroxide Organ weight (absolute weight) Decreased: Brain, thymus, heart, lung, liver, kidney, spleen, pituitary, thyroid, adrenal, prostate, testis, uterus, ovary, submaxillary gland Histopathological changes** Ovary, uterus, testis, prostate, seminal vesicle, pituitary gland	(Seki *et al*., 1997)
SD rat (male and female, 35 days old)	2 years	45-75%	Survival rate Increased (main causes of death were neoplasms) Body weight and body weight gain Decreased Hematology and blood chemistry Increased: ALP, Cl Decreased: WBC, NEUT, LYMPH, PLT, TP, ALB, TC, TG, HDL, Ca Urinalysis Increased: pH Decreased: Volume, protein	(Hubert *et al*., 2000)
Dog (male, 7 months old)	4 weeks	23-50%	Body weight and body weight gain Decreased Water consumption Decreased Electrocardiography Increased: PR interval Decreased: heart rate Hematology and blood chemistry Increased: ALT, GGT, BIL, TBA, BUN, CRE, Cl Decreased: Reticulocyte, WBC, NEUT, LYMPH, MONO, EOS, BASO, ALP, GLUC, TP, ALB, Ca, IP, amylase, phospholipid Urinalysis Decreased: Volume, Na, K excretion Bone marrow cells Increased: M/E ratio, myeloid cell, monocyte Decreased: Nucleated cell, erythroid cell Organ weight (absolute weight) Increased: Adrenal Decreased: Thymus, heart, lung, liver, kidney, spleen, testis, prostate Histopathological changes** Bone marrow, thymus, seminiferous tubule, stomach	(Takamatsu *et al*., 2015)

Table 1. (Continued).

Animal (sex, age*)	Duration of food restriction	Supplied food (% of control)	Toxicological parameters changes with food-restriction	References
Dog (male, 9 months old)	12 weeks	50-67%	Body weight and body weight gain Decreased Electrocardiography Increased: PR interval Decreased: heart rate Hematology and blood chemistry Increased: RBC, HGB, HCT, GGT, FFA Decreased: Reticulocyte, WBC, NEUT, LYMPH, MONO, EOS, AST, LDL	(Morita *et al*., 2015)
Cynomolgus monkey (male, 45-51 months old)	2 weeks	25%	Body weight and body weight gain Decreased Hematology and blood chemistry Increased: AST, ALT, GGT, BIL, BUN, CRE, TKB, GLDH Decreased: MCV, reticulocyte, TG, Fe, TIBC, UIBC Urinalysis Increased: Volume Decreased: Specific gravity, Na, K, Cl excretion Bone marrow cells Increased: M/E ratio Decreased: Nucleated cell, erythroid cell, myeloid cell Organ weight (absolute weight) Decreased: Thymus, liver Histopathological changes** Heart, liver, thymus, pancreas, bone marrow, adrenal	(Fujisawa *et al*., 2024)

*at the initiation of food restriction, **Tissues/organs where findings were observed
Abbreviations: SD - Sprague-Dawley, RBC - red blood cells, HGB - hemoglobin, HCT - hematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, WBC - white blood cells, NEUT - neutrophils, LYMPH - lymphocytes, MONO - monocytes, EOS - eosinophils, BASO - basophils, PLT - platelets, PT - prothrombin time, APTT - activated partial thromboplastin time, FIB - fibrinogen, AST - aspartate aminotransferase, ALT - alanine aminotransferase, ALP - alkaline phosphatase, BIL - bilirubin, TBA - total bile acids, GGT - gamma-glutamyl transferase, GLUC - glucose, TP - total protein, ALB - albumin, GLB - globulin, CK - creatine kinase, TC - total cholesterol, TG - triglycerides, PL - phospholipid, BUN - blood urea nitrogen, CRE - creatinine, Na - sodium, K - potassium, Cl - chloride, Ca - calcium, IP - inorganic phosphorus, Fe - iron, TIBC - total iron binding capacity, UIBC - unsaturated iron binding capacity, HDL - high-density lipoprotein, LDL - low-density lipoprotein, FFA - free fatty acids, TKB - total ketone bodies, GLDH - glutamate dehydrogenase, NAG - N-acetyl-β-D-glucosaminidase.

