Exp. Anim. 49(3), 153–161, 2000

Establishment and Characteristics of Four Sub-Strains of F344 Rats Reared on Various Low Protein and Low Energy Diets

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Abstract: *Four sub-strains, reared by sib-mating and having for their origin the F344/DuCrj strain of rats, were established by feeding with different levels of low protein and low energy diets, and their characteristics investigated. The amounts of crude protein (CP) and digestible energy (DE) in the four diets were 17.6%–3.0 kcal, 10.5%–2.5 kcal, 8.4%–2.0 kcal, and 10.5%–2.5 kcal, respectively, and the four sub-strains established here were provisionally designated as F344/Tig1, F344/Tig2, F344/Tig3 and F344/Tig4, respectively. Intakes of nitrogen-corrected metabolizable energy (MEn) did not differ, and a large intake of digestible crude protein (DCP) was observed in F344/Tig1 rats. The body weight of rats provided with lower-nutrient diets showed a tendency to decrease until the F2 generation, but no change among the generations was seen subsequently, and the same compiled differences in protein content were maintained. Similar transitions were observed in the lifetime rearing test. Though F344/Tig3 rats, which were reared on minimum nutrients, showed a tendency to delayed puberty, we were easily able to breed four pairs in every generation using procedures similar to those used for other strains of rats. There were no differences among the F344/Tig1 to -3 strains of rats in body length, digestive tract length, or organ weight per body weight, and all the rats had a normal range of biochemical values. But the F344/Tig4 showed a high glutamic-oxaloacetic transaminase (GOT), and a tendency to decreased liver function and a shorter lifespan. These sub-strains of F344 rats clarified differences in fatty acid compositions, such as docosahexaenoic acid (DHA) in serum, liver and the brain. The rats were intended to be useful animal models for the study of nutritional environments and their influence on the memory and learning.*

Key words: *animal breeding, animal model, low protein and low energy diet, nutritional environment, sub-strains of F344 rat*

(Received 4 October 1999 / Accepted 21 February 2000)

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Introduction

Diet may be the most important environmental factor influencing the ability of animals to attain their genetic potential for longevity. Quantitative nutrient requirements for most species of domestic animals change with various stages of the life cycle, i.e., growth, reproduction and maintenance, but the nutritional requirements of rodents throughout their reproductive life span have not been determined. Current formulas for laboratory animal diets are a natural progression from those for farm animals, but the objectives and conditions for raising laboratory and farm animals are often quite different. Indeed, the practice of *ad libitum* feeding current laboratory animal formulas may actually have deleterious effects that can be reduced or eliminated by restricting food intake [4].

Formulas fed to rodents involved in long-term survival studies may have to be reevaluated, since some formulas now used were formulated for production colonies, such as the breeding diets containing about 24% crude protein (CP) and 3.5 kcal/g energy. But chronical nephrosis and other problems have resulted from the excess intake of protein in long-term experiments in aging research under *ad libitum* feeding.

Sonaka *et al.* [17] have described how protein restriction in male F344 rats delays the aging effects of nitrogen loss and reduced cardiovascular function. Norido *et al.* [10] concluded that rats could be maintained into old age with no signs of nutritional inadequacy on diets with lower energy (2 kcal/g) and lower protein (10% crude protein) content than that in general use.

We have tried to develop low-protein and low-energy diets by using natural ingredient for aging research. We carried out breeding tests on three generations of Wistar/Tig and F344/DuCrj strains of rats to evaluate the suitability of an experimental animal diet [14]. The result, an 8% crude protein containing diet, posed no difficulty in continuous breeding. We then continued brother and sister mating and established four substrains, F344/Tig1, F344/Tig2, F344/Tig3 and F344/Tig4, which were research animal models for different nutritional environments.

We report here on the establishment of four substrains of F344 rats, the breeding data, and some physiological characteristics of the rats.

Materials and Methods

All experiments were performed with the consent of the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology (TMIG).

Animals: An original strain of rats (F344/DuCrj) were purchased from Charles River Japan, Inc. (Yokohama, Japan) and brother and sister mating was continued in each strain on several diets under different nutritional conditions.

The rats were housed in polysulfone cages (SEOBIT Inc., Tokyo) measuring 26.5 (W) × 42.5 (D) × 20.0 (H) cm with wood chips on the cage floor in an air-conditioned room maintained at a temperature of 24 ± 1˚C and a humidity of 55 ± 5% with 12-hr artificial light (8:00 to 20:00).

