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Body weight is not always a good predictor of longevity in mice

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Abstract

There have been some observations that low body weight and a low level of some hormones (e.g. IGF-1) during the first half of life are predictors of longer life in mice. However, contradictions in the available data on the biomarkers of aging and predictors of longevity have shown that the research in these fields has become a controversial pursuit. In our study we addressed the following questions: (i) Can particular physiological parameters (body weight, food intake, estrus function, body temperature, incidence of chromosome aberrations in bone marrow cells) measured at the age of 3 and 12 months be a predictor of longevity and the rate of tumor development in five strains of mice? (ii) Can a heavy body weight at the age of 3 and 12 months be a predictor of longevity and high tumor risk in five strains of mice? Mice of five strains—CBA, SHR, SAMR, SAMP and transgenic HER-2/neu (FVB/N)—were under observation from the age of 2–3 months until natural death. Body weight and temperature, food consumption, and estrous cycle were longitudinally studied in all animals. Tumors discovered at autopsy were studied morphologically. We calculated the life span's parameters (mean, maximum, mortality rate, mortality rate doubling time) as well as their correlation with other parameters studied. The longest living CBA mice have the lowest body weight at the ages of 3 and 12 months, the lowest food consumption, body temperature, incidence of chromosome aberrations and spontaneous tumor incidence. In comparison with all other mouse strains they also have the latest disturbances in estrus function and highest body weight gain. The shortest living transgenic HER-2/neu mice have the lowest weight at the ages of 12 months, the lowest body weight gain, maximal body temperature, the most rapid disturbances in estrus function and the highest incidence of chromosome aberrations and tumor incidence in comparison to all other mouse strains. Our findings have shown that heavier body weight at the age of 12 months is a predictor of longevity in female CBA and SAMP mice but not in SHR, SAMR and HER-2/neu mice. Excessive body weight at the ages of 3 or 12 months is not a predictor of increased tumor risk in the strains studied. In general, the existence and direction of a significant correlation between body weight and life span depends upon the animals' age and genotype.

Keywords: Life span; Longevity; Spontaneous tumors; Biomarkers; Predictors; Mice

1. Introduction

A number of works provide examples of physiological traits, measurable in the first half of the life span, that can provide significant prognostic information about life expectancy in animals (Harper et al., 2003; Ingram et al., 2001; Miller et al., 2002, 2003; Piantanelli et al., 2001). In contrast to the observed positive correlation between body weight (BW) and life span (LS) across mammalian species (Economos, 1980b), within species higher relative BW is thought to be associated with decreased LS. Epidemiological observations provide evidence on increased mortality associated with excessive BW and obesity (Lew and Garfinkel, 1979; Stunkard, 1983; Lee et al., 2001). There are several reports where differences in body size among individuals within a species are associated with differences in longevity. In each case, superior longevity is associated with smaller body size. Within a species, the differences in body size depend on differences in single genetic loci, as in the df/df Ames dwarf mice (Brown-Borg et al., 1996; Bartke et al., 2003) and the urokinase knockout α-MUPA mice (Miskin and Masos, 1997). Calorie restriction (30–40%) reduces BW and substantially increases mean and maximum

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life span in research populations or cohort of rodents (Weindruch and Walford, 1988). In monkeys, calorie restriction reduces body size and slows down age-related changes in numerous parameters (Roth et al., 1999a,b, 2002). Miller et al. (2000) examined longevity in a series of 15 mouse stocks that were selected over 22 generations for their different rate of body weight gain and found that the BWs at 3, 6 and 12 months are significant predictors of longevity (among stocks). Miller et al. (2002) have shown that low body weight at a young age (two months) is a predictor of longer life span in mice. In this paper, we report data on the predictor efficacy of some physiological parameters in mice of various strains, focusing on the BWs at young and middle age as predictors of longevity.