ly expected to decrease after food restriction. Examples are proteins, carbohydrates, fats, vitamins, and minerals. Among these, parameters generally measured in non-clinical toxicity studies include glucose, total protein, albumin, total cholesterol, triglycerides, calcium, and inorganic phosphorus, all of which have been commonly reported to decrease after food restriction across all animal species (Table 1). On the other hand, there is also a report that glucose increases after food restriction (Levin *et al*., 1993). Stress activates the hypothalamic-pituitary-adrenal axis, which releases glucocorticoids from the adrenal cortex. Simultaneously, stress also activates the sympathetic nervous system, releasing catecholamines, and the synergistic action of glucocorticoids and catecholamines promotes gluconeogenesis, leading to hyperglycemia (Nirupama *et al*., 2018). This stress-induced promotion of gluconeogenesis is one factor that could explain why glucose increased or was maintained after food restriction in more than half of the studies in Table 1 (Asanuma *et al*., 2011; Fujisawa *et al*., 2024; Hubert *et al*., 2000; Levin *et al*., 1993; Morita *et al*., 2015; Ogawa *et al*., 1985; Umeya *et al*., 2024).

In all rats, dogs, and monkeys, there have been numerous reports of a decrease in erythroid cells in the bone marrow and circulating reticulocytes. Under food restriction, the involvement of iron availability in this hematopoietic suppression was considered in two papers (Asanuma *et al*., 2011; Fujisawa *et al*., 2024). Total iron binding capacity (TIBC) indicates the total amount of iron that can bind to transferrin in the blood, therefore a similar parameter to transferrin. Transferrin is a plasma protein produced in the liver and is an important indicator of malnutrition in clinical settings. In fact, just as blood transferrin decreases in malnutrition in humans, TIBC decreased during food restriction in rats and monkeys (Asanuma *et al*., 2011; Fujisawa *et al*., 2024). The decrease in TIBC lead to a decrease in the amount of iron available in the bone marrow, which suppressed eryth-

roid production there. However, there were no reports of a decrease in circulating erythrocytes; instead, increases in erythrocytes, hemoglobin, and hematocrit were commonly reported, mainly in rats (Table 1). In most cases, increases in creatinine, blood urea nitrogen (BUN), sodium, and chloride, and/or a decrease in urine volume were observed without accompanying kidney damage, leading to the conjecture that these were caused by hemoconcentration resulting from dehydration (Asanuma *et al*., 2011; Levin *et al*., 1993; Moriyama *et al*., 2008; Oishi *et al*., 1979; Umeya *et al*., 2024). It is known that eating can activate physiological signals that elicit drinking behavior (Kraly, 2004), and indeed, several studies have shown that food restriction decreases water intake (Asanuma *et al*., 2011; Oishi *et al*., 1979; Takamatsu *et al*., 2015; Umeya *et al*., 2024). Another possibility is that because the life span of erythrocytes is long, the decrease in erythrocyte count during the food restriction period did not exceed the speed of hematopoiesis, and the impact of bone marrow suppression was not reflected peripherally (Fujisawa *et al*., 2024; Levin *et al*., 1993). Note that the average erythrocyte life span in each animal is 45-68, 100-115, 86-105, and 110-120 days in rats, dogs, monkeys, and humans, respectively (Rodnan *et al*., 1957; Stingo, 2019).

In most reports, leukocytes (especially neutrophils and lymphocytes) and platelets decreased in rats and dogs. While changes in leukocytes were not obvious in monkeys, there seemed to be a tendency for a decrease in lymphocytes, though there was initially a large individual difference (Fujisawa *et al*., 2024). Since circulating lymphocytes are often used as an indicator of nutritional status clinically, this change is extrapolatable to humans. The mechanism is thought to be a consequence of stress due to food restriction leading to glucocorticoid release from the adrenal cortex, then to thymic atrophy, and finally to a decrease in lymphocytes. Indeed, histopathologic changes in the thymus and adrenal gland have been reported in many studies (Table 1) (Fujisawa *et al*., 2024; Levin *et al*., 1993; Moriyama *et al*., 2008; Takamatsu *et al*., 2015; Umeya *et al*., 2024). The decrease in neutrophils, monocytes, and platelets is thought to be due to bone marrow depression, similar to the erythrocyte-related parameters. To support this, a decrease in myeloid and other cells in bone marrow tests or bone marrow histopathology has been reported (Fujisawa *et al*., 2024; Levin *et al*., 1993; Moriyama *et al*., 2008).

