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A group of five parameters as a new biological marker on F344/N rats

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Abstract

The National Institute for Longevity Sciences (NILS) established an aging farm (A/F) for producing aging/aged laboratory rodents at the Experimental Animal Facility Wing under the NILS A/F Guide planned by the Laboratory Animal Research Facilities (LARF). Five parameters, the average life span, the number of days of 75, 50, and 25% survival points, and average of the top 10 longest life span among laboratory strains of rodents at NILS-A/F, were reproducible for F344/N rats specifically by strain and sex under the LARF A/F guide. These five parameters may serve as an effective and practical biological marker, especially in aging science including longevity science, to evaluate characteristics of strains of laboratory rodents. The five parameters can identify clear substrain differences between F344/N and F344/Du and breeder differences between F344/DuCrj and F344/DuCrl. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Biological marker; F344/Du; F344/N; Survivability

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1. Introduction

NILS started an aging farm (A/F) project to produce aging/aged laboratory rodents F344/N rats and C57BL/6 mice in June 1996 (Tanaka et al., 2000b). The NILS A/F was established according to the NILS A/F Guide prepared by LARF, and an internal supply of aging/aged laboratory rodents began for research of both aging and longevity science in May 1999.

In the process of the establishment of NILS A/F, an interesting peculiarity in the shape of survival curves and a similarity between average life span and 50% survival were found specifically by strain and sex (Tanaka et al., 2000b). Additionally, individual days of 75, 50 and 25% survival seemed to be reproducible according to the strain and sex.

The current study was conducted to confirm whether this similarity was reproducible with more extensive data and to compare the five parameters consisting of average life span, the number of days of 75, 50, and 25% survival points, and the average of the top 10 longest life span under NILS A/F with comparable data provided in the external literature.

2. Materials and methods

Four-week-old colonies of F344/NSlc rats were purchased from SLC Co. Ltd., Hamamatsu, Japan. New colonies of the same age and strain were added every month.

Animals were raised in accordance with the NILS A/F Guide with a focus on a decrease in the number of individual cohorts (Tanaka et al., 2000b).

3. Results

This report deals with only the survival of F344/N rats (Table 1, Figs. 1 and 2). The first purpose is to evaluate the efficiency of the NILS Aging Farm Guide for six groups of animals that are divided by the date of birth (Group 1: June 25, 1996; Group 2: July 23, 1996; Group 3: October 1, 1996; Group 4: November 26, 1996; Group 5: December 31, 1996; and Group 6: January 28, 1997). The second purpose is to confirm the reproducibility of the five parameters. When they are reproducible, they can serve as a new biological marker to monitor the aging process of aging/aged laboratory rodents at A/F.

Table 1 summarizes a comparison as of April 1, 2000 in terms of the average life span, 75, 50 and 25% survival points, and average number of days of the top 10 longest life span.

3.1. Average life span

For Group 1 males ($n = 80$), the first death appeared on the 467th day of age,

Table 1
Survival of F344/N rats in the NILS aging farm[a]

