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Life Span Study of SPF Fischer 344 Male Rats Fed *Ad Libitum* or Restricted Diets: Longevity, Growth, Lean Body Mass and Disease

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A life-span study was carried out on longevity, pathologic lesions, growth, lean body mass and selected aspects of muscle of barrier-maintained SPF Fischer 344 rats fed either *ad libitum* (Group A) or 60% of the *ad libitum* intake (Group R). Food restriction was as effective in prolonging the life of already long-lived SPF rats as previously shown for rats maintained in conventional facilities. Food restriction not only increased the mean length of life but also acted to extend life span since more than 60% of the Group R rats lived longer than the longest lived Group A rat. Renal lesions occurred at an earlier age in Group A rats than in Group R rats and progressed more rapidly. Death of most Group A rats was associated with severe renal lesions while few Group R rats showed such lesions at death. Food restriction was also found to delay or prevent interstitial cell tumors of the testes, bile duct hyperplasia, myocardial fibrosis and myocardial degeneration. Gastrocnemius muscle mass declined in advanced age and food restriction delayed this decline. Interestingly, however, lean body mass did not progressively decline with increasing age but rather decline occurred only after the onset of the terminal disease process.

Key Words: Food restriction, Longevity, Lean body mass, Chronic nephropathy, Leydig cell tumors, Bile duct hyperplasia, Nutrition and aging, Gastrocnemius muscle mass

IN recent years, there has been an increasing use of the specific pathogen-free (SPF) rat as a model for the study of aging (Cohen, 1979). When the SPF status is sustained throughout life, the longevity of the population is increased compared to rats of the same strain and sex housed in conventional facilities (Weisbroth, 1972). Housing in a so-called barrier facility can maintain SPF status; the increase in population longevity occurs presumably by preventing infectious disease. The commercial availability of barrier-maintained SPF male Fischer 344 rats and the genetic homogeneity of these rats have led to the extensive use of this strain in aging studies (Masoro, 1980).

Food restriction has proved to be the most effective and reproducible procedure in experimental gerontology for enhancing the longevity of laboratory rodent populations. However, there have been no studies on the effects of food restriction on the already long-lived barrier-maintained SPF rat populations or on the Fischer 344 rat strain.

A broad-based investigation has been carried out in our laboratory on aging characteristics of barrier-maintained (SPF) male Fischer 344 rats either fed *ad libitum* or approximately 60% of the *ad libitum* intake. Because of the scope of this work, the reporting of the findings has involved the publication of several papers: responsiveness of adipocytes to hormones (Bertrand et al., 1980b; Yu et al., 1980); lipid metabolism (Liepa et al., 1980); adipose mass and cellularity (Bertrand et al., 1980a); skeletal muscle structure and function (McCarter et al., 1979); and vascular smooth muscle function (Herlihy & Yu, 1980). Data on longevity, pathologic lesions, growth and lean body mass of these rats are of importance both for a fundamental consideration of the action of

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food restriction on aging and for a meaningful integration of the individually published components of our research. Such data comprise the substance of this paper.

METHODS

Male SPF Fischer 344 weanling rats (28 days old, n = 531) were delivered in a single shipment from the Charles River Laboratories. They were singly-housed in bonneted cages in a barrier facility.

The maintenance and operation of the barrier facility were carried out in the following way. A flow of air entered the barrier through a mechanical filter (HEPA) and air was exhausted without recycling. Air pressure within the barrier was maintained about 5 mmHg above the outside environment. The temperature was maintained at 25-27C, relative humidity ranged between 40 and 50% and a 12-hour light/12-hour dark cycle was used. Drinking water entered the barrier through a Millipore sterilizing system (Model 29300) after which it was acidified to pH 2.5-3.0 with HCl. Materials, except for food, entered the facility either after being autoclaved in a pass-through autoclave or after treatment with Zephiran chloride solution (aqueous solution 1:750). All personnel put on sterilized uniforms plus masks, hoods, shoe covers and two pairs of gloves before entering the barrier through a specially designed series of air locks.

The SPF status of the rat population maintained in the barrier facility was monitored in the following manner. Sera from eight rats sacrificed immediately after arrival and from five rats during the first year of the study were submitted to Microbiological Associates (Walkersville, MD) for the serologic detection of rat and mouse viruses. Fresh tissues from these same rats were also submitted for identification of *Mycoplasma pulmonis* using PPLO agar. Fixed lung tissue from the same rats and on occasion others that spontaneously died or were sacrificed was examined for lesions caused by this microorganism. In addition, a broad survey of microbial contamination was carried out on a weekly basis; culture swabs were made of equipment, cages, bonnets, food, drinking devices including on occasion excreta to detect fungi, aerobic bacteria and anaerobic organisms.

Until 6 weeks of age, all rats were fed *ad libitum* a semisynthetic diet of the following composition: 21% casein, 15% sucrose, 43.65% dextrin, 3% Solka-Floc, 10% corn oil, 0.15% DL-methionine, 0.2% choline chloride, 5% Ralston-Purina mineral mix and 2% Ralston-Purina vitamin mix. The composition of the mineral mix and vitamin mix has been described previously (Bertrand et al., 1980a). At 6 weeks of age, 260 rats were randomly selected to comprise the population of Group A and another 260 rats were selected to comprise Group R. Group A rats continued to be fed *ad libitum* the diet described above while Group R rats were provided, in daily allotments, approximately 60% of the mean amount of diet consumed by the Group A population (see Fig. 1 for the mean food intake of the Group A rats). The nature of the diet fed Group R rats was identical to that fed Group A rats except that the vitamin concentration in the diet was increased in order to provide both groups with the same vitamin intake.

At 6 weeks of age, both Group A and Group R rats were further divided into three subgroups: a longevity study group, a group for the longitudinal study of lean body mass, and a group for use in cross-sectional measurements of various physiological and morphological characteristics.