Rats were weaned at four weeks, and four pairs in each group were mated at 16 weeks of age in each generation.

Body (head, body and tail) lengths were measured under ethyl ether anaethesia, and after killing by bleeding from the abdominal aorta, the digestive tract (small intestine and large intestine) lengths and organ weights of the brain, pituitary, thyroids, thymus, heart, lungs, liver, kidneys, spleen, adrenals and gonads (testes and ovaries) were examined in the F7 and F8 generations of the rats at 7, 15 and 30 weeks of age.

Food consumption and growth curves were examined every five weeks of the F9 generation of each strain of rats. Urinary analysis with Uro-Hema-Combistix (Bayer-Sankyo Co., Ltd., Tokyo) was carried out every five weeks after 90 weeks of age. Biochemical values were examined at 15, 30, 54, 104 and 130 weeks of age by collecting blood from the tip of the tail. The serum was collected after centrifugation and then stored at 4˚C until use. Survival curves were examined in the F9 and F10 generations of each strain of rats.

Experimental diets: The nutritional components of each experimental diet are shown in Table 1. The compositions of experimental diets are shown in Table 2. The analytical components and digestible nutrients of the experimental diets are shown in Table 3. Evaluation of the energy intakes of the rats was performed by measuring nitrogen-corrected metabolizable energy (MEn), a method first reported by Suzuki *et al.* [18]. Digestion trials of the four diets were carried out on

Table 1. Nutritional components in the experimental diets

Diet Group	CP [a] %	DCP [b] %	DE [c] k cal/g	Low-protein and Low-energy Diet
1	17.6	14.7	3.0	commercial diet for long-term rearing
2	10.5	8.0	2.5	low-protein and low-energy diet
3	8.4	5.9	2.0	minimum low-protein and low-energy diet
4	10.5	7.1	2.5	high-fiber-formulated diet

[a] crude protein. [b] digestible crude protein. [c] digestible energy.

Table 2. Compositions of experimental diets (g/100g diet)

Ingredient	1 [a]	2	3	4
Ground yellow shelled corn	+	10.00	20.00	24.50
Ground whole barley	+			
Ground whole wheat	+	10.00		
Ground whole oats		33.00	28.22	30.00
Wheat flour			10.00	
White fish meal	+			
Defatted soybean meal	+			
Defatted rice bran		7.00		1.43
Animal fat		2.57		
Dry heated soybeans	+			
Wheat bran	+	7.00	7.00	7.00
Millet bran		10.00	30.00	3.50
Alfalfa meal, dehydrated				30.00
Corn gluten feed		15.95		
Brewer's yeast	+			
Sodium chloride	+	0.45	0.45	0.45
Calcium carbonate	+	0.86	0.50	
Calcium phosphate	+	1.25	1.93	1.80
Choline chloride		0.20	0.20	0.20
Lysine		0.49	0.80	0.26
Methionine		0.43	0.40	0.36
Cellulose powder		0.30		
NIH vitamin mixture [b]		0.25	0.25	0.25
NIH mineral mixture [c]		0.25	0.25	0.25
Commercial vitamin mixture	+			
Commercial mineral mixture	+			

[a] Closed formula of commercial diet for rodent maintenance: "+" denotes ingredients used, percentage not reported.

[b] Mineral premix of NIH-31 autoclavable diet (mg/kg diet): Cobalt (cobalt carbonate), 0.44; Copper (copper sulfate), 4.40; Iron (iron sulfate), 66.20; Manganese (manganese oxide), 110.00; Zinc (zinc oxide), 11.00; Iodine (calcium iodate), 1.65.

[c] Vitamin premix of NIH-31 autoclavable diet (per kg diet): Stabilized vitamin A palmitate, 24,300.00 IU; Vitamin D_3 (D-activated animal sterol), 4,190.00 IU; Vitamin K (menadione activity), 22.10 mg; All-*rac*-α-tocopheryl acetate, 16.50 mg; Choline chloride, 772.00 mg; Folic acid, 1.10 mg; Niacin, 22.10 mg, Ca-*d*-pantothenate, 27.60 mg; Pyridoxine-HCl, 2.21 mg; Riboflavin supplement, 5.51 mg; Thiamine mononitrate, 71.7 mg; *d*-Biotin, 0.13 mg; Vitamin B_{12} supplement, 0.015 mg.