2. Material and methods

2.1. Animals

Female CBA and outbred Swiss-derived SHR mice were purchased from the 'Rappolovo' Animal Farm of the Russian Academy of Medical Sciences. Colonies of senescence accelerated mice SAMP (prone) and SAMR (resistant) mice were established from several pairs of breeders of 105th generation SAMP-1 and 96th generation SAMR-1 mice obtained from the Department of Embryology, Moscow State University and bred at the Department of Carcinogenesis and Oncogerontology, N.N. Petrov Research Institute of Oncology. For this report, we observed animals of the 110–111th generations of SAMP-1 and the 101st–102nd generations of SAMR-1. Female homozygous HER-2/neu transgenic FVN/B mice were obtained from Charles River (Hollister, CA) by the Italian National Research Center for Aging and were housed and bred at the Department of Carcinogenesis and Oncogerontology, N.N. Petrov Research Institute of Oncology.

2.2. Longevity study

The mice were kept in polypropylene cages ($30 \times 21 \times 9$ cm^3), five mice in a cage at a temperature of 22 ± 2 °C. A regimen of 12 h of light and 12 h of dark was followed. The animals received sterilized standard laboratory chow (Anisimov et al., 2003) and tap water ad libitum. The animals were checked daily by animal care personnel and weekly by a veterinarian. The study was carried out in accordance with the regulations for ensuring the humane treatment of animals under the approval of the Committee on Animal Research of the N.N. Petrov Research Institute of Oncology.

Weights were recorded to the nearest gram at monthly intervals from 2 or 3 months of age until the death of the animals. Once every 3 months vaginal smears were taken daily for 2 weeks from the female animals and cytologically examined to estimate the phases of their estrus functions. In the same period, rectal body temperatures of the mice were measured with an electronic thermometer, TPEM (KMIZ, Russia). We observed the animals right up to their natural death and then registered the date of each death. We could then calculate the mean life span, the age by which 90% of the animals died, and the maximal life span.

2.3. Cytogenetic study

Chromosomal aberrations in bone marrow cells were studied using the modified Ford's method described in Rosenfeld et al. (2001). Mice were killed under ether anaesthesia. Both femurs from each mouse were dissected and the bone marrow cells flushed gently with 0.56% KCl solution into a centrifuge tube. The cells were treated for 20 min with hypotonic solution and fixed with ethanol: acetic acid mixture (3:1). Slides were stained with 4% acetoorseine and 20–30 well-spread anaphases were analyzed for each animal. Cells with chromosome breaks, acentric fragments, and other aberrations were then evaluated at $1000\times$ magnification with a light microscope (Leitz, Germany).

2.4. Pathomorphological examination

We autopsied every animal that died or that was killed when moribund, examining their skin and internal organs. Neoplasia were classified according to the recommendations of the International Agency of Research on Cancer (IARC) as 'fatal' (i.e. those directly causing the death of the animal) or 'incidental' (for cases where the animal died of a different cause) (Gart et al., 1986). All tumors, as well as tissues and organs with suspected tumors, were excised and fixed in 10% neutral formalin. After routine histological processing, the tissues were embedded in paraffin. Thin, 5–7 μm histological sections were then stained with hematoxylin-eosine and microscopically examined. The experimental group to which the mouse belonged was blinded. We classified tumors according to IARC recommendations (Turusov and Mohr, 1994).

2.5. Statistics

The experimental results were statistically processed using variation statistics methodology (Goubler, 1978). We defined the significance of discrepancies according to Student's t-criterion, Fischer's exact method, χ^2-analysis, and the non-parametric criterion of Wilcoxon–Mann–Whitney (Goubler, 1978; Weisberg, 1980). Spearman-rank correlation coefficients and multivariate regression were calculated according Goubler (1978). In order to calculate the discrepancies in neoplasm incidence, an IARC method of combined contingency tables was calculated individually for the fatal and incidental tumors (Gart et al., 1986). We used Cox's method (Cox and Oakes, 1996) for survival

analysis. All the reported test values used in survival analyses of data are two-sided.

2.6. Mathematical models and estimations

The mathematical model used to describe survival is the Gompertz model with the survival function

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}[\exp(\alpha x) - 1]\right\}$$

where parameters α and β are associated with the population rate of aging and initial mortality rate, respectively (Anisimov et al., 2001). Parameter α is often characterized by the value of mortality rate doubling time (MRDT), calculated as $\ln(2)/\alpha$. Parameters for the model were estimated from data using the maximum likelihood method implemented in the Gauss statistical system (Gauss System and Graphic Manual, 1994). We calculated confidence intervals for the aging rate parameter estimates using log-likelihood functions (Cox and Oakes, 1996).