For the longevity study, a total of 230 rats, (115 Group A rats and 115 Group R rats) were used. Since these rats were weighed every 14 days, life span information on growth and weight changes were obtained.

For the longitudinal study of lean body mass, 14 Group A rats and 16 Group R rats

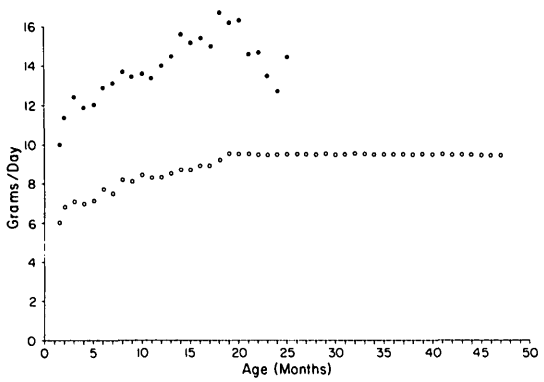

Fig. 1. Food intake by Group A and Group R Rats. The filled circles are for the Group A rats and the open circles for Group R rats. The caloric value of the diet was 4.1 Kcal per gram.

were used. Lean body mass was determined several times during the life of each rat by subtracting total fat mass from the directly measured total body mass. Fat mass was indirectly determined by measuring the uptake of cyclopropane by the rat according to the method of Lesser et al. (1973). The equilibrium concentration of cyclopropane in the chamber housing the rat was measured by gas chromotography using Chromosorb W (80/100 mesh) coated with 3% OV-101 (Applied Science Lab) as a liquid phase maintained at 29C. Triangulation was used for quantitation of the recorded peak with a retention time identical to that of a standard cyclopropane source.

For cross-sectional studies, 10 Group A rats were killed by decapitation at each of the following ages: 6, 12, 18, 24 months and 9 were killed at 27 months of age; 10 Group R rats were killed at each of the following ages: 6, 12, 18, 24, 30 and 36 months. The data collected on the gastrocnemius muscle and on the pathologic lesions will be reported in this paper. The many biochemical and physiological studies carried out on tissues from these rats have been or will be published elsewhere (Bertrand, 1980a, b; Herlihy & Yu, 1980; Liepa et al.,1980; McCarter et al., 1979; Yu et al., 1980).

The gastrocnemius muscles were excised and their mass was measured gravimetrically. The composition of the muscle was analyzed as follows: Dry weight was measured gravimetrically after removing the water content by heating at 60C in a vacuum oven. The protein content of the muscle was measured by the procedure described by Lowry et al. (1951) for insoluble proteins. Collagen content was determined by Method II of Jackson and Cleary (1967). Lipids were quantitatively extracted from the muscle by the method of Masoro et al. (1964). The cholesterol content of this extract was measured by the method of Rudel and Morris (1973) and the lipid phosphorus content by the method of Bartlett (1959).

Pathologic analyses were carried out on the rats killed for cross-sectional studies and on rats that died spontaneously. This involved gross inspection and selected histopathologic analyses of selected tissues for rats in which postmortal changes did not preclude such analyses. For the microscopic analyses, tissues were fixed in 10% formaldehyde and embedded in paraffin. Sections were prepared and stained with hematoxylin-eosin and in some cases additionally with periodic acid-Schiff stain.

RESULTS

Food intake throughout life is recorded in Fig. 1. In the case of the Group A rats, the intake gradually increased until 18 months of age and decreased after 20 months of age. Group R rats were provided approximately 60% the amount of food eaten by the Group A rats until 20 months of age, but from 20 months of age on, each Group R rat received 9.5g of food per day. With rare exceptions, Group R rats ate all of their daily food allotment.

In Fig. 2a, body weight is related to chronologic age. Body weight peaks at about 18

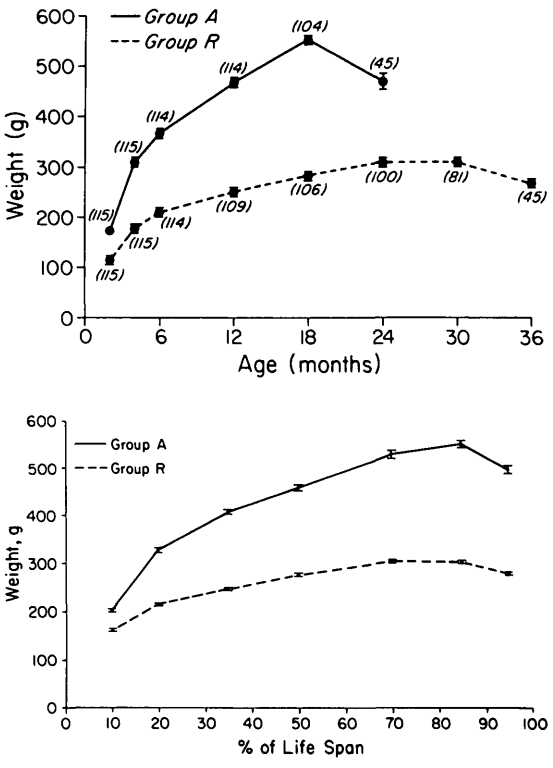

Fig. 2. Changes in Body Weight During Life Span. The solid line refers to the Group A rats and the broken lines to the Group R rats. Fig. 2a: The data are mean values ± SE for all animals alive at a given chronologic age. The numbers in parenthesis are the number of animals so analyzed. Fig. 2b: The data are mean values ± SE derived from the weight of each rat at the percent of its life span indicated on the X axis. Of the 115 rats in each group, only those that lived longer than 1 year are included since death before 1 year of age ended life during a rapid growth period. For the Group A rats, the n = 114 and for the Group R rats the n = 110.

months of age in Group A rats and from 24 to 30 months of age in Group R rats. In Fig. 2b, body weight is related in the case of each rat to percent of that rat's life span (e.g., the weight at death would be 100% of that rat's life span). This mode of expression provides information on life span changes in body weight not biased by selective mortality. In the case of Group A rats, the peak weight occurred at 85% of the life span which for most of these rats was approximately 20 months of age. In the case of the Group R rats, maximum weight was reached by 70% of the life span with a plateau in body weight through 85% of the life span which for most rats means that maximum body weight was maintained between the ages of 25 and 30 months.