Table 3. Analytical components and digestible nutrients in the experimental diets

		1	2	3	4
Moisture	%	7.2	7.5	6.5	7.9
Crude Protein	%	18.4	12.0	10.4	12.0
Crude Fat	%	4.2	6.3	4.0	4.2
Crude Fiber	%	6.5	11.7	14.7	12.3
Crude Ash	%	6.9	6.6	8.0	6.3
NFE [a]	%	56.8	55.9	56.4	57.3
Energy [b]	k cal/g	3.39	3.28	3.03	3.15
DCP [c]	%	15.4	8.7	6.2	7.2
DE [d]	k cal/g	2.959	2.813	2.306	2.580
MEn [e]	k cal/g	2.612	2.431	2.086	2.362

[a] nitrogen-free extract. [b] sum of decimal fractions of protein, fat, and carbohydrates (NFE) × 4, 9, 4 Kcal/g, respectively. [c] digestible crude protein. [d] digestible energy. [e] nitrogen-corrected metabolizable energy.

another group of one and two-year old F344/DuCrj rats in a TMIG aging colony. The mean MEns for the four diets were calculated as shown in Table 3.

Statistical analysis: All results were tested for statistical difference by two-way analysis of variance (ANOVA) with Stat View Ver. 5.0 [3]. Statistical significance was determined at 5% or less.

Results

The body weight decreased between the P and F2 generations in each group at a level that corresponded to the protein content of each diet, and the resulting weight differences were maintained in subsequent generations (data not shown). Early mean body weight changes in each group in the early stage (at 5 to 16 weeks of age) in the F2 to F20 generations are shown in Table 4. All groups showed significant differences, except groups 2 and 4 at all ages in both sexes.

Table 4. Mean body weight changes for each group in the F2 to F20 generations

Diet Group		Weeks of Age				
		5	7	10	13	16
male	1	80.7 ± 13.8	141.1 ± 16.0	220.0 ± 11.4	263.8 ± 10.9	292.0 ± 10.7
	2	43.0 ± 7.6	77.8 ± 11.2	141.7 ± 15.3	191.0 ± 15.8	225.7 ± 14.9
	3	30.9 ± 4.8	53.7 ± 9.2	97.6 ± 18.0	146.2 ± 20.9	183.1 ± 22.9
	4	47.7 ± 11.9	84.4 ± 17.4	151.5 ± 19.6	201.9 ± 19.2	230.3 ± 16.5
female	1	72.5 ± 9.4	109.8 ± 7.4	142.7 ± 6.1	161.5 ± 5.9	172.7 ± 5.8
	2	43.2 ± 7.1	74.2 ± 9.4	113.9 ± 6.9	134.3 ± 6.6	145.5 ± 6.4
	3	31.5 ± 5.0	55.3 ± 9.1	95.4 ± 10.8	122.4 ± 9.2	137.2 ± 10.1
	4	46.8 ± 11.3	78.4 ± 13.1	122.6 ± 9.6	142.7 ± 8.3	154.0 ± 8.8

* all groups were significant at all ages for the p=0.0001 level except groups 2 and 4.

Mean litter sizes and weaning rates of the P to F20 generations are shown in Fig. 1. Mean litter sizes and parturition rates (in parenthesis) for groups 1 to 4 were 8.9 ± 1.4 (97.7%), 8.8 ± 0.9 (97.6%), 8.3 ± 1.2 (98.8%), and 8.4 ± 1.9 (97.6%), respectively. Mean weaning rates were decreased by 50% to 60% in F3 and F4 in the third group, but the mean weaning rate and reproductive index by mean litter size × weaning rate (in parenthesis) were similar in all four groups, 96.8% (8.62), 93.2% (8.20), 89.6% (7.44), and 98.2% (8.25), respectively.

Energy intake, expressed as MEn per metabolic body weight (MBW: body weight $kg^{0.75}$) showed characteristic, age-dependent curves and showed no differences among diet groups and age groups of both sexes (Fig. 2). On the other hand, protein intake, expressed as digestible crude protein (DCP) per MBW, showed a characteristic, age-dependent curve and high levels in the control group of group 1 (F344/Tig1), along with small differences among the other groups according to the protein content of each diet (Fig. 3).

