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Effect of Low Protein and Low Energy Diet on Physiological Status and Digestibility of F344 Rats

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Abstract: *A long-term raising study was carried out on male F344/DuCrj rats with three low protein (Crude Protein (CP); 14.5, 11.5, 8.5%) and low energy (Digestible Energy (DE); 2.0 kcal/g) diets from 4 to 104 weeks of age. In rats fed the 8.5% CP diet, body weight and digestible crude protein (DCP) consumption at 10 weeks of age were lower ($P<0.05$) but the body weight at 50 weeks of age was higher ($P<0.05$) than in the other groups. In rats fed the 8.5% CP diet the crude fat digestibility was higher ($P<0.05$), and the CP/nitrogen-corrected metabolizable energy (MEn) ratio was low. On the other hand, the mean survival time at 80 weeks of age was shorter in rats fed the 8.5% CP diet ($P<0.05$).*

Key words: *aging, DCP, digestibility, F344/DuCrj rat, MEn*

Introduction

Rats and mice are usually given about 24% crude protein (CP) and 3.5 kcal/g of digestible energy (DE) *ad libitum* in long-term raising and aging experiments. The nutritional excess seems to cause more frequently, spontaneous diseases of the kidney and liver, resulting in shortening life span, and McCay *et al.* [8] reported that dietary restriction prolonged the life of laboratory rodents. Ross and Bras [10] and Harrison and Archer [5] mentioned the effects of restricting the diet quantity on longevity, and French *et al.* [4] reported that the life span of male Wistar rats fed 23% crude fat and 5 kcal/g was significantly shorter than that of rats fed 4% crude fat and 3.9 kcal/g. The occurrence of chronic nephropathy was reported to be lower in CP 18% than in CP 24% diet in four rat strains in a long-term raising study [7]. The National Institute on Aging [16], Tokyo Metropolitan Institute of Gerontology [14], and the National Institute for Longevity Sciences [19] have been using a low protein (18%) diet for aging/aged laboratory rodents.

In our previous report [6] on a long-term raising study on male F344/DuCrj rats using diets differing in crude protein and digestible energy contents, few pathological findings were detected in the kidney and liver with the lower protein and energy diet group. In this study, the long-term effects of three low protein and low energy diets on the digestibility of nutrients, life span and physiological status were investigated in F344/DuCrj rats.

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Table 1. Ingredients and formulation (%) of the low protein diets

Ingredient	Diet		
	A	B	C
Corn	6.0	13.8	20.0
Barley	–	–	28.2
Wheat flour	10.0	10.0	10.0
Wheat bran	40.0	40.0	–
Millet bran	22.8	23.4	30.0
Defatted soybean meal	10.3	1.5	–
Salt	0.5	0.5	0.5
Calcium carbonate	1.8	1.6	0.5
Calcium phosphate	0.4	0.6	1.9
Choline chloride	0.2	0.2	0.2
Lysine	0.1	0.4	0.8
Methionine	0.4	0.5	0.4
Vitamin mixture (AIN-76)	2.5	2.5	2.5
Mineral mixture (AIN-76)	5.0	5.0	5.0

Table 2. Composition of the low protein diets

Component	Diet		
	A	B	C
Dry matter (DM) (%)	88.6	88.2	90.5
Crude protein (% of DM)	16.7	13.6	10.1
Crude fat (% of DM)	2.9	2.9	3.4
Crude fiber (% of DM)	14.6	10.4	12.7
Crude ash (% of DM)	12.5	11.6	11.0
Digestible Energy§ (kcal/kg of DM)	2257	2268	2210

§: From the swine data in the Standard Table of Feed Composition in Japan [17].

Materials and Methods

All experiments were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

Diets

Three diets A, B and C differing in CP content (14.5, 11.5 and 8.5%) were used, and the DE content was designed to be 2.0 kcal/g. Pelleted diets 12 mm in diameter were prepared mainly from practically non-purified ingredients, as shown in Table 1, and the AIN (American Institute of Nutrition)-76 mixture was used for vitamins and minerals [20]. The diets were analyzed in duplicate for moisture, nitrogen, diethyl ether extracts, crude fiber and crude ash (Association of Official Analytical Chemists [1]), and the results are shown in Table 2. DE was calculated by adding the individual DE values of the ingredients [17].