The lean body mass was measured at several different times during the life span (Figs. 3 & 4) for each of the rats in the subgroups used for the longitudinal study of body composition. Because it is life span changes that are of interest, the times at which these measurements were made during the life of each rat are expressed in terms of the percent of total length of life of that rat (designated as % of the life span) rather than in terms of chronologic age. The age at death for each rat is reported in the legends to the figures which readily permits the data to be considered in terms of chronologic age if so desired. In the case of the 6 longest lived Group A rats, the lean body mass continued to increase for the first 75% of the life span and no loss in lean body mass was observed until well after 90% of the life span. For most of the 8 shortest lived Group A rats, lean body mass increased for the first 90% of the life span; no age-related decrease was observed with these rats, but few measurements were made after 95% of the life span. With the 8 longest lived Group R

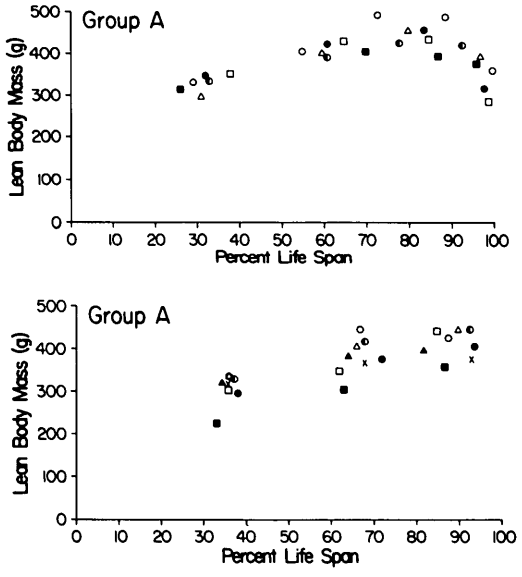

Fig. 3. Changes in Lean Body Mass During the Life Span of the 14 Group A Rats of the Longitudinal Study. Fig. 3a contains the data for the six longest lived rats for which four or more measurements were made and Fig. 3b for the eight shortest lived rats for which only three measurements were made. The X axis is expressed in terms of percent of its life span that the rat had lived at the time the measurement was made. For Fig. 3a, the following is the length of life in days of each rat as identified by symbol: ■, 842; △, 743; □, 733; ○, 811; ◐, 784; ●, 740. For Fig. 3b, the following is the length of life in days for each rat as identified by symbol: ■, 693; ▲, 732; ●, 649; □, 711; X, 646; ◐, 672; ○, 704; △, 692.

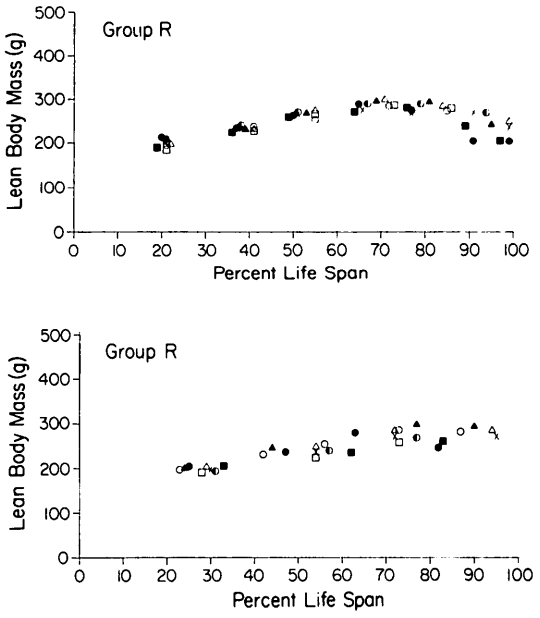

Fig. 4. Changes in Lean Body Mass During the Life Span of 16 Group R Rats of the Longitudinal Study. Fig. 4a contains the data for the eight longest lived rats and Fig. 4b for the eight shortest lived rats. The X axis is expressed in terms of percent of its life span that the rat had lived at the time the measurement was made. For Fig. 4a, the following is the length of life in days for each rat as identified by symbol: ■, 1245; ▲, 1170; ●, 1224; □, 1087; X, 1226; ◐, 1201; ○, 1101; △, 1130. For 4b, the following is the length of life in days for each rat as identified by symbol: ■, 737; ▲, 1055; ●, 979; □, 822; X, 859; ◐, 809; ○, 1086; △, 863.

rats, lean body mass increased until about 70% of the life span and began to decrease after 85% of the life span. The lean body mass of the 8 shortest lived Group R rats also increased until about 70% of the life span and in most cases did not change after that; however, only in the case of three of the rats were measurements made after 90% of the life span.

Data on the mass of the gastrocnemius muscle from the rats used in the cross-sectional study are displayed in Fig. 5. At all ages studied in common, the mass of the gastrocnemius muscles of the Group A rats was greater than that of the Group R rats. The gastrocnemius muscle mass of Group A rats reached a maximum by 12 months of age and markedly declined after 18 months of age while the gastrocnemius muscle mass of the Group R rats did not significantly decline until after 30 months of age.