Body weight changes in the F9 generation are shown in Fig. 4. The body weight of each group corresponded to the amount of protein in the diets. Organ weights at 7, 15 and 30 weeks of age also reflected the differences ($p<0.05$) between the diet groups and age groups of both sexes. Several organ weights per body weight are shown in Fig. 5. The low testes weight in group 3 (F344/Tig3) at seven weeks of age indicates delayed sexual maturity, but the other organs indicate normal growth. Furthermore, the body length and digestive tract length parallel body weight (data not shown).

Urobilinogen, occult blood, protein, glucose and pH of the urine were similar (data not shown). Biochemical values at 15, 30, 54, 104 and 130 weeks of age were shown in Fig. 6. The GOT (glutamic-oxaloacetic transaminase) values in group 4 (F344/Tig4) were sig-

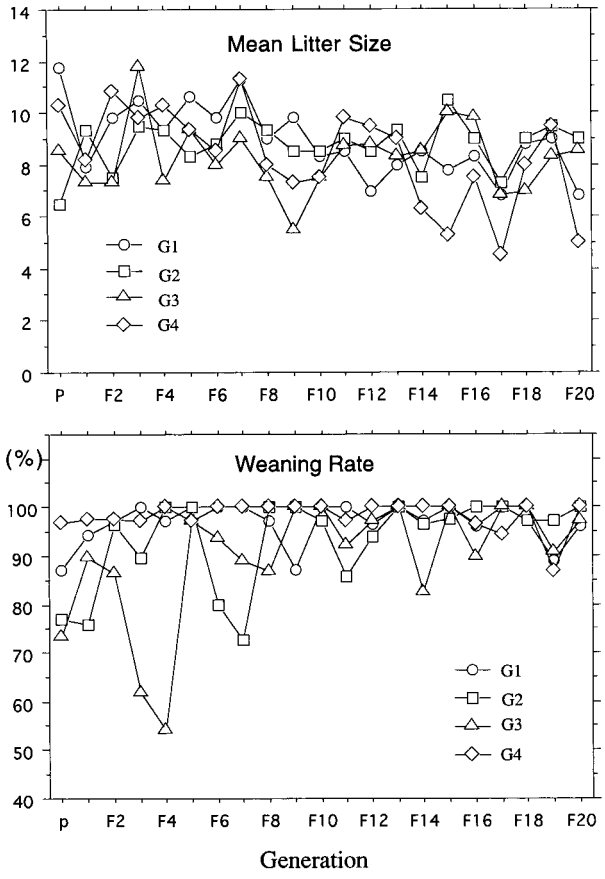

Fig. 1. Reproductive performance in the four sub-strains of F344 rats under different dietary conditions.