Digestion trial

Digestion trials were performed using male F344/DuCrj rats, five each at 1 and 2 years of age. They were given a commercial diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) after weaning, and their average body weights were 406 g and 424 g, at one and two years of age, respectively. The rats were individually housed in polycarbonate cages (220 × 220 × 180 mm), and first given the experimental diet, diet A, and thereafter diets B and C for 12 days each, and feed consumption was measured every day. Feces and urine were collected for the last four days, and dried by a vacuum freeze dryer (FREEZVAC-7S, Tozai Tsusho Co., Ltd., Tokyo). The feces were analyzed for moisture, nitrogen, diethyl ether extracts and crude fiber, and the urine was analyzed for nitrogen [1]. The gross energy of diets, feces and urine were measured by an autocalculating bomb calorimeter (CA-4P, Shimadzu Corporation, Kyoto), and the digestibility and nitrogen-corrected metabolizable energy (MEn) values of the diets were calculated [18].

Animals

One hundred and twenty, 4-week-old male F344/DuCrj rats were purchased from Charles River Japan, Inc. (Yokohama). The rats were housed in groups of four in polycarbonate cages (265 × 425 × 200 mm) with bedding of wood chips (Charles River Japan, Inc.). All animals were maintained in a barriered room controlled at 23 ± 2°C and 50–60% relative humidity with a 12/12 h light/dark cycle.

Observation

The experimental diets were given *ad libitum* from 4 to 104 weeks of age. Tap water was also freely available. Every Monday, the bedding was changed, and feed consumption and body weight were measured. Feed consumption was calculated by subtraction of residue from supply without collecting feed spills. Digestible crude protein (DCP) and MEn consumption were calculated from feed consumption, the DCP and MEn values of the diets being estimated from the results of the digestion trials. At 52 and 104 weeks of

Table 3. Digestibility of the low protein diets in F344 rats

Component	Age	Diet		
		A (n=5)	B (n=5)	C (n=5)
Dry matter (DM) (%)	1y	61.3	61.3	58.8
	2y	63.0	58.4	59.5
Crude protein (%)	1y	84.2 [a, *)]	82.3 [a, *)]	77.1 [b, *)]
	2y	72.8 [a)]	66.0 [b)]	66.4 [b)]
Crude fat (%)	1y	69.8 [a, *)]	66.3 [a, *)]	83.8 [b)]
	2y	79.2 [a)]	72.9 [b)]	87.3 [c)]
Crude fiber (%)	1y	6.5 [a, *)]	4.5 [a, b)]	1.6 [b)]
	2y	12.6 [a)]	6.3 [b)]	5.1 [b)]
MEn§ (kcal/kg of DM)	1y	2206 *)	2252	2322
	2y	2457	2285	2488

§: nitrogen-corrected metabolizable energy. [a, b, c]:Values in the same line bearing different superscripts differ significantly (P<0.05). *: Significantly different (P<0.05) from those of two-year old rats.

age, blood was sampled from 4 rats randomly selected, one per group, under anesthesia after overnight fasting (17:00 to 9:00) with freely available water.

Sera were examined for total cholesterol, triglyceride, β-lipoprotein, leucine aminopeptidase (LAP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (AL-P), choline-esterase, blood urine nitrogen (BUN), lactate dehydrogenase (LDH), total serum protein and albumin/globulin (A/G) ratio by Mitsubishi Chemical BCL Co., Ltd. (Tokyo).

Statistical analyses

All results were tested for statistical difference by an analysis of variance [15] and Duncan's multiple-range test [3] with SAS [13].

Results

In the digestion trials, as shown in Table 3, the age and dietary CP levels affected the digestibility of CP, crude fat and crude fiber (P<0.01), while neither age nor dietary CP levels affected the digestibility of dry matter. The age also affected MEn (P<0.01).