3. Results

3.1. Strain differences in parameters of life span, biomarkers of aging and tumor incidence in mice

There was a significant variability between the different mouse strains under observation with regard to the life span parameters as well as the parameters of body weight, food consumption, estrus function, body temperature and spontaneous tumorigenesis (Table 1). From all the strains studied, the mean life span was the longest in female CBA and the shortest in female HER-2/neu mice: the difference in the mean life span between these two strains was 57%. A ranking according to mean life span would be: CBA > SAMP > SAMR > SHR > HER-2/neu. However, the mean life span of the last 10% survivors and the maximal life span were similar among all strains except for HER-2/neu, for which these parameters are lower than for the other strains. Fig. 1 clearly shows the differences in survival patterns of these mouse strains.

The rate of population aging (evaluated as α in the Gompertz equation) could be ranked as follows: HER-2/neu > CBA > SAMP > SAMR > SHR.

The mean body weight at the age of 3 months was maximal in SHR mice (26.5 g) and minimal (21.4 g) in CBA mice, a difference of 28.5% ($p < 0.05$) However, at the age of 12 months, the difference between these two strains was only 16.8%. At the same time, the CBA mice had the maximum mean body weight at this age (35.7 g) and HER-2/neu mice the minimum (25.0 g) (-30%, $p < 0.05$). The body weight gain (from the third to the 12th month of life) was maximal in female CBA mice (91%) and minimal in HER-2/neu mice (4.6%). According to the mean body weight at this age, the strains could be ranked as follows: SHR > SAMP > SAMR > HER-2/neu > CBA. The ranking according to body weight gain would be CBA > SAMP > SAMR > SHR > HER-2/neu. It is worth noting that a ranking according to mean body weight parameters in the last 10% survivors would produce almost the same order (Table 1).

Food consumption at the age of 3 months was the highest in HER-2/neu mice (5.1 g) and the lowest in CBA mice (2.3 g) (-55%, $p < 0.05$). The ranking according to this parameter is: HER-2/neu > SAMR > SHR > SAMP > CBA. Figures for the age of 12 months produce the same ranking (Table 1).

The mean body temperature at the age of 12 months was maximal in HER-2/neu (38.88 °C) and SAMP-1 mice, and minimal in CBA mice (37.60 °C) (-3.3%, $p < 0.05$). Ranking the strains according to this parameter produces the following: HER-2/neu > SAMR > SAMP > SHR > CBA (Table 1).

The mean length of the estrous cycle was maximal in HER-2/neu females (5.96 days) and minimal in SAMP mice (3.88 days) (-34.9%, $p < 0.05$). This parameter remained practically constant between the third and the 12th months of life in CBA, SHR, SAMR and HER-2/neu mice and increased slightly in SAMP mice. The mice of the HER-2/neu and SAMP strains showed more disturbances in estrus function than CBA and SAMR females. An evaluation of the rate of age-related disturbances in estrus function shows that according to this parameter the strains could be ranked as follows: HER-2/neu > SAMP > SAMR > SHR > CBA (Table 1).

The incidence of chromosome aberrations (ChA) in bone marrow cells at the age of 3 months was maximal in HER-2/neu cells (5.2%) and minimal in SHR mice (2.9%) (-44.2%; $p < 0.05$); the ranking was as follows: HER-2/neu > SAMP > SAMR > CBA > SHR. There was a significant age-related increase in the incidence of ChA in all strains studied, but the ranking of the strains at the age of 12 months was practically the same (Table 1).

Total spontaneous tumor incidence was different in the mouse strains studied, reaching 81.7% in HER-2/neu mice compared with 30% in CBA mice. According to this parameter, the ranking is: HER-2/neu > SAMP > SAMR > SHR > CBA. The data on tumor site and type are given in Table 2.