(Date of Birth)	Sex	Number of animals	Range (days)	Average (days)	75% survival		50% survival		25% survival		Average of the longest live decade
					Months of age	Days of age	Months of age	Days of age	Months of age	Days of age	
(1996/6/25)	Male	80	467–992	754.6 ± 121.4[b]	21.97	668	24.63	749	28.28	860	936.9 ± 25.3
(1996/7/23)	Male	55	483–942	742.4 ± 118.9	21.64	658	24.27	738	27.72	843	909.8 ± 22.7
(1996/10/1)	Male	109	462–1024	764.5 ± 128.4	22.30	678	24.73	752	27.95	850	985.5 ± 19.6
(1996/11/26)	Male	55	583–968	759.7 ± 127.2	21.54	655	25.49	775	28.28	860	920.0 ± 28.6
(1996/12/31)	Male	52	532–1027	760.4 ± 123.3	21.24	646	24.37	741	28.28	860	935.3 ± 45.3
(1997/1/28)	Male	55	497–1010	738.9 ± 115.1	21.60	657	23.94	728	27.39	833	907.6 ± 43.7
(1996/6/25)	Female	80	585–1201	873.6 ± 136.3	25.29	769	28.35	862	32.19	979	1100.8 ± 45.0
(1996/7/23)	Female	55	553–1112	835.6 ± 144.9	24.43	743	28.02	852	30.85	938	1036.0 ± 69.3
(1996/10/1)	Female	109	441–1133	845.7 ± 146.3	24.43	743	28.64	871	31.24	950	1065.5 ± 41.3
(1996/11/26)	Female	55	574–1185	880.0 ± 139.4	26.31	800	28.58	869	32.32	983	1078.6 ± 45.3
(1996/12/31)	Female	50	637–1173	877.6 ± 121.6	25.32	770	29.00	882	31.93	971	1034.4 ± 56.1
(1997/1/28)	Female	55	525–1144	840.5 ± 161.3	23.28	708	27.85	847	30.25	920	1084.9 ± 49.6

[a] As of April 1, 2000.
[b] Mean ± S.D.

Table 2
Maximum difference in survival months and days of F344/N rats among six groups in the NILS aging farm[a]

Average survival		75% survival		50% survival		25% survival		Average of the longest live decade	
Sex	Days	Months of age	Days of age	Months of age	Days of age	Months of age	Days of age	Months	Days
Male	25.6	1.05	32	1.55	47	0.89	27	2.56	77.9
Female	44.4	3.03	92	1.15	35	2.07	63	2.16	65.6

[a] As of April 1, 2000.

and the last death on the 992nd day of age. For females in the same group ($n = 80$), the first death occurred on the 585th day of age, and a few rats were still alive beyond 1155 days of age. The average life span of Group 1 males was 754.6 ± 121.4 (mean $\pm$ S.D.) days, while that of Group 1 females was 873.6 ± 136.3 days.

For Group 2 males ($n = 55$), the first death appeared on the 483rd day of age, and the last death on the 942nd day of age. For females in the same group ($n = 55$), the first death occurred on the 553rd day of age, and the last death on the 1112th day of age. The average life span of Group 2 males was 742.4 ± 118.9 days, while that of Group 2 females was 835.6 ± 144.9 days.

For Group 3 males ($n = 109$), the first death appeared on the 462nd day of age, and the last death on the 1024th day of age. For females in the same group ($n = 109$), the first death occurred on 441st day of age, and the last death on the 1133rd day of age. The average life span of Group 3 males was 764.5 ± 128.4 days, while that of Group 3 females was 845.7 ± 146.3 days.

For Group 4 males ($n = 55$), the first death appeared on the 583rd day of age, and the last death on the 968th day of age. For females in the same group ($n = 55$), the first death occurred on 574th day of age, and the last death on the 1185th day of age. The average life span of Group 4 males was 759.7 ± 127.2 days, while that of Group 4 females was 880.0 ± 139.4 days.

For Group 5 males ($n = 52$), the first death appeared on the 532nd day of age, and the last death on the 1027th day of age. For females in the same group