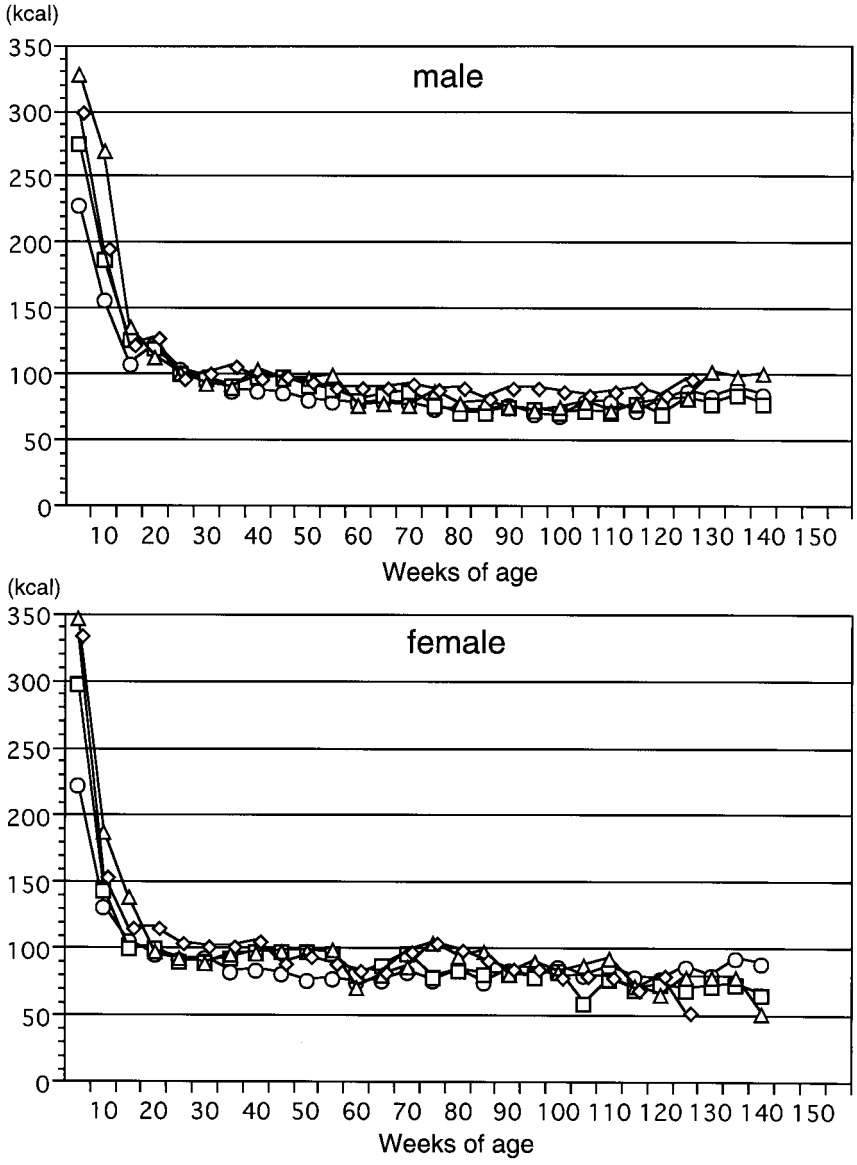

Fig. 2. Energy intake (MEn/MBW) in the four sub-strains of F344 rats under differrent dietary conditions. Each group is represented as follows: ○-G1, □-G2, △-G3, ◇-G4.

nificantly higher than in the other groups at 30 weeks. The BUN (blood urea nitrogen) values tended to be higher according to the protein content of each diet, and all data were within normal limits. TCH (total cholesterol) tended to increase in a normal aging process, and GL (blood glucose) decreased with age-dependent changes.

The survival data for each group are shown in Table 5. Group 4 (F344/Tig4) had a short life span, but the other groups did not differ from each other. The longest life span was 1,196 days old, a male in group 3 (F344/Tig3).

Discussion

We established four sub-strains, F344/Tig1, -2, -3 and -4, under different nutritional environments with four low-protein and low-energy diets. The body weight of each group corresponded to amount of protein in the diet. The F344/Tig3 strain of rats reared with the low-

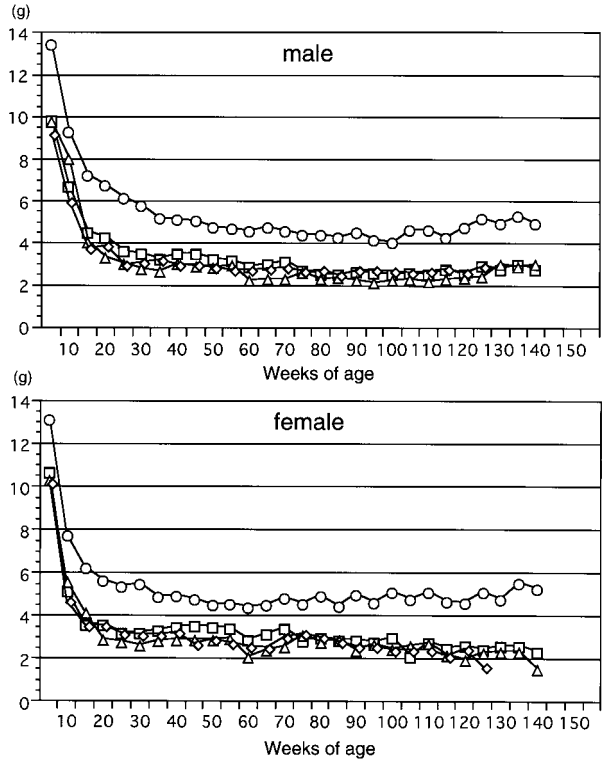

Fig. 3. Protein intakes (DCP/MBW) in the four sub-strains of F344 rats under differrent dietary conditions. Each group is represented as follows: ○-G1, □-G2, △-G3, ◇-G4.