Spearman rank correlations were calculated to examine the relationships between the parameters as evaluated for all five mouse strains and the mean life span and spontaneous tumor incidence (Table 3). Mean life span significantly correlated with body weight gain in the last 10% of survivors and negatively correlated with food intake at the age of 3 months. Tumor incidence negatively correlated with maximum life span and body weight gain between the age of 3 and 12 months, and positively correlated with food intake, dynamics of age-related disturbances in estrus function, and body temperature.

Table 1
Parameters of life span, body weight, food consumption, estrus function, body temperature and tumor incidence for female mice of different strains

Parameters	Mouse strain				
	CBA	SHR	SAMR-1	SAMP-1	HER-2/neu (FVB/N)
Number of mice	50	95	40	42	60
Life span parameters					
Mean LS, days	685 ± 9.24	457 ± 20.74*	514 ± 21.8*	557 ± 18.55*	294 ± 5.54*
Mean LS of last 10% survivors, days	736 ± 1.28	747 ± 5.37	725 ± 11.36	700 ± 13.27*	386 ± 6.69*
Maximum LS	740	772	766	749	431
Mortality rate, α, days^{-3}	19.0 (16.6; 20.3)	5.04* (4.14; 6.07)	8.1* (6.60; 10.5)	11.2* (8.76; 14.8)	19.1 (16.7; 23,8)
MRDT, days	37 (34; 42)	138* (114;167)	86* (66;107)	62* (47; 79)	36 (29; 41)
Body weight (BW) parameters of all mice					
BW at 3 mo (g)	21.4 ± 0.25	26.5 ± 0.3*	24.8 ± 0.27*	25.0 ± 0.21*	23.9 ± 0.28*
BW at 12 mo (g)	29.7 ± 0.78	35.7 ± 0.94*	32.3 ± 0.46*	32.5 ± 0.53*	25.0 ± 0.45[a]*
BW gain (3–12 mo) (%)	40.9 ± 3.29	32.2 ± 2.98	28.8 ± 2.24*	29.7 ± 2.07*	12 ± 1.25[b]*
Body weight (BW) parameters of the last 10% survivors					
BW at 3 mo (g)	20.7 ± 0.42	26.6 ± 0.62*	25.2 ± 0.73*	25.0 ± 0.84*	22.9 ± 0.52*
BW at 12 mo (g)	29.7 ± 1.36	32.5 ± 2.92	32.8 ± 1.53	34.6 ± 1.33*	25.7 ± 0.92[a]*
BW gain (3–12 mo) (%)	43.2 ± 4.47	24.8 ± 10.66	30.8 ± 8.08	38.4 ± 2.75**	14.0 ± 1.4[b]*
Food consumption (FC)					
FC, g/day/mouse					
At 3 mo	2.3 ± 0.19	4.3 ± 0.23*	4,80 ± 0,0*	3,75 ± 0,12*	5.1 ± 0.28*
At 12 mo	3.1 ± 0.12	3.8 ± 0.42	4,20 ± 0,0*	4,05 ± 0,16*	5.0 ± 0.35[a]*
Estrous function parameters					
Length of estrus cycle (days)					
At 3 mo	4.80 ± 0.30	5.96 ± 0.21*	4.57 ± 0,33	3.88 ± 0,19*	5.5 ± 0.31
At 12 mo	4.86 ± 0.25	5.32 ± 0.30	5.75 ± 0,34*	4.66 ± 0,20	6.3 ± 0.35[a]*
No. of mice with regular estrous cycles (%)					
At 3 mo	100	93	80	100	83
At 12 mo	100	87	100	67*	50[a]*
Body temperature					
Body temperature (°C) at 12 mo	37.6 ± 0.09	37.67 ± 0.09	38.8 ± 0.17*	37.96 ± 0.26	38.88 ± 0.17*
Incidence of chromosome aberrations (ChA) in bone marrow cells					
ChA (%)					
At 3 mo	3.1 ± 0.15	2.9 ± 0.20	3.5 ± 0.12	8.1 ± 0.10*	5.3 ± 0.14*
At 12 mo	8.9 ± 0.24	8.5 ± 0.14	10.9 ± 0.09*	19.1 ± 0.16*	8.5 ± 0.12
Tumorigenesis					
No. of tumor-bearing mice	15 (30%)	39 (41.1%)	27 (67.5%)*	31 (73.8%)*	46 (76.7%)*
No. of fatal tumor-bearing mice	3 (6%)	35 (36.8%)*	27 (67.5%)*	31 (73.8%)*	46 (76.7%)*

*The difference with the relevant parameter in CBA mice is significant, $p < 0.05$. **The difference with the corresponding parameter for total mice of the same strain is significant, $p < 0.05$.