The digestibility of CP tended to decrease in response to a decrease in the CP content of the diet, being the highest with diet A and lowest with diet C (P<0.05). The CP digestibility was higher at one year of age (P<0.05). The digestibility of crude fat was the highest with diet C (P<0.05) at both one and two years of age, and it was lower at one year of age with diets A and B (P<0.05). The digestibility of crude fiber was lower with diet C than with diet A at one and two years of age (P<0.05), and it was lower with diet A at one year (P<0.05). Among diets A, B and C, no significant differences were observed in MEn, though rats fed diet A at two years of age had significantly higher MEn (P<0.05) than 1-year-old rats.

The consumptions of feed, DCP and MEn per metabolic body weight at 10 to 100 weeks of age are shown in Tables 4 to 6. The DCP and MEn consumptions from 4 to 75 and from 80 to 100 weeks of age were measured using the digestibility estimates obtained in the digestion trials at one and two years of age, respectively.

From 30 to 80 weeks of age feed consumption was lower in rats fed diet C than those fed diets A and B (P<0.05). The DCP consumption was significantly different among the groups from 10 to 80 weeks of age (P<0.05), whereas the MEn consumption was not.

As shown in Table 7, body weight was lower in rats fed diet C at 10 weeks of age (P<0.05) but was the highest at 50 weeks of age (P<0.05). All cases showed a peak weight at 80 weeks of age.

As shown in Figs 1 and 2, the serum concentrations of total cholesterol, β-lipoprotein, choline-esterase, LDH and total serum protein were significantly higher at 104 weeks of age than at 52 weeks of age (P<0.01). At 52

Table 4. Feed intake of the low protein diets in F344 rats

Age	Diet		
	A	B	C
10 w	80.7[§, a]	70.4[b]	76.9[a]
30 w	46.1[a]	42.8[b]	38.7[c]
50 w	43.5[a]	42.3[a]	39.7[b]
80 w	39.1[a]	40.4[a]	35.9[b]
100 w	39.1[a, b]	35.2[a]	43.1[b]

§: Mean value (g/kg$^{0.75}$/day) for seven days.
a, b, c: Values in the same line bearing different superscripts differ significantly (P<0.05).

Table 5. DCP intake in F344 rats given the low protein diets

Age	Diet		
	A	B	C
10 w	10.3[§, a]	7.1[b]	5.4[c]
30 w	5.9[a]	4.3[b]	2.7[c]
50 w	5.6[a]	4.3[b]	2.8[c]
80 w	4.3[a]	3.3[b]	2.2[c]
100 w	4.3[a]	2.9[b]	2.7[b]

§: Mean value (g/kg$^{0.75}$/day) for seven days.
a, b, c: Values in the same line bearing different superscripts differ significantly (P<0.05).

Table 6. MEn intake in F344 rats given the low protein diets

Age	Diet		
	A	B	C
10 w	177.9[§, a]	158.4[b]	174.7[a]
30 w	101.6[a]	96.3[b]	88.0[c]
50 w	96.0[a]	95.2[a]	90.2[b]
80 w	96.0	92.4	89.3
100 w	96.0[a]	80.4[b]	107.2[c]

§: Mean value (kcal/kg$^{0.75}$/day) for seven days.
a, b, c: Values in the same line bearing different superscripts differ significantly (P<0.05).

Table 7. Body weight (g) of F344 rats given the low protein diets

Age	Diet		
	A	B	C
10 w	192.1[a]	186.2[b]	139.9[c]
30 w	328.4[a, b]	332.2[a]	323.2[b]
50 w	355.2[a]	362.6[a]	381.6[b]
80 w	378.8[a]	391.7[b]	405.4[c]
100 w	360.1[a]	363.9[a]	386.8[b]

a, b, c: Values in the same line bearing different superscripts differ significantly (P<0.05).