Transaminases (ALT, AST) varied in increase or decrease even within the same species depending on the study. Across all animal species, a trend was observed where transaminases increased with shorter periods of food restriction (within 4 weeks) and decreased with longer periods (more than 4 weeks). The increases or decreases in transaminases are thought to be caused by different mechanisms. The initial increase in transaminases due to food restriction is speculated to be due to stress-induced release of glucocorticoids and the subsequent promotion of gluconeogenesis and fatty acid metabolism (Fujisawa *et al*., 2024; Kobayashi *et al*., 2020). Indeed, it has been reported that in mice with caloric restriction, gluconeogenesis and protein catabolism are induced, and transaminase activity in the liver increases subsequently (Dhahbi *et al*., 1999; Hagopian *et al*., 2003). On the other hand, decreases in transaminases are thought to be associated with changes accompanying the atrophy of the small intestinal mucosa (Kobayashi *et al*., 2020). However, the authors say this applies to the initial period of food restriction (1-7 days), and there have been no reports of histopathological findings in the small intestine in the studies shown in Table 1. The cause of the decrease in transaminase after more than 4 weeks of food restriction is still not clear.

Alkaline phosphatase (ALP) is a well-known biomarker of protein malnutrition (Waterlow, 1970). There is no doubt that food restriction decreased circulating ALP activity because of reduced ALP protein synthesis (Asanuma *et al*., 2011; Oishi *et al*., 1979; Takamatsu *et al*., 2015; Umeya *et al*., 2024). However, some reports in rats have shown that ALP increases with food restriction (Hubert *et al*., 2000; Levin *et al*., 1993; Seki *et al*., 1997). As the main source of ALP is bone in rodents, the author concluded that food restriction suppressed a decrease in bone-derived ALP associated with growth, leading to higher ALP levels compared to the control group.

Increases in bilirubin, total bile acids, and GGT were observed in all animal species (Fujisawa *et al*., 2024; Levin *et al*., 1993; Moriyama *et al*., 2008; Takamatsu *et al*., 2015). The primary cause of these changes is the accumulation of bile due to decreased food intake, which suppressed bile excretion and increased the amount of bile components in the blood.

Moreover, in all animal species, many studies reported an increase in BUN due to food restriction (Asanuma *et al*., 2011; Fujisawa *et al*., 2024; Levin *et al*., 1993; Moriyama *et al*., 2008; Takamatsu *et al*., 2015; Umeya *et al*., 2024). This can be attributed to two mechanisms: hemoconcentration due to dehydration, and enhanced protein catabolism caused by malnutrition. Although some papers reported an increase in BUN and creatinine, none of the papers showed any histopathological findings related to the kidney, suggesting that the increase was not due to renal toxicity.

Table 2. Changes in toxicological parameters due to restricted feeding across different animal species (22-33% of normal food-supply for 2-4 weeks).