The protein and lipid composition of the gastrocnemius muscle are recorded in Table 1. The dry weight:wet weight ratio did not change with age. There was also no progressive change with age in either phospholipid or cholesterol per gram of muscle. However, a significant decline in protein content occurred between 12 and 27 months of age in Group A rats and between 12 and 30 months of age in Group R rats. After 12 months of age, the collagen content per mg muscle dry weight increased with increasing age and the rate of this increase was greater in the muscles of the Group A rats than in those of the Group R rats.

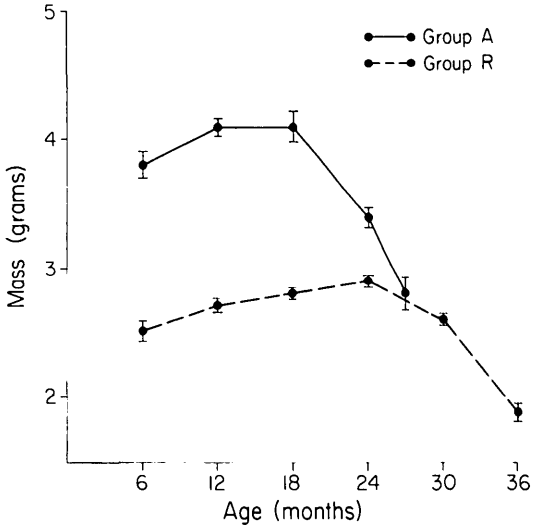

Fig. 5. Mass of the Gastrocnemius Muscle. The Y axis refers to the combined mass of the two gastrocnemius muscles. The values reported are means ± SE. The n = 10 except for 27-month-old Group A rats where the n = 9. The solid line refers to Group A rats and the broken line refers to Group R rats.

Table 1. Protein and Lipid Composition of Gastrocnemius Muscles.*

Age	Dry Weight (mg/g wet wt)		Lipid Phosphorus (μmoles/g wet wt)		Cholesterol (μmoles/g wet wt)		Protein (mg/100 mg dry wt)		Collagen[a] (μg hydroxypoline/mg dry wt)	
(mos)	Group A	Group R	Group A	Group R	Group A	Group R	Group A	Group R	Group A	Group R
6	258 ± 1 (n = 10)	250 ± 1 (n = 9)	11.8 ± 0.2 (n = 9)	10.5 ± 0.4 (n = 9)	1.82 ± 0.07 (n = 9)	1.63 ± 0.05 (n = 9)	79.5 ± 1.3 (n = 10)	77.8 ± 1.6 (n = 10)	9.13 ± 0.24 (n = 10)	8.52 ± 0.22 (n = 10)
12	263 ± 1 (n = 10)	254 ± 1 (n = 10)	12.3 ± 0.2 (n = 10)	11.5 ± 0.1 (n = 10)	2.20 ± 0.06 (n = 10)	2.20 ± 0.10 (n = 10)	80.5 ± 2.0 (n = 10)	82.6 ± 3.9 (n = 10)	8.49 ± 0.20 (n = 9)	7.56 ± 0.35 (n = 8)
18	260 ± 1 (n = 10)	254 ± 0 (n = 10)	12.8 ± 0.3 (n = 10)	12.7 ± 0.5 (n = 10)	1.81 ± 0.14 (n = 10)	2.10 ± 0.14 (n = 10)	73.3 ± 1.2 (n = 10)	75.5 ± 1.4 (n = 10)	8.93 ± 0.23 (n = 9)	8.41 ± 0.12 (n = 8)
24	258 ± 1 (n = 10)	256 ± 1 (n = 10)	9.0 ± 0.2 (n = 10)	9.3 ± 0.2 (n = 10)	1.92 ± 0.09 (n = 10)	1.69 ± 0.04 (n = 10)	70.9 ± 0.7 (n = 10)	73.2 ± 1.0 (n = 10)	11.96 ± 0.36 (n = 10)	9.78 ± 0.15 (n = 10)
27	260 ± 3 (n = 7)		11.2 ± 0.2 (n = 9)		2.40 ± 0.09 (n = 7)		65.1 ± 2.6 (n = 9)		16.29 ± 1.15 (n = 6)	
30		252 ± 1 (n = 10)		8.6 ± 0.4 (n = 10)		2.04 ± 0.05 (n = 10)		65.9 ± 1.5 (n = 10)		14.72 ± 0.87 (n = 10)
36		248 ± 2 (n = 10)		11.7 ± 0.1 (n = 10)		2.44 ± 0.08 (n = 10)		72.5 ± 1.8 (n = 10)		16.13 ± 0.37 (n = 10)

*Data are expressed as means ± SE.
[a]To avoid including varying amounts of tendon, collagen content was assayed only for the center (belly) of the muscle.

Table 2. Age-specific Death Rates of Group A and Group R Rats.

Age Range (mo)	1-4	4-8	8-12	12-16	16-20	20-24	24-28	28-32	32-36	36-40	40-44	44-48
Group A	0.01	0	0	0.02	0.1	.54	.93	1.0	—	—	—	—
Group R	0	0.03	0.02	0.04	0.03	0.05	0.08	0.21	0.37	0.62	0.82	1.0

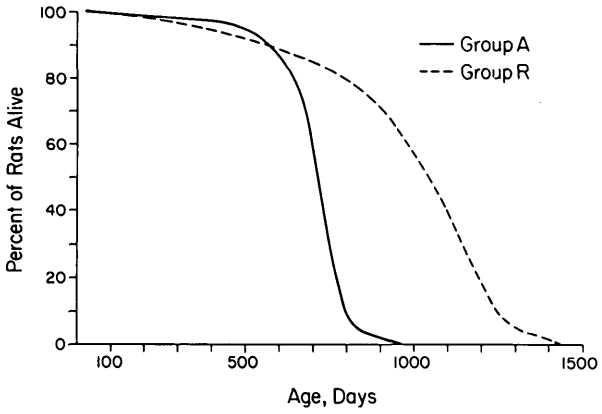

Fig. 6. Survival Curve for Group A and Group R Rats. The solid line refers to Group A rats (n = 115) and the broken line to Group R rats (n = 115). For Group A, the mean length of life ± SE is 701 ± 10 days, the median length of life is 714 days and the maximum length of life is 963 days. For Group R, the mean length of life ± SE is 986 ± 25 days, the median length of life is 1047 days and the maximum length of life is 1435 days.