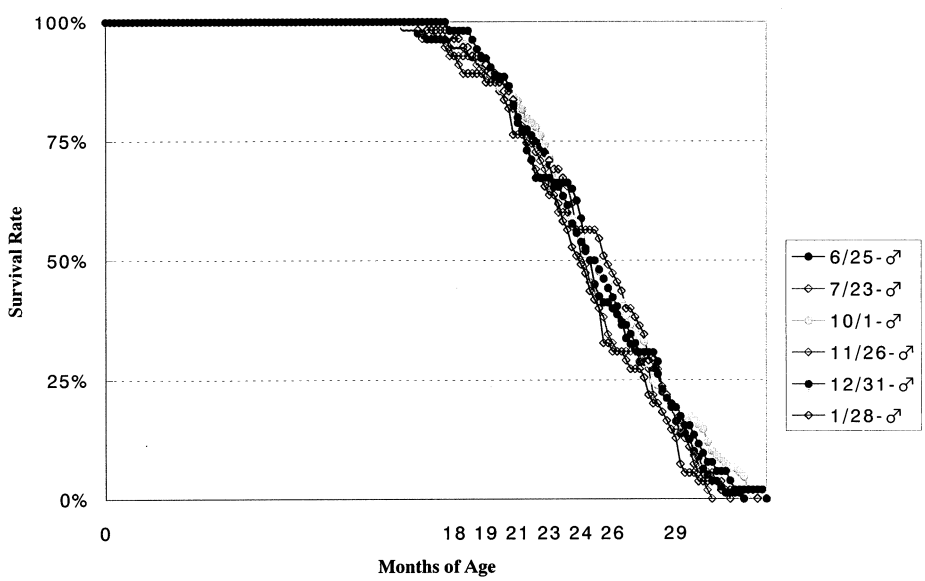

Fig. 1. Survival of male F344/N at NILS aging farm.

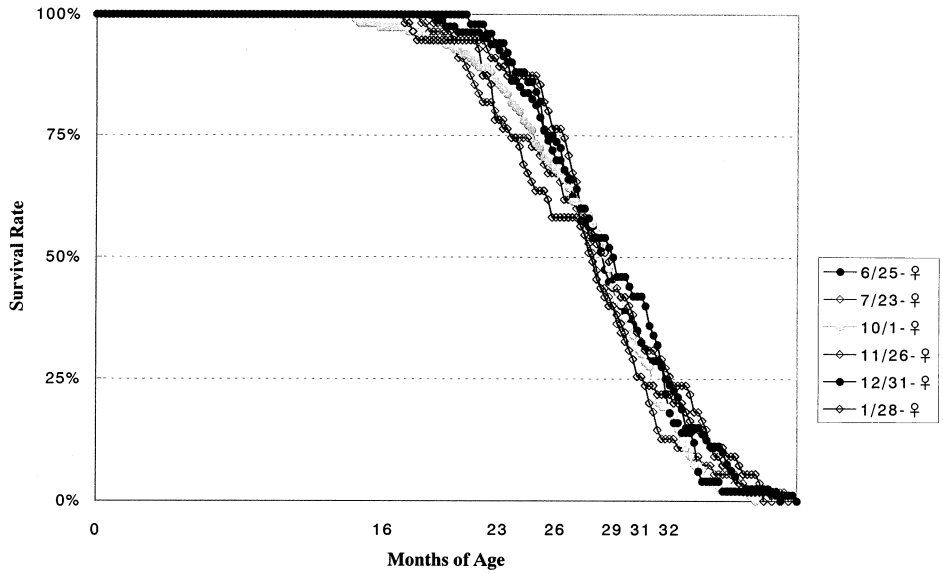

Fig. 2. Survival of female F344/N at NILS aging farm.

($n = 50$), the first death occurred on 637th day of age, and the last death on the 1173rd day of age. The average life span of Group 5 males was 760.4 ± 123.3 days, while that of Group 5 females was 877.6 ± 121.6 days.

For Group 6 males ($n = 55$), the first death appeared on the 497th day of age, and the last death on the 1010th day of age. For females in the same group ($n = 55$), the first death occurred on the 525th day of age, and the last death on the 1144th day of age. The average life span of Group 6 males was 738.9 ± 128.4 days, while that of Group 6 females was 840.5 ± 161.3 days.

The maximum difference among six groups was 25.6 days, less than 1 month in males and 44.4 days, about 1.5 months, in females (Table 2).

3.2. 75% survival point

In Group 1, the 75% survival point for male was marked on the 668th day of age, and that for female, the 769th day. In Group 2, 3, 4, 5 and 6, the 75% survival point for males was the 658th, 678th, 655th, 646th and 657th day, and that for females, the 743rd, 743rd, 800th, 770th and 708th day, respectively.