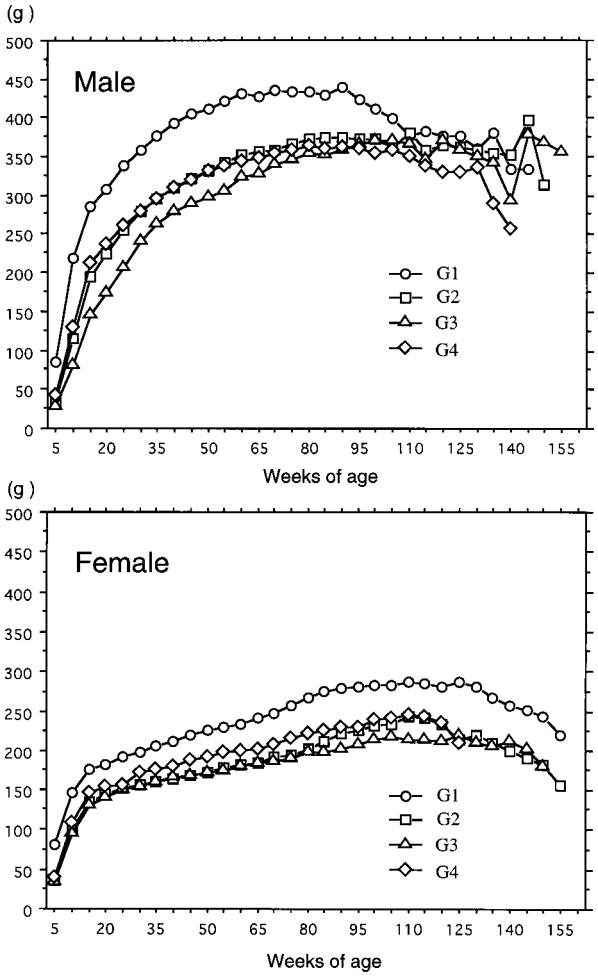

Fig. 4. Body weight changes in the four sub-strains of F344 rats under different dietary conditions.

est nutrients had delayed puberty, but their reproductive performance was unchanged. All rats had normal physiological states as indicated by the results of biochemical and other analyses. Survival data were similar to those for rats in the TMIG supply facilities, specifically the 50 percent survival time and the longest life span in the F344/DuCrj were 122.1 and 162.3 weeks of age in males, and 128.7 and 168.4 weeks of age in females [5].

Estimated nutrient requirements based on digestible protein for the maintenance, growth and reproduction of rats are 5%, 15% and 15%, respectively [8]. Ross [12] reported that the life span had been found to be influenced not only by quantitative dietary restriction but also by the ratios of the protein and carbohydrate components in the diets, and showed a maximum length of life under the 8% protein-content diet.

Nakagawa and Masana [7] examined the effect of protein nutrition on growth and lifespan in female Donryu rat by using 10%, 18% and 27% protein diets. The tail length and body weight of rats fed a 10%-casein diet were lower than those of the other groups, but the differences among all the groups became more significant later in the growth period. The estrus cycle appeared later in the 10% casein-fed rats and seemed to be more irregular than in the other groups. Neither the difference in diet nor the variation in littermates had a statistically significant effect on the lifespan.

Sheehan *et al.* [13] found that a dietary protein concentration averaging four percent was required for 12-month-old Sprague-Dawley female rats. Baldwin and Griminger [1] were able to maintain nitrogen balance in 12- and 24-month-old male F344 rats with an amino acid mixture simulating casein provided in the diet at 4.5 percent to 6 percent. Turner *et al.* [19]