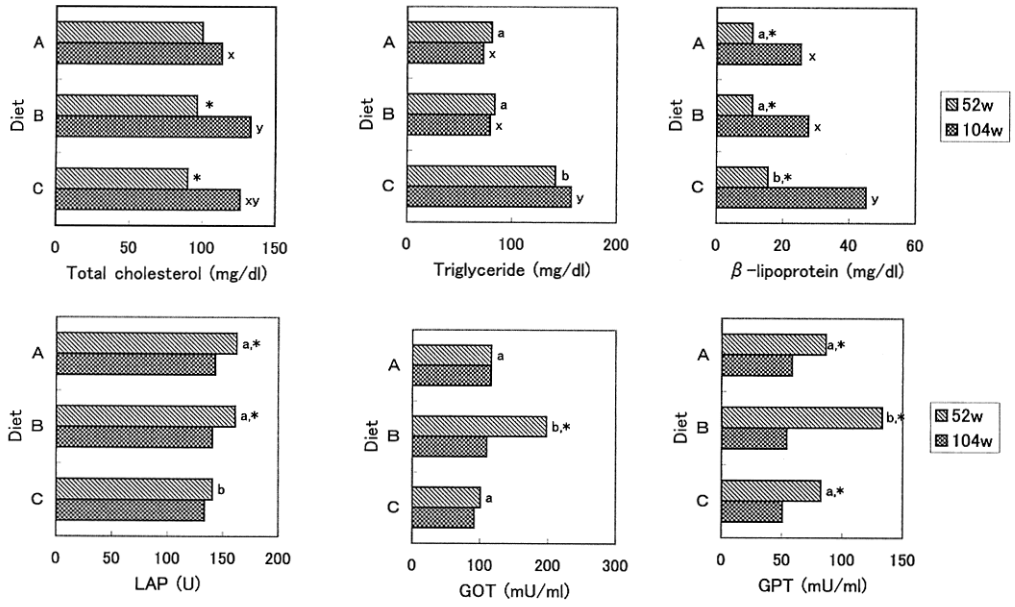

Fig. 1. Total Cholesterol, triglyceride, β-lipo protein, LAP, GOT and GPT in F344 rats given the low protein diets at 52 and 104 weeks of age. a, b, x, y: Significantly different (P<0.05) between a and b or between x and y. *: Significantly different (P<0.05) from 104 weeks of age.

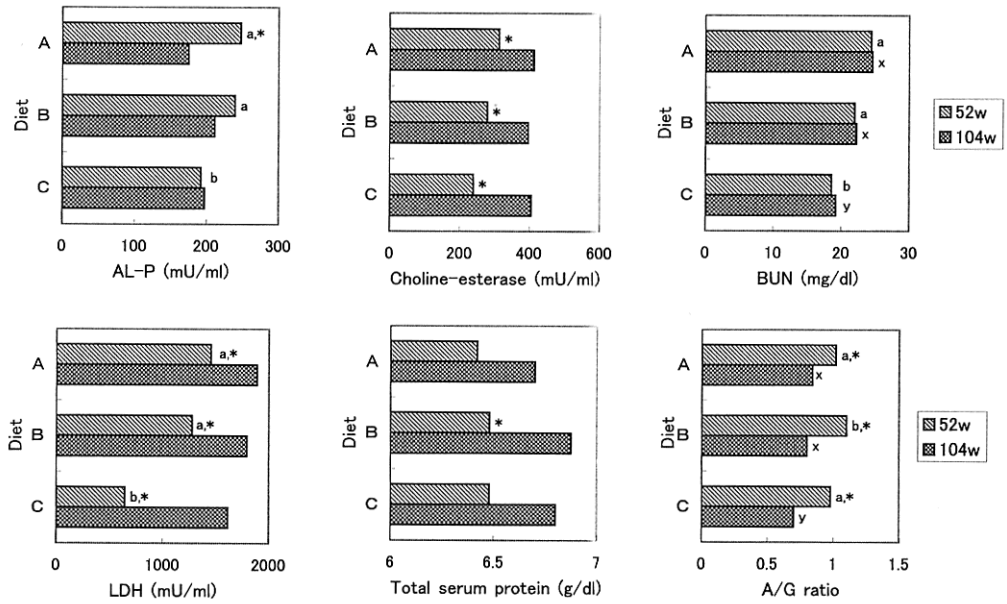

Fig. 2. AL-P, choline-esterase, BUN, LDH, total serum protein and A/G ratio in F344 rats given the low protein diets at 52 and 104 weeks of age. a, b, x, y: Significantly different (P<0.05) between a and b or between x and y. *: Significantly different (P<0.05) from 104 weeks of age.