The maximum difference among six groups was 32 days, about 1 month, in males and 92 days, about 3 months, in females. This 3 month difference was the largest under the current comparison (Table 2).

3.3. 50% survival point

In Group 1, the 50% survival point for male was attained on the 749th days of age, and that for female, the 862nd day. In Group 2, 3, 4, 5 and 6, the 50% survival point for males was the 738th, 752nd, 775th, 741st and 728th day, and that of females, the 852nd, 871st, 869th, 882nd and 847th day, respectively.

The maximum difference among six groups was 47 days, about 1.5 months, in males and 35 days, about 1 month, in females (Table 2).

3.4. 25% survival point

In Group 1, the 25% survival point for males was attained on the 860th day of age, and that for females, the 979th day. In Group 2, 3, 4, 5 and 6, the 25% survival point for males was the 843rd, 850th, 860th, 860th and 833rd day, and females, the 938th, 950th, 983rd, 971st and 920th day, respectively.

The maximum difference among six groups was 27 days, less than 1 month, in males and 63 days, about 2 months, in females (Table 2).

3.5. Average days of the top 10 longest life span

In Group 1, the average days of the top 10 longest life span of the male animals were 936.9 ± 25.3 days, and those of the females were 1100.8 ± 45.0 days. In Group 2, 3, 4, 5 and 6, the average days of the longest male decade were the 909.8 ± 22.7, 985.5 ± 19.6, 920.0 ± 28.6, 935.3 ± 45.3 and 907.6 ± 43.7 days, respectively, and those of the females were 1036.0 ± 69.3, 1065.5 ± 41.3, 1078.6 ± 45.3, 1034.4 ± 56.1 and 1084.9 ± 49.6 days, respectively.

The maximum difference among six groups was 77.9 days, about 2.5 months, in males and 65.6 days, about 2 months, in females (Table 2).

3.6. Differences in each parameter among six groups of rats (Table 2)

The largest difference in the average life span was 25.6 days (less than 1 month) for the male groups and 44.4 days (1.5 months) for female groups, and that in 75% survival was 32 days (1.05 months) and 92 days (3.03 months). It was 47 days (1.55 months) and 35 days (1.15 months) in 50% survival, 27 days (0.89 month) and 63 days (2.07 months) in 25% survival, and 77.9 days (2.56 months) and 65.6 days (2.16 months) in the average of the top 10 longest life span for male and female groups, respectively.

3.7. Comparison of average life span (Table 3)

According to Sprott and Austad (1995), the median life span of F344 rats, fed an NIH31 diet ad libitum at the National Institute on Aging (NIA), Baltimore, was 103 weeks (721 days) in males and 116 weeks (812 days) in females. Their maximum life span was 124 weeks (868 days) for males and 148 weeks (1036 days) for females.

Table 3
Comparison of average life span of rats reported as "F344"

Insitute	Male average (months)	Female average (months)	Citation of strain	Breeder	Reference	Judged	Remarks
Amrican Medical[a]	23.84	22.19	Fischer from Dunning		Jacobs and Huseby, 1967	F344/Du	Not barriered
Harvard[b]		23.9	Fischer from Dunning	CRL	Davey and Moloney, 1970	F344/Du	Blood sampling
Harvard[b]		25	Fischer	CRL	Moloney et al., 1970	F344/Du	Blood sampling
?	31	29	F344	?	Festing, 1979	Not identified	Miss citation?
San Antonio[c]	23.05		F344	CRL	Yu et al., 1982	F344/Du	ad lib
San Antonio[c]	32.42		F344	CRL	Yu et al., 1982	F344/Du	DR
TMIG[d]	28.18	29.46	F344/DuCrj	CRJ	TMIG Data, 1985	F344/Du	
San Antonio[c]	24.00		F344	CRL	Iwasaki et al., 1988	F344/Du	ad lib
San Antonio[c]	25.32		F344	CRL	Shimokawa et al., 1993	F344/Du	ad lib
NIA[e]	23.70	26.70	F344	HSD	Sprott and Austad, 1995	F344/N	
Toho University	29		F344	CRJ	Tanaka et al., 2000a	F344/Du	
NILS	24.77[f]	28.24[f]	F344/N	SLC	Tanaka et al., 2000a,b	F344/N	

[a] American Medical Center.
[b] Children's Cancer Research Foundation and Harvard Medical School.
[c] University of Texas Health Science Center.
[d] Tokyo Metropolitan Institute for Gerontology.
[e] National Institute on Aging.
[f] Average of six groups in Table 1.