Animals	Rat				Dog	Monkey
Duration of food restriction	2 weeks	2 weeks	2 weeks	4 weeks	4 weeks	2 weeks
Supplied food (% of control)	25%	25%	33%	22%	23%	25%
References	(Levin *et al*., 1993)	(Moriyama *et al*., 2008)	(Asanuma *et al*., 2011)	(Oishi *et al*., 1979)	(Takamatsu *et al*., 2015)	(Fujisawa *et al*., 2024)
Body weight	↓	↓	↓	↓	↓	↓
(% of control)	(38-42%)	(58-70%)*	(50%)*	(33%)*	(75%)	(88%)
Water consumption	NE	NE	↓	↓	↓	NE
Hematology						
RBC-related parameters (RBC, HGB, HCT)	↑	↑	↑	-	-	-
Reticulocytes	↓	↓	↓	NE	↓	↓
WBC-related parameters (WBC, NEUT, LYMPH, MONO, EOS, BASO)	↓	↓	↓	↓	↓	-
PLT	↓	↓	-	NE	-	-
Blood chemistry						
AST, ALT	↑	↑	NE	↓	↑	↑
ALP	↑	-	↓	↓	↓	-
BIL, TBA, GGT	↑	↑	NE	NE	↑	↑
GLUC	↑	↓	NE	↓	↓	-
TP, ALB, GLB	↓	↓	-	↓	↓	-
TC, TG	↓	↓	NE	↓	-	↓
BUN, CRE	↑	↑	↑	-	↑	↑
Na, Cl	↑	↑	NE	↑	↑	-
K	↓	↓	NE	-	-	-
Ca, IP	↓	↓	NE	↓	↓	-
Fe	NE	NE	↑	NE	NE	↓
TIBC, UIBC	NE	NE	↓	NE	NE	↓
Urinalysis						
pH	NE	↓	NE	NE	-	-
Volume	NE	-	NE	↓	↓	↑
Na, K, Cl excretion	NE	NE	NE	NE	↓	↓
Bone marrow cell						
M/E ratio	NE	NE	↑	NE	↑	↑
Nucleated cell	NE	NE	↓	NE	↓	↓
Myeloid cell	NE	NE	-	NE	↑	↓
Erythroid cell	NE	NE	↓	NE	↓	↓
Lymphocyte	NE	NE	↓	NE	-	-
Monocyte	NE	NE	-	NE	↑	-
Organ weight						
Thymus, liver	↓	↓	NE	↓	↓	↓
Heart, spleen, kidney	↓	↓	NE	↓	↓	-
Reproductive organs	↓	↓	NE	↓	↓	-
Adrenal	↓	-	NE	↓	↑	-

↑: increased, ↓: decreased, -: no apparent change, NE: not examined, *: approximate value

Another change considered to be caused by food restriction is a decrease in heart rate. In both of the dog studies, food restriction was reported to lead to a decrease in heart rate and prolongation of the PR interval (Morita *et al*., 2015; Takamatsu *et al*., 2015). This is considered to be due to a decrease in circulating blood volume caused by a decrease in water intake (Takamatsu *et al*., 2015). Another possible mechanism is that the decrease in body weight led to a decrease in sympathetic nervous activity and an increase in parasympathetic nervous activity, which prolonged the PR interval and decreased the heart rate (Morita *et al*., 2015). As previously mentioned, mental stress conversely increases sympathetic nervous activity, so the heart rate reduction and PR prolongation after food restriction may depend on the balance of the sympathetic/parasympathetic nervous activity and the timing of data acquisition.

Conclusion

In non-clinical toxicity studies of drug candidates, food intake often decreases, which causes various parameter changes. Understanding which parameters can change and what mechanisms can cause these changes due to food restriction is crucial to improving the quality of safety tests. Moreover, differences in these parameter changes due to variations in animal species and food restriction conditions can also provide important information for considering human translatability.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Asanuma, F., Miyata, H., Iwaki, Y. and Kimura, M. (2011): Feature on erythropoiesis in dietary restricted rats. J. Vet. Med. Sci., **73**, 89-96.

Dhahbi, J.M., Mote, P.L., Wingo, J., Tillman, J.B., Walford, R.L. and Spindler, S.R. (1999): Calories and aging alter gene expression for gluconeogenic, glycolytic, and nitrogen-metabolizing enzymes. Am. J. Physiol., **277**, E352-E360.

Fujisawa, N., Matsushita, T., Matsuo, S., Hiranuma, M., Azabu, H., Saito, R., Komatsu, S.I., Kato, A., Toyota, N., Taketo, J. and Suzuki, H. (2024): Effects of two weeks of food restriction on toxicological parameters in cynomolgus monkeys. Exp. Anim., **73**, 73-82.