Table 3. Relationship Between Growth, Body Weight and Length of Life of Group A Rats.

	r	P
Parameter[a]		
weight at 2 mos. of age	−0.14	n.s.
weight at 4 mos. of age	−0.15	n.s.
increase in weight between 2 & 4 mos. of age	−0.16	n.s.
growth rate coefficient* (k)	−0.12	n.s.
growth rate at 4 mos. of age*	−0.35	<0.001
duration of growth*	0.12	n.s.
maximal exponential weight* (Wasym)	−0.21	<0.05

[a] n = 114 rats

*based on exponential growth model:

$W(t) = [W(2\text{ mo}) - Wasym]e^{-kt} + Wasym$

W(t) = weight at an experimental time (t)

W(2 mos) = weight at 2 mos of age

Wasym = maximal exponential weight

Data from the longevity study are presented in Fig. 6 and Table 2. The mean length of life of the Group R rat population is significantly longer than that of the Group A population. Analysis of the survival curves by the method of Breslow (1970) indicates that the curve of Group A differs significantly from the curve of Group R. The barrier maintenance resulted in almost a rectangular curve for Group A rats (n = 115); i.e., most lived to near the maximum length of life. Food restriction clearly acted to extend the life span since more than 60% of the Group R rats (n = 115) lived longer than the longest lived Group A rat. Age-specific death rates (Table 2) show that the highest rates occur between 28 and 32 months of age in Group A rats and between 44 and 48 months of age in Group R rats.

Group R rats grew less rapidly but for a longer duration of time and reach a lower maximum body weight than Group A rats (Fig. 2). There was sufficient range in growth rate and maximum weight attained for the Group A rats to permit an analysis of this group in regard to correlations between growth or body weight and length of life. The results are reported in Table 3. There was no significant correlation between length of life and weight at 2 or 4 months of age or the increase in body weight between 2 and 4 months of age or the maximal weight actually attained (data not shown). Analysis of the growth in terms of an exponential growth model did, however, reveal a statistically significant negative correlation between length of life and the growth rate at 4 months of age as well as with the maximal weight these animals would have attained if growth were solely exponential.

The kidneys of both Group A and Group R rats were analyzed for pathologic lesions. Chronic nephropathy was the most common renal disease. The system for grading chronic nephropathy and the results of the analysis are presented in Table 4. The data from the rats sacrificed for cross-sectional studies indicate that renal lesions start at an earlier age in Group A rats than in Group R rats and clearly show that the rate of progression of these lesions is markedly slowed by food restriction. The data from the rats that died

Table 4. Incidence of Spontaneous Renal Disease.*

Rat Group	Age Range mos.	Sacrificed or Spontaneous Death[a]	No. of Rats Analyzed	Number of Rats With Grade of Chronic Nephropathy[b]					
				0	1	2	3	4	E
A	5½ to 6½	sacrificed	9	4	5	0	0	0	0
A	11½ to 12½	sacrificed	10	0	1	8	1	0	0
A	17½ to 18½	sacrificed	10	0	0	0	9	1	0
A	23½ to 24½	sacrificed	10	0	0	0	2	8	0
A	26½ to 27½	sacrificed	8	0	0	0	2	2	4
A	1½ to 18	spontaneous	5	1	0	0	2	2	0
A	18 to 24	spontaneous	80	0	0	0	3	21	56
A	24 to 30	spontaneous	49	0	0	0	0	11	38
A	30 to 36	spontaneous	1	0	0	0	0	1	0
R	5½ to 6½	sacrificed	10	8	2	0	0	0	0
R	11½ to 12½	sacrificed	10	3	7	0	0	0	0
R	17½ to 18½	sacrificed	10	0	3	7	0	0	0
R	23½ to 24½	sacrificed	10	0	0	10	0	0	0
R	29½ to 30½	sacrificed	9	0	0	8	1	0	0
R	35½ to 36½	sacrificed	10	0	0	2	8	0	0
R	1½ to 18	spontaneous	0	0	0	0	0	0	0
R	18 to 24	spontaneous	3	0	0	1	2	0	0
R	24 to 30	spontaneous	13	0	0	7	6	0	0
R	30 to 36	spontaneous	20	0	0	2	16	2	0
R	36 to 42	spontaneous	16	0	0	0	11	5	0
R	42 to 48	spontaneous	3	0	0	0	0	3	0

*In addition to the data reported in the Table, 4 of Group A rats in the 18 to 30 month age range had acute pyelitis or pyelonephritis, 1 had retention cysts, and hydronephrosis and leukemic infiltration were observed in 1 rat from each Group in this age range.

[a]In the kidneys of many of the rats that died spontaneously autolysis occurred. In less than 10% of the Group A rats and in about 60% of Group R, the extent of autolysis was too great to permit accurate lesion analysis.

[b]Histopathological grading of the chronic nephropathy.

Grade 0: No Lesions

Grade 1: Little or no changes in glomeruli; some thickening of mesangial matrix and glomerular capillary basement membrane (GBM). Swollen and vacuolated cortical tubular cells. Minimal hyperplasia of tubular epithelium. Few hyaline casts in corticomedullary junction. Rare changes in interstitial tissue. All lesions are minimal and focal.

Grade 2: Occasional changes in glomeruli; thickening of mesangial matrix and GBM. Scattered hyaline casts and dilated tubules with atrophic epithelium in the region of corticomedullary junction, but less frequent in cortical and papillary tubules. Pigment in tubular epithelium. Occasional small foci of lymphocytic infiltration in interstitial tissue.