[a] At the age of 6 months.

[b] BW gain, 2–6 months.

3.2. Dependency of parameters of life span and spontaneous tumor incidence in light and heavy 3- and 12-month-old mice of different strains

The data on the survival and tumorigenesis parameters in five strains of mice subdivided according to their BW at a young or adult age are given in Table 4.

It can be seen that the mean life span of CBA mice which were lighter (<21 g) at the age of 3 months was higher than that of mice which were heavier (>21 g) at the same age ($p < 0.05$). However, in all of the other studied strains there were no statistically significant differences in the mean life spans of lighter and heavier mice (at the age of 3 months). There was no statistically significant intraspecies difference between heavier and lighter animals in relation to any parameter of aging (α, MRDT) or spontaneous tumor incidence; this was true for body weights measured at the age of 3 months as well as those at 12 months. One exception was the increase in the mortality rate (α) and MRDT in heavier SAMP mice as compared with lighter ones. Also, the mean life span of SAMP mice which were heavier at the age of 12 months was higher than that of mice of this strain which were lighter at this age.

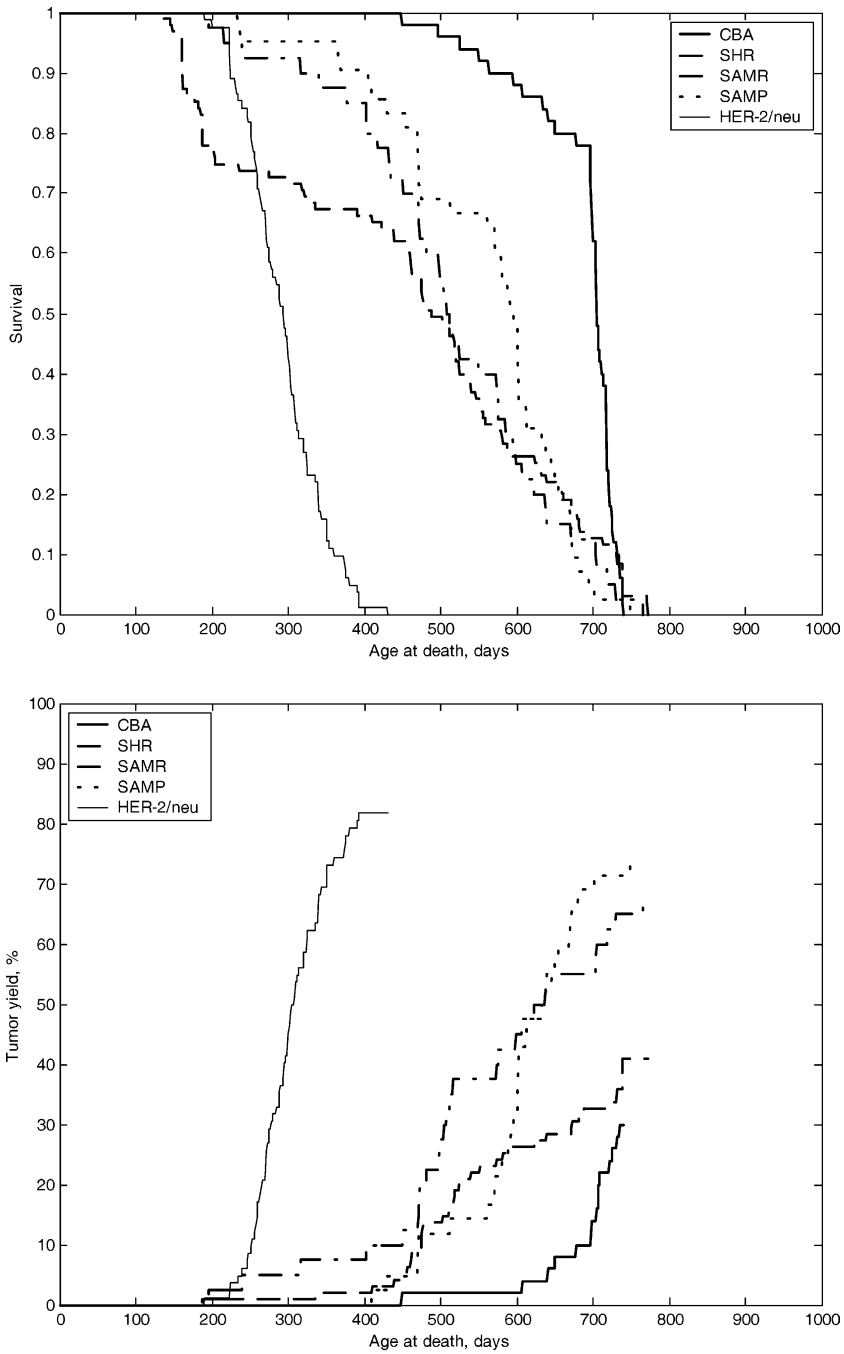

Fig. 1. Survival curves (upper graph) and tumor yield curves (%, lower graph) for female mice of five different strains.

Further, dependencies are observed when the correlation between BW gain and mean LS between the age of 3 and 12 months is taken as a predictor of longevity. In three mouse strains (CBA, SHR and SAMP), animals which gained more body weight lived statistically significantly longer than those in the subgroup that gained less body weight (Table 4). The results of log-rank statistical treatment of the differences between the survival curves of light and heavy female mice and the parameters of regression vs. age at death (Figs. 2 and 3) show that there is no uniform pattern for any five mouse strains studied (Figs. 2 and 3, Table 5).