Table 8. Mean survival time of F344 rats given the low protein diets

Age	Diet		
	A	B	C
80 w	558.2[§, a]	560.0[a]	532.0[b]
100 w	682.0[a, b]	689.8[a]	646.4[b]

§: Days. [a, b]: Values in the same line bearing different superscripts differ significantly (P<0.05).

and 104 weeks of age triglyceride and β-lipoprotein were at higher levels in rats fed diet C (P<0.05), while LAP was lower at 52 weeks of age (P<0.05). In rats fed diet B, the concentrations of GOT and GPT were higher (P<0.05) at 52 weeks of age but not at 104 weeks of age. In all cases, GPT was lower at 104 weeks than at 52 weeks of age (P<0.05).

As presented in Fig. 2, the serum AL-P levels at 52 weeks of age were lower in rats fed diet C (P<0.05) but not at 104 weeks of age. In rats fed diet C, BUN was lower at 52 and 104 weeks of age (P<0.05), and LDH was lower at 52 weeks of age (P<0.05). With aging, total serum protein tended to increase with decreased A/G ratio (P <0.01).

As shown in Table 8, the mean survival time at 80 weeks of age was shorter in rats fed diet C than others (P<0.05), but at 100 weeks of age no significant difference was seen between rats fed diet C and those fed diet A. At 100 weeks of age, the survival time was shorter in rats fed diet C as compared to those fed diet B (P<0.05).

Discussion

The digestibility of crude protein decreased with aging, while that of crude fat increased, showing that the digestible components used as an energy source at one year of age differed from those used at two years of age. The high digestibility of crude fat at two years of age might have resulted in an increase in adiposity, perhaps shortening the life span.

The digestibility of crude fiber also increased with aging. As the digestion of dietary fiber is known to depend in rats on the active microbial flora in the cecum [11], the age-dependent increase in digestibility of crude fibers might have been due to cecal cellulolytic microorganisms. The higher CP content in diet A than in diet C might have enhanced the activity of the microorganisms in the cecum raising the crude fiber digestibility.

In rats excess protein (nitrogen) was reported to be secreted as nonprotein nitrogen into the digestive tract and utilized by microorganisms [9, 12]. The secretion of nonprotein nitrogen is considered to be much greater in two year-old rats with more active microorganisms, increasing fecal nitrogen in relation to the decreased apparent digestibility of crude protein and the increased digestibility of crude fibers.

The differences in the digestibility of nutrients between groups might be due to the different chemical compositions, ingredients and formulations of the diets. The higher digestibility of crude protein in rats fed diet A may be attributed to the defatted soybean meal used as a protein source, while the lower digestibility of crude fibers in those fed diet C may have been affected by barley as a source of fibers.

At 10 weeks of age MEn consumption in rats fed diet C was equivalent to that in those fed diet A, but was higher than in those fed diet B, though the body weight and DCP consumption were lower. Serum BUN greatly depends on the diet composition, and increases when protein intake is large. In this study, the serum BUN was related to the protein content of the diet and was lower in rats fed diet C with the lowest CP content at both 52 and 104 weeks of age. As the lower level of BUN in rats fed diet C is considered to be due to the smaller intake of DCP and growing animals require a high value of DCP, diet C would be insufficient for feeding younger rats.

In rats fed diet C body weight surpassed that in the other groups at 45 weeks of age and it was significantly higher after 50 weeks of age, probably related to the higher digestibility of crude fat in diet C, while the CP/MEn ratio was lower. The higher concentrations of serum triglyceride and β-lipoprotein at 52 and 104 weeks of age were considered to be due to the higher digestibility of crude fat.

The mean survival time at 80 weeks of age was shorter in rats fed diet C than those fed the other diets. The first deaths occurred at 72, 79 and 20 weeks with diets A, B and C, respectively. This might be explained by the lower DCP consumption and body weight gain in rats fed diet C until 10 weeks of age. Berg and Simms [2] reported that longevity with restricted feeding was related to the inhibition of adiposity. The high digestibility of crude fat and more body weight gain in rats fed diet C at 50 or more weeks of age, might result in the short life span.

From the results, diets composed of non-purified ingredients containing 11.5% to 14.5% CP and 2.0 kcal/g DE are recommended for long-term raising of F344 rats.

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