Grade 3: Further thickening of GBM, increased mesangial matrix or the appearance of segmental sclerosis in glomeruli. Mild thickening of Bowman's capsule. Eosinophilic fluid in dilated Bowman's spaces. Some adhesions of glomerular tufts to Bowman's capsule. Prominent hyaline casts in cortical, medullary and papillary dilated tubules. Scattered foci of lymphocytic infiltration with mild interstitial fibrosis.

Grade 4: More prevalent segmental sclerosis or diffuse sclerosis in glomeruli. Thickening of Bowman's capsule with some crescent appearance. Frequent adhesions of glomerular tuft to Bowman's capsule. More prominent dilated tubules with numerous hyaline casts over entire kidney. Atrophy or hyperplasia of tubular epithelium. Lymphocytic infiltration with moderate fibrosis in interstitial tissue. Most lesions are severe and diffuse.

Grade E: End Stage A widespread glomerulosclerosis. Obsolescence of glomeruli. Marked tubular dilation with numerous hyaline casts. Diffuse interstitial fibrosis with abundant lymphocytic infiltration. Frequent calcification. Lack of normal morphological evidence of renal tissue.

spontaneously support these conclusions and further show that the death of most Group A rats is associated with Grade 4 or end-stage renal lesions while such is not the case with Group R rats. Indeed, an end-stage grade lesion was never seen in kidneys from Group R rats.

Data on the lesions in the testes of Group A and Group R rats are presented in Table 5. Interstitial cell tumors were found to occur with increasing age in both Group A and Group R rats but delayed to older age by food restriction. The presence of interstitial cell hyperplasia increased until middle age after which it declined; the peak incidence appears to be at about 18 months of age in Group A rats and 24 months of age in Group R rats. Atrophy of the seminiferous tubules also occurred with increasing age with a similar time course of incidence in both groups. Periarteritis was often found in Group A rats, but was infrequently seen in Group R rats.

The histopathologic analysis of liver is presented in Table 6. Bile duct hyperplasia

Table 5. Incidence of Testicular Lesions.[a]

Rat Group	Age Group (mos)	Sacrificed or Spontaneous Death	No. of Rats Analyzed	Atrophy* of Seminiferous Tubules	Intratubular Calcium Deposits	Periarteritis	Interstitial Cell Hyperplasia[b]	Interstitial Cell Tumors[b]
A	5½ to 6½	sacrificed	9	0	0	0	0	0
A	11½ to 12½	sacrificed	9	1	0	0	4	0
A	17½ to 18½	sacrificed	10	3	3	0	10	7
A	23½ to 24½	sacrificed	10	6	0	1	8	9
A	26½ to 27½	sacrificed	8	6	5	3	4	5
A	1½ to 18	spontaneous	8	0	0	0	4	0
A	18 to 24	spontaneous	84	39	16	24	45	35
A	24 to 30	spontaneous	47	34	22	2	13	30
A	30 to 36	spontaneous	1	1	0	0	0	1
R	5½ to 6½	sacrificed	9	0	0	0	0	0
R	11½ to 12½	sacrificed	10	1	2	0	2	0
R	17½ to 18½	sacrificed	9	4	3	0	5	1
R	23½ to 24½	sacrificed	10	4	2	0	8	6
R	29½ to 30½	sacrificed	9	4	2	0	5	6
R	35½ to 36½	sacrificed	10	10	6	1	1	9
R	1½ to 18	spontaneous	0	0	0	0	0	0
R	18 to 24	spontaneous	5	3	3	0	1	0
R	24 to 30	spontaneous	24	8	3	0	4	9
R	30 to 36	spontaneous	44	35	19	0	3	25
R	36 to 42	spontaneous	44	27	18	1	1	36
R	42 to 48	spontaneous	5	1	0	0	0	3

*In about 70% of the rats from both groups with atrophy of seminiferous tubules, there is also the occurrence of an interstitial cell tumor.

[a]Cysts, mesotheliomas, proliferation, interstitial edema, calcification of the arterial wall are seen only very occasionally and are therefore not included in the table.

[b]It is difficult to distinguish nodular interstitial cell hyperplasia and small interstitial cell tumor histologically. In this study, those nodules with diameters of 1 mm or more and which compressed surrounding seminiferous tubules were considered to be tumors. With age, these tumors increased in size.

Table 6. Incidence of Hepatic Lesions.[a]

Rat Group	Age Range (mos)	Sacrificed or Spontaneous Death	No. of Rats Analyzed	Bile Duct Hyperplasia	Periductal Fibrosis	Bile Duct Dilation	Cystic Spaces	Fatty Change
A	5½ to 6½	sacrificed	9	0	0	0	0	0
A	11½ to 12½	sacrificed	10	3	1	3	0	3
A	17½ to 18½	sacrificed	10	8	3	7	1	5
A	23½ to 24½	sacrificed	9	4	1	5	1	7
A	26½ to 27½	sacrificed	9	6	5	2	2	6
A	1½ to 18	spontaneous	7	0	0	0	0	1
A	18 to 24	spontaneous	54	15	16	2	1	13
A	24 to 30	spontaneous	36	8	6	0	2	11
A	30 to 36	spontaneous	1	0	0	0	0	0
R	5½ to 6½	sacrificed	9	0	0	0	0	2
R	11½ to 12½	sacrificed	10	0	0	0	0	3
R	17½ to 18½	sacrificed	10	1	1	0	0	2
R	23½ to 24½	sacrificed	10	0	0	0	0	3
R	29½ to 30½	sacrificed	9	3	2	1	0	1
R	35½ to 36½	sacrificed	10	5	4	1	1	3
R	1½ to 18	spontaneous	0	0	0	0	0	0
R	18 to 24	spontaneous	3	0	0	0	0	1
R	24 to 30	spontaneous	14	2	0	0	1	3
R	30 to 36	spontaneous	32	2	3	0	1	12
R	36 to 42	spontaneous	26	6	7	0	5	6
R	42 to 48	spontaneous	4	1	1	0	1	1

[a]Lymphocytic infiltration, focal liver cell necrosis, nodular hyperplasia, leukemic infiltration, and hepatic degeneration are seen only very occasionally and are therefore not included in the table.

is a common finding and its incidence and severity increased with age. However, the occurrence of this lesion was delayed until older ages in Group R rats. The occurrence of periductal fibrosis and cystic spaces was also delayed by food restriction. Bile duct dilation was much more common in Group A rats than Group R rats, but there was no obvious difference between the two groups in regard to fatty change.