Festing (1979) summarized various biological characteristics of laboratory strains, including F344 rats, and cited many biological characteristics as well as the survivability on this strain of rats. For example, the median life span of F344 was about 31 months in males and 29 months in females (the cited literature and its author were not identified), 675 days in females and 725 days in males (Jacobs and Huseby, 1967) and 25 months in females (Moloney et al., 1970).

Yu et al. (1982), Iwasaki et al. (1988) and Shimokawa et al. (1993) reported in detail the survival data on male F344 fed ad libitum at the University of Texas Health Science Center at San Antonio, 701 days (23.05 months), 730 days (24.00 months) and 110 weeks (25.32 months), respectively.

According to the data book of the Tokyo Metropolitan Institute for Gerontology (TMIG), the median life span of male F344/DuCrj rats was 762 days (28.18 months). In the presentation by the Toho University at the 22nd annual meeting of Japan Society for Biomedical Gerontology, it was 29 months (Tanaka et al., 2000a).

There are two major substrains in F344 rats, which are sublines at NIH (National Institutes of Health, Bethesda) and Dunning (Festing, 1979; Tanaka et al., 2000a). These substrains can be identified to be either F344/N or F344/Du from individual history or breeders purchased from. Table 3 shows the breakdown of F344 substrains. Additionally, F344/Du available from Charles River Japan (CRJ) in Japan can be subdivided as F344/DuCrj, and from Charles River Laboratories (CRL) in the USA as F344/DuCrl.

3.8. Identification of F344 substrains (Table 3)

In terms of the substrain, F344 rats used at NIA by Sprott and Austad (1995) were identified from the description to be F344/N. Substrains by Jacobs and Huseby (1967), Davey and Moloney (1970), and Moloney et al. (1970) might be F344/Du and by San Antonio (1982, 1988, 1993) to be F344/DuCrl, and by Toho F344/DuCrj (Tanaka et al., 2000a).

4. Discussion

4.1. Sex difference in survivability

A clear sex difference was noticeable in the survivability of F344/N (Table 1). When compared in terms of the five parameters, females survived longer than males. Miyaishi et al. (2000) pointed out a higher and earlier incidence of leukemia in males than females. The earlier and more severe incidence of leukemia in males may explain in part their shorter survivability.

4.2. Reproducibility

Survival curves of the six F344/N groups closely overlapped each other within the same sex (Figs. 1 and 2, Tanaka et al., 2000b, unpublished observation). Since

the five parameters showed similar patterns within the same sex, the maximum difference in each parameter per sex was very small. Individual differences observed in each parameter were less than 100 days (3.28 months). Although survivability is known to be modified by certain factors associated with housing conditions, including diet, microbiological protection and others, the five parameters cited in this report are reproducible. These reproducible five parameters may serve as a new biological marker for laboratory rodents in aging and longevity science.

Thus, if there is a difference of more than 100 days in survivability, it may imply the presence of factors that can modify a survivability value specific to the housing conditions or the type of inbred strain of rats. The inbred strain in this case, F344/N, was established under tight genetic regulations to contribute to the constant reproduction of phenotypes. When a certain inbred strain of rats was different from F344/N on the survivability by more than 100 days, we can deduce the presence of modifying factors as consisting of not only the housing conditions but also its genetic background.