Green, C.L., Lamming, D.W. and Fontana, L. (2022): Molecular mechanisms of dietary restriction promoting health and longevity. Nat. Rev. Mol. Cell Biol., **23**, 56-73.

Hagopian, K., Ramsey, J.J. and Weindruch, R. (2003): Caloric restriction increases gluconeogenic and transaminase enzyme activities in mouse liver. Exp. Gerontol., **38**, 267-278.

Hubert, M.F., Laroque, P., Gillet, J.P. and Keenan, K.P. (2000): The effects of diet, ad libitum feeding, and moderate and severe dietary restriction on body weight, survival, clinical pathology parameters, and cause of death in control Sprague-Dawley rats. Toxicol. Sci., **58**, 195-207.

Kobayashi, A., Suzuki, Y. and Sugai, S. (2020): Specificity of transaminase activities in the prediction of drug-induced hepatotoxicity. J. Toxicol. Sci., **45**, 515-537.

Kraly, F.S. (2004): Eating provides important physiological signals for satiety and drinking. Physiol. Behav., **82**, 49-52.

Levin, S., Semler, D. and Ruben, Z. (1993): Effects of two weeks of feed restriction on some common toxicologic parameters in Sprague-Dawley rats. Toxicol. Pathol., **21**, 1-14.

Mattison, J.A., Colman, R.J., Beasley, T.M., Allison, D.B., Kemnitz, J.W., Roth, G.S., Ingram, D.K., Weindruch, R., de Cabo, R. and Anderson, R.M. (2017): Caloric restriction improves health and survival of rhesus monkeys. Nat. Commun., **8**, 14063.

Morita, J., Izumi, T., Sunouchi, M., Arima, K. and Tsutsumi, S. (2015): Effects of reduced food intake on the parameters of toxicity evaluation in dogs. J. Toxicol. Sci., **40**, 523-533.

Moriyama, T., Tsujioka, S., Ohira, T., Nonaka, S., Ikeda, H., Sugiura, H., Tomohiro, M., Samura, K. and Nishikibe, M. (2008): Effects of reduced food intake on toxicity study parameters in rats. J. Toxicol. Sci., **33**, 537-547.

Nirupama, R., Rajaraman, B., and Yajurvedi, H.N. (2018): Stress and glucose metabolism: A review. Imaging J. Clin. Medical. Sci. **5**, 008-012.

Ogawa, Y., Matsumoto, K., Kamata, E., Ikeda, Y. and Kaneko, T. (1985): Effect of feed restriction on the peripheral blood and bone marrow cell counts of Wistar rats. Jikken Dobutsu, **34**, 407-416.

Oishi, S., Oishi, H. and Hiraga, K. (1979): The effect of food restriction for 4 weeks on common toxicity parameters in male rats. Toxicol. Appl. Pharmacol., **47**, 15-22.

Rodnan, G.P., Ebaugh, F.G. Jr. and Fox, M.R. (1957): The life span of the red blood cell and the red blood cell volume in the chicken, pigeon and duck as estimated by the use of Na2Cr51O4, with observations on red cell turnover rate in the mammal, bird and reptile. Blood, **12**, 355-366.

Seki, M., Yamaguchi, K., Marumo, H. and Imai, K. (1997): Effects of food restriction on reproductive and toxicological parameters in rats--in search of suitable feeding regimen in long-term tests. J. Toxicol. Sci., **22**, 427-437.

Stingo, V. (2019): Erythrocytes of the rhesus and cynomolgus monkeys. Eur. J. Histochem., **63**, 3076.

Takamatsu, K., Yamashita, H., Satake, S., Kazusa, K., Tabata, H. and Nishikata, T. (2015): Effects of four-week feed restriction on toxicological parameters in beagle dogs. Exp. Anim., **64**, 269-280.

Umeya, N., Okada, K. and Nishimura, N. (2024): Effects of food restriction for 3 or 7 days on toxicity-related parameters in rats. Fundam. Toxicol. Sci., **11**, 57-67.

Waterlow, J.C. (1970): Enzyme changes in malnutrition. J. Clin. Pathol. Suppl. (Assoc. Clin. Pathol.), **4**, 75-79.