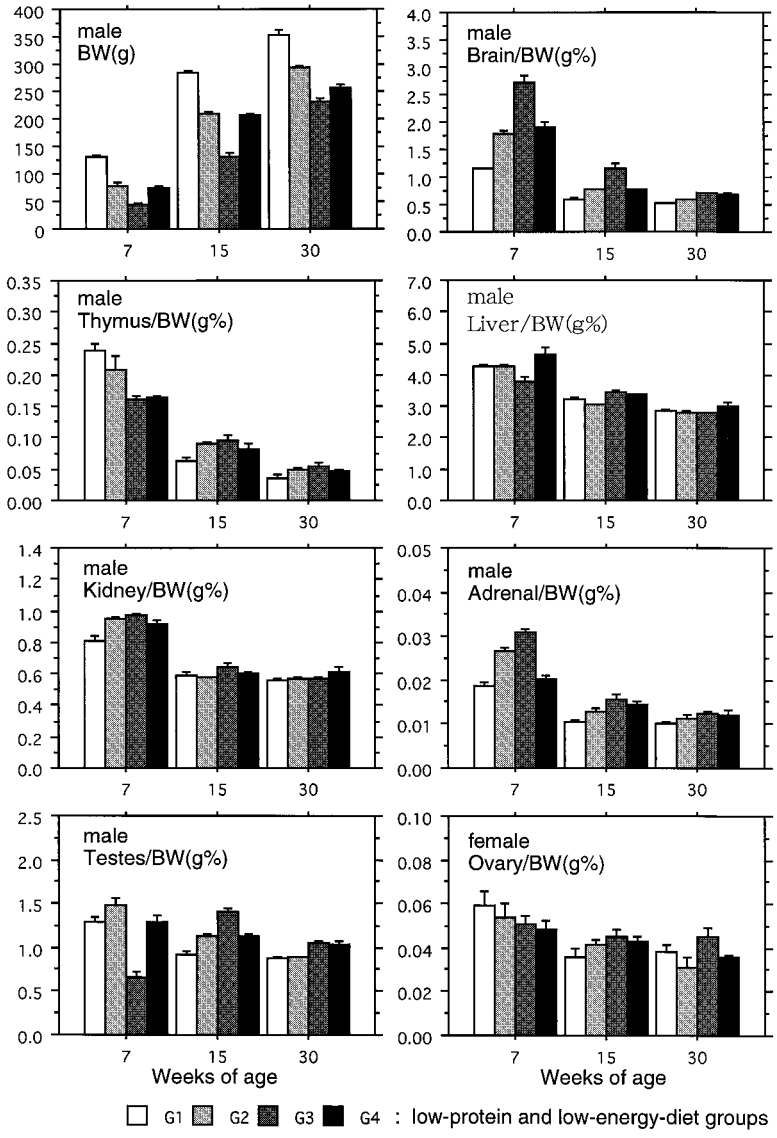

Fig. 5. Characteristic growth of organ weight percentage per body weight, in grams.

compared the protein requirements for growth and reproduction in female Sprague-Dawley rats. The protein intake of 8.6 percent (in whole-egg powder) delayed puberty, but subsequent reproductive function was normal except for altered pup weight. Linley *et al.* [6] examined the effect of age on protein requirements for body maintenance in male Sprague-Dawley rats aged 11 months and 18 months. Body weight changes were maintained in older rats at 6.52% dietary protein and in younger animals at 4.98% dietary protein.

These data show that the maintenance requirement is about five percent protein when the source is of a high quality. In a natural-ingredient diet, a concentration of seven percent crude protein is suggested [8]. Furthermore, signs of protein deficiency are described as follows. Protein deficiency in young rats results in reduced growth, anemia, hypoproteinemia, depletion of body protein, muscular waste, emaciation, and if sufficiently severe, death. In adults, a loss of weight and body nitrogen occurs, and chronic deficiency may lead to edema. Estrus becomes irregular and may cease, fetal resorption occurs, and newborns are weak or dead. A lack of protein for pregnant and lactating rats may result in offspring that are stunted in growth and have