Cardiac lesions, myocardial fibrosis and myocardial degeneration, were found beginning at about 18 months of age in Group A rats and at about 30 months of age in Group R rats (data not shown). Histopathologic analysis of the lungs (data not shown) revealed little disease and no evidence for the presence of pneumonia due to *Mycoplasma pulmonis* or any other microorganism.

Discussion

The data reported in this paper clearly establish that food restriction is as effective in prolonging life of already long-lived barrier-maintained SPF rats as it has previously been shown to be with rats maintained in conventional facilities (Masoro, 1980). It is also demonstrated that the Fischer 344 male rats respond to food restriction in a fashion similar to that previously observed for other rat strains. Analysis of survival curves as well as age-specific death rates establishes that mortality characteristics of the Group R rats and Group A rats are significantly different (Breslow, 1970) with the curve of the Group R rats being shifted to the right with a less steep slope than the curve for the *ad libitum* fed rats. This finding is in agreement with the conclusion of Sacher (1977) that food restriction prolongs life by decreasing the rate of aging.

It is of interest that the median length of life of the *ad libitum* fed rats in our study was 23½ months while Coleman et al. (1977) reported the median length of life of SPF male Fischer 344 rats maintained at Charles River Laboratories to be 29 months. In a study involving more than 500 male Fischer 344 rats housed in conventional facilities, Chesky and Rockstein (1976) found a median length of life of approximately 22 months. Although the difference between the findings of Chesky and Rockstein and the other two groups may be explained on the basis of conventional maintenance versus barrier maintenance, this explanation cannot be the basis for the difference in median length of life between the rats maintained in our laboratory and those maintained at the Charles River Laboratories.

Some insight as to the reason for this difference in median length of life may be gained by considering the differences in body weight and growth of these two male SPF Fischer 344 rat populations. Baskin et al. (1979) report that the male Fischer 344 rat maintained at the Charles River Laboratories grows until 6 months of age after which the weight plateaus at approximately 400 grams until 24 months of age and then declines at older ages. In contrast, the rats maintained in our laboratory increased in body weight until 20 months of age when their weight was approximately 550 g after which their body weight decreases. Moreover, our rats also have a greater body weight than the rats maintained at Charles River Laboratories at 2 months of age and at 4 months of age (Masoro, 1980). The rat maintenance procedures in our laboratory were identical to those at Charles River Laboratories except for two purposeful changes. At Charles River, more than one rat is housed per cage while because of the nutritional design in our study there was only one rat per cage. At Charles River Laboratories, the rats were fed the Charles River 4RF Rat-Mouse diet which contains 5% fat and a minimum of 26% protein while our diet contains 10% corn oil and 21% protein. One or both of these animal maintenance factors probably cause the differences in growth observed between these two populations.

Since the rat maintenance procedures appear to influence the pattern of growth and the maximum size so markedly, it is not surprising that median length of life is also affected. Indeed, in many studies with rodents, significant correlations have been found between growth rate (Goodrick, 1977) or duration of growth (Ross et al., 1976) or body weight (Ross et al., 1976) and longevity. Moreover, in the present study, a significant negative correlation was found in the Group A rats between the growth rate as well as maximal exponential weight attained and the length of life (Table 2). Since the rats maintained at the Charles River Laboratories have smaller maximum size, it is perhaps not surprising

that they also have a greater median length of life.

Although growth is a determinant of length of life, it is probably not the major factor for the life-prolonging action of food restriction. Stuchlikova et al. (1975) have shown that food restriction started at 12 months of age prolongs the life of male Wistar strain rats and Weindruch et al. (1980) find that food restriction started after mice have reached full growth prolongs life.

Coleman et al. (1977) evaluated the kidneys of 144 SPF male Fischer 344 rats maintained at Charles River Laboratories. All but one of these rats had some degree of renal pathology and that one rat was less than 6 months of age. They reported a highly significant correlation between aging and severity of the renal disease and further found that death was often related to renal disease. The findings with our Group A rats confirm the results of Coleman et al. (1977). In addition, food restriction was found to markedly delay the age-related increase in severity of these renal lesions and possibly the age of onset of initial lesions. It is most striking that the death of most Group A rats was associated with severe renal lesions (Grades 4 or end-stage) while few of the Group R rats showed such lesions at death. In a study of male and female Sprague-Dawley rats, Berg and Simms (1960) reported that the age of onset of renal disease was delayed by moderate food restriction and that the extent of increase in severity of the renal lesions with age was less in the food-restricted rats than in those fed *ad libitum*. Similar findings with male Sprague-Dawley rats were reported by Bras and Ross (1964) and by Saxton and Kimball (1941) with male Osborne-Mendel rats. Thus, food restriction influences the age-related development of renal disease in a similar fashion in all rat strains studied in this regard.