There is a negative correlation between body weight at the age of 3 months and age of death in CBA and SHR mice, and a positive correlation in SAMR and SAMP mice. There is a positive significant ($p < 0.05$) correlation between body weight at the age of 12 months and age at death in CBA, SAMR and SAMP mice and a negative one in SHR mice. On the other hand, there is a positive correlation between body weight gain and age of death for all five strains of mice studied (Figs. 2 and 3).

Survival curves and tumor yield curves for heavier and lighter mice at the age of 3 or 12 months, as well as for

Table 2
Spontaneous tumor incidence, localization and type in female mice of different strains

Parameters	Mouse strain				
	CBA	SHR	SAMR-1	SAMP-1	HER-2/neu (FVB/N)
Number of mice	50	95	40	42	60
No. of tumor-bearing mice	15 (30%)	39 (41.1%)	27 (67.5%)	31 (73.8%)	46 (76.7%)
No. of fatal tumor-bearing mice	3 (6%)	35 (36.8%)	27 (67.5%)	31 (73.8%)	46 (76.7%)
Total number of tumors	20	45	29	33	181
Localisation and type of tumors					
Mammary gland: adenoma	1	–	–	–	–
Adenocarcinoma	5 (3)*	25 (23)*			181 (46)*
Lungs: adenoma	11 (10)	1	–	–	–
Adenocarcinoma	–	1	–	–	–
Leukemia/lymphoma	–	13	27	31	–
Uterus: polyp	–	3	–	–	–
Adenocarcinoma	–	1	–	–	–
Skin: papilloma	–	1	–	–	–
Vessel wall: haemangioma	3	–	–	–	–
Malignant fibrous histiocytoma	–	–	2	3	–

*Some mice have more than 1 tumor of this localisation.

mice which rapidly or slowly gained body weight are given in Figs. 4–8 for all five different strains. Statistical analysis (log-rank test) shows that there was a significant shift to the right in the survival curves of CBA and SAMP mice which were heavier at the age of 12 months, as well as those which gained more body weight (Fig. 4a). Also, lighter SAMP mice died earlier than heavier mice of this strain (Fig. 7b).

The analysis of tumor yield curves shows that only one strain exhibits a trend: in female SAMP mice, heavier mice with tumors live longer than lighter ones. No statistically significant differences were found between tumor yield curves in any of the other strains (subdivided into heavy and light subgroups).