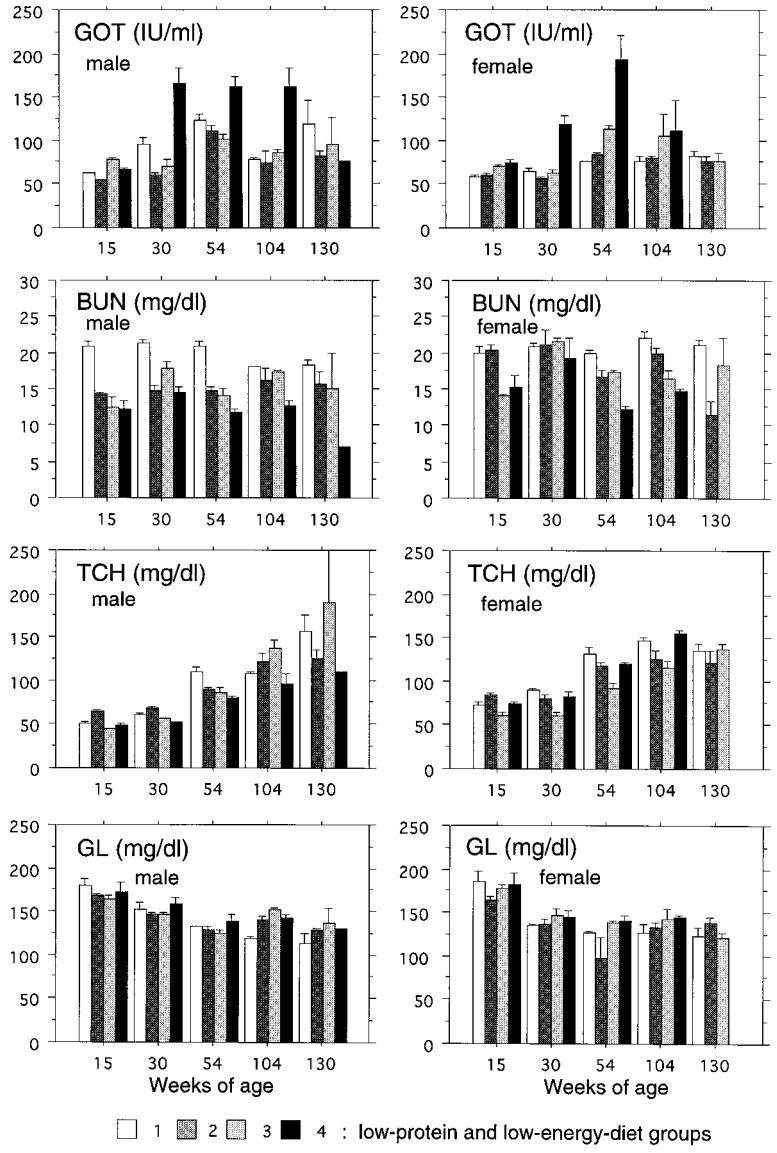

Fig. 6. Biochemical values in the four sub-strains of F344 rats under different dietary conditions.

reduced concentrations of DNA and RNA in various tissues. Low-protein diets result in reduced food intake. The reproductive capacity of the male is impaired by consumption of diets with inadequate concentrations of protein.

Although the four sub-strains of F344, developed here and given the four low-protein and low-energy diets, had limited weight gain in accordance with the protein content of each diet, and delayed puberty was observed in F344/Tig3 rats reared with minimum nutrients of 8% crude protein diets, reproductive performance was unchanged in the other strains of F344 rats and generations were successively maintained without difficulty. Furthermore, the biochemical values were not only normal but also the levels of the growth hormone (GH) and Thyroxin (T_4) at 30 weeks of age were normal (data not shown).

The F344/Tig4 strain had reduced liver function and shortened life, which were supposed to be caused by a formula abundant in alfalfa meal (30%). It is well known that alfalfa contains toxic naturally occurring, physiologically-active saponins [2, 11] and strychnine

Table 5. Lifespan parameters expressed as mean ± SEM weeks of age

Group		25%	50%	75%	mean	max (day)
male	1	103.7 ± 5.0	110.3 ± 20.6	134.4 ± 2.9	121.2 ± 7.1	150.7 (1055)
	2	115.7 ± 6.6	118.9 ± 18.0	149.3 ± 4.3	132.0 ± 6.7	153.9 (1077)
	3	120.1 ± 9.1	128.1 ± 6.0	142.7 ± 18.7	134.0 ± 8.5	170.9 (1196)
	4	114.7 ± 7.6	128.7 ± 4.7	129.7 ± 0.6	125.6 ± 3.4	140.6 (984)
female	1	100.7 ± 15.3	132.1 ± 18.8	154.4 ± 3.7	134.5 ± 8.7	157.9 (1105)
	2	108.3 ± 14.5	131.0 ± 11.7	144.0 ± 9.2	134.0 ± 7.5	157.7 (1104)
	3	114.3 ± 15.9	125.7 ± 9.2	137.3 ± 12.7	129.5 ± 7.2	155.3 (1087)
	4	120.7 ± 12.3	124.9 ± 2.9	126.3 ± 1.2	120.3 ± 4.1	127.7 (894)

[9]. Further investigation is needed.

Detailed data on organ weights, hematological and biochemical findings, composition of fatty acids in the serum, brain and liver, postmortem examination, and pathological findings are under preparation [15, 16].

Acknowledgments

We are grateful to Dr. Hatsue Waki, Dr. Yasukazu Tanaka and Dr. Susumu Ando, of the TMIG Department of Membrane Biochemistry, for characterizing of the fatty acid composition, and Dr. Yoshitake Ito, of the Institute for Medical Science of Aging, Aichi Medical University, for his pathological examination.

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