Although Coleman et al. (1977) found that male Fischer 344 rats have a relatively low incidence of malignant tumors, they did find that testicular Leydig cell tumors commonly occur in an age-related fashion with a 100% incidence by 30 months of age. Our results with Group A rats are similar and also show that food restriction delays the age of onset of this tumor. Other types of tumors have also been shown to be prevented or time of occurrence delayed by food restriction but not all tumors are so influenced (Ross, 1976); indeed, the occurrence of certain types of tumors is increased by food restriction (Ross, 1976). In contrast to the findings with Leydig cell tumors, the course of seminiferous tubular atrophy was similar in Group A and Group R rats making it unlikely that these two histopathologic changes are related.

Coleman et al. (1977) reported that bile duct hyperplasia was common in the Fischer 344 rats they studied and that it increased in incidence and severity with age. Since this lesion had not previously been described, they suggested that an environmental influence in their study was responsible for it. The Group A rats in our study showed a similar pattern of occurrence of bile duct hyperplasia and the occurrence of this lesion was delayed to older ages in Group R rats. Clearly, this is a nutritionally modulated lesion.

The major cardiac lesions were also delayed by food restriction. The general conclusion to be drawn from our study and previous studies is that most but not all age-related pathologic processes are delayed by food restriction.

Lesser and his colleagues (1973, 1980) reported the lean body mass remains stable in male Sprague-Dawley rats into senescence. This was a most unexpected finding since the concept that a loss in lean body mass is a basic characteristic of aging is generally accepted (Shock, 1955). However, our findings on lean body mass of the Fischer 344 male rats also show that there is not a progressive fall in lean body mass during adult life in either the Group A or Group R rats, but rather lean body mass increased until about 75% of the life span and remained unchanged for most of the remainder of the life of the rat. Lesser et al. (1973) suggest that the fall in lean body mass that occurs just before death is the result of terminal disease; our findings are in agreement with this view. It is of interest that total fat mass markedly declines in Group A and Group R rats after 70% of the life span (Bertrand, 1980a).

One of the reasons for the belief that lean body mass declines with age in rats and other mammals is the evidence for a decline in muscle mass with age (Gutmann & Hanzlikova, 1976). In rats, a loss of mass in individual muscles with age has been clearly demonstrated (Tauchi et al., 1971). Indeed, in both

Group A and Group R rats, a loss in the mass of the gastrocnemius muscle occurred with advancing age. However, most of the individual muscles which showed a loss of mass with age are hindleg muscles such as the gastrocnemius muscle. In the case of the Group A and Group R rats of our study, the mass of lateral omohyoideus muscle did not change with age (McCarter et al., 1979) and Lesser et al. (1980) report that the mass of the psoas muscle and the total muscle mass of male Sprague-Dawley rats do not change with age. Thus, the age-related loss in mass of individual muscles such as the gastrocnemius muscle must be compensated for either by an increase in the mass of other muscles as implied by the findings of Lesser et al. (1980) or by an increase in the mass of other organs such as viscera or by an increase in extracellular fluid volume or by a combination of these.

It should be noted that although there was an age-related loss in gastrocnemius muscle mass with age in both the Group A and Group R rats, this loss occurred at a much later age in the Group R rats than in the Group A rats. This is another example of food restriction delaying age-related changes in structure and function.

The changes in mass of the gastrocnemius muscle with age were not due to changes in hydration of the muscle since there was no change in the dry weight:wet weight ratio of the muscle with age. However, there was a progressive decline with age in the amount of protein per unit dry weight of gastrocnemius muscle. Mohan and Radha (1975) found a decrease in total protein per unit wet weight in red and white skeletal muscle with increasing age in Wistar strain rats, but Lesser et al. (1980) reported no change with age in the protein content of the psoas muscle or of the total muscle mass with age in Sprague-Dawley rats. A decrement of protein content per unit mass of muscle may only occur in those muscles that lose mass with age.

The collagen content of many organs (Deyl et al., 1971) including rat skeletal muscle (Mohan & Radha, 1980) is known to increase with increasing age. This was also found to be the case for the gastrocnemius muscle of the Fischer 344 rats; to make certain that varying amounts of tendon would not influence the results, muscle samples totally free of tendon were analyzed. Deyl et al. (1971) found that food restriction delayed the age-related increase in the collagen content of lungs, liver and kidneys; in our study, food restriction was found to also decrease the rate of age-related increase in collagen content of the gastrocnemius muscle.

Hrachovec and Rockstein (1962) reported that in male rats of the CFN and the Sprague-Dawley strains the cholesterol concentration in their hindlimb muscles progressively increased with increasing age while the phospholipid concentration progressively decreased. In contrast, in our study with Fischer 344 rats, there was no progressive change in the gastrocnemius cholesterol or phospholipid with age in either Group A or Group R rats. The reasons for this discrepancy are unknown. One possible reason is that our rats were fed a semisynthetic diet essentially free of cholesterol while Hrachovec and Rockstein fed a commercial, pelleted rat food which may have contained appreciable amounts of cholesterol leading to a progressive replacement of phospholipid by cholesterol in the muscle membranes.

In this paper, data on longevity, pathologic lesions, growth and lean body mass are presented for a population of male SPF Fischer 344 rats fed *ad libitum* or 60% of the *ad libitum* intake that had been used in a broad spectrum physiologic and biochemical research. The general conclusion from the total study is that the life-prolonging effect of food restriction is associated with a marked delay in the occurrence of age-related diseases and of age-related changes in physiological functions — in some instances, it even appeared that their occurrence was prevented. Since loss of physiological capacity and disease undermine the ability of animals to cope with commonly occurring challenges, it is logical to conclude that the modulation of these events by food restriction is causally related to the life-prolonging action of this nutritional manipulation. Even though age-related disease and physiological deterioration play an important role in longevity, they probably are reflections of rather than the basic aging process or processes modulated by food restriction. The nature of these processes remains to be defined. Although food restriction appears to have promise as an experimental tool for learning about the basic nature of aging in mammals, its use in this context remains to be exploited.

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