The comparison by mean and maximal life span alone cannot detect changes correctly in survival curves. The same mean life span does not always mean the same 75% and 25% survival. A longer 75% survival point does not necessarily result in a longer 25% survival point. These characteristics are specifically reproducible among inbred strains of mice (unpublished observation). This also supports an association of genetic backgrounds with survivability mentioned above.

4.3. Differences in survivability among F344 substrains (Tables 3 and 4)

While the five parameters were so reproducible for F344/N that they may serve as a biological parameter at least for NILS, they had been initially assumed to show different results among other institutes. When compared in terms of the average life span in months, the survivability of F344/N was nearly the same between NILS (24.57 months: 24.29–25.13) and NIA (23.70 months) but clearly contrasted with F344/DuCrj at Toho University and TMIG. The average life span of male F344/DuCrj was 29 months of age in Toho University and 28.18 in TMIG. It was also nearly the same at Health Science Center, San Antonio, as shown in 23.05, 24.00 and 25.32 months for F344/DuCrl and at American Medical Center as 23.84 months for F344/Du (Table 3) in the USA.

Table 4
Relationship among "F344" substrains deduced from the average life span

Japan	F344/DuCrj	$\neq$ [a]	F344/NSlc
	$\neq$ [b]		$\fallingdotseq$ [c]
USA	F344/DuCrl	$\fallingdotseq$ [d]	F344/NHsd[e]

[a] Based on the comparison between TMIG and NILS.
[b] TMIG and San Antonio.
[c] NIA and NILS.
[d] San Antonio and NILS.
[e] Harlan Sprague Dawley.

The current proposal that the survivability reproducible within 100 days (2.18 months) may serve as a new marker seems to be generalized for substrains specifically.

The values of average life span from Toho University and TMIG were larger than those of NILS and NIA by more than 100 days (2.18 months). This implies the presence of certain substrain differences, detectable through survivability, between F344/Du at Toho University and TMIG, and F344/N at NILS and NIA (Table 4). These substrains should be compared in detail, especially for their genomes.

Similar differences detected through average life span were found between F344/DuCrj by 29 or 28.18 months in Japan and F344/DuCrl by 23.05, 24.00 and 25.32 months in San Antonio. Although F344/DuCrj stemmed from F344/DuCrl (Tanaka et al., 2000a), F344/DuCrj survived for longer time than F344/DuCrl, by about 4 months. This difference due to breeders, CRL and CRJ, suggests the presence of a certain genetic difference between these sibling substrains (Table 4). These sibling substrains should also be compared in detail.

Interestingly, F344/DuCrl could survive with a lower incidence of leukemia for 32.42 months only under dietary restrictions (DR in Table 3, Yu et al., 1982; Iwasaki et al., 1988; Shimokawa et al., 1993). The biological characteristics of F344 rats, its substrains, F344/Du and F344/N, and its sibling substrains within F344/Du, F344/DuCrl and F344/DuCrj, detected through the average life span and DR seem to be considerably different from each other. While other elements of the difference detectable through the five parameters, except for the incidence of leukemia (Festing, 1979; Miyaishi et al., 2000), are unclear, biologists should pay attention to the type of F344 substrains and to its breeder.

Similar to F344/N rats, the survivability of C57BL/6 mice was also reproducible (Tanaka et al., 2000b, unpublished observation). This suggests that the survivability of inbred strains is basically reproducible. This may be partly due to the high incidence of leukemia characterizing F344/N rats and lymphoma characterizing C57BL/6 mice.

In 1998, CRL started to sell both substrains of F344 rats, F344/Du and F344/N, with catalogue names, "Fischer Rats: CDF (F-344)/CrlBR (CDF: Caesarian Section Derived Fischer, BR: Barrier Reared)" and "F344 Rats: F344/CrCrlBR (Cr: Cancer Research Institute)", respectively. This means that the substrains cannot be identified from breeders alone. Users should cite the name of a strain and the name and location of a breeder.

By paying due attention to differences associated with substrains, including the five parameters and leukemia incidence, researchers can improve the reliability of their data on F344/N or F344/Du rats, especially, for aging and longevity science.

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