4. Discussion

Our observations have shown that the longest-living CBA mice had minimal body weight at the ages 3 and 12 months, the lowest food consumption, body temperature, incidence of chromosome aberrations and spontaneous tumors, and the latest disturbances in estrus function, but maximal body weight gain in comparison with all other mouse strains. The shortest-living transgenic HER-2/neu mice had the lowest body weight at the age of 12 months, the lowest body weight gain, maximal body temperature, the fastest disturbances in estrous function and highest incidence of chromosome aberrations and tumor incidence in comparison to all other mouse strains.

Table 3
Correlations between parameters tested, longevity and tumor incidence in female mice of five strains

Parameter	Mean life span		Tumor incidence	
	Spearman's rank correlation	*p*-Value	Spearman's rank correlation	*p*-Value
Mean life span	–	–	−0.6	0.285
Maximum LS	0.3	0.624	−0.9	0.037
BW at 3 mo	−0.3	0.624	0.1	0.873
BW at 12 mo	−0.3	0.624	0.1	0.873
BW gain, 3 − 12 mo	0.7	0.188	−0.9	0.037
BW at 3 mo (10%)	−0.4	0.505	0	1
BW at 12 mo (10%)	0.4	0.505	0	1
BW gain, 3 − 12 mo (10%)	1	0	−0.6	0.285
Food intake at 3 mo	−0.9	0.037	0.7	0.188
Food intake at 12 mo	−0.7	0.188	0.9	0.037
Dynamics of regular cycle disturbances	−0.6	0.285	1	0
Body temperature at 12 mo	−0.7	0.188	0.9	0.037
ChA at 3 mo	0	1	0.8	0.104
ChA at 12 mo	0.2	0.747	0.6	0.285
Spontaneous tumor incidence	−0.6	0.285	–	–

Tabulated values are Spearman-rank correlation coefficients for all five mouse strains considered against mean life span and tumor incidence separately.

Table 4
Body weight at the age of 3 and 12 months, parameters of life span and spontaneous tumor incidence in female mice of various strains

Parameters	Body weight at the age of 3 months								BW at the age of 2 mo	
	CBA		SHR		SAMR		SAMP		HER-2/neu	
	<21 g	>21 g	<27 g	>28 g	<25 g	>26 g	<24 g	>25 g	<22 g	>23 g
Number of mice	31	19	60	35	26	14	16	26	31	29
Mean LS ($M \pm m$), days	699 ± 9.1	661 ± 18.3*	482 ± 24.8	414 ± 36.2	535 ± 23.6	474 ± 43.7	529 ± 39.9	574 ± 17.1	289 ± 8.65	304 ± 10.85
Maximum LS	740	735	772	772	766	704	749	695	393	431
Mortality rate, α, days^{-2}	1.7 (1.6; 2.0)	1.8 (1.4; 2.1)	5.7 (4.4; 8.3)	4.0 (2.8; 6.1)	8.5 (7.0; 11.3)	7.6 (4.6; 13.4)	8.1 (5.3; 14.6)	14.7* (11.4;19.5)	19.7 (17.4;27.6)	17.4 (14.3;25.6)
MRDT, days	41 (34; 43)	38 (33; 54)	121 (93; 156)	172 (114; 250)	82 (61; 99)	91 (52; 152)	86 (48; 130)	47* (35; 61)	35 (25; 40)	40 (27; 49)
Mean BW ($M \pm m$), g	20 ± 0.2	23 ± 0.3*	25 ± 0.3	29 ± 0.2*	24 ± 0.27	26 ± 0.2*	24 ± 0.12	26 ± 0.21*	21 ± 0.17	24 ± 0.2*
No.of total tumor-bearing mice	10 (32.3%)	5 (26.3%)	30 (50.0%)	9 (25.7%)	16 (61.5%)	11 (78.6%)	12 (75%)	19 (73.03%)	24 (77.4%)	22 (81.5%)
	Body weight at the age of 12 months									
	<29 g	>29 g	<33 g	>33 g	<31 g	>32 g	<31 g	>32 g	<25 g	>26 g
Number of mice	6	18	18	46	18	17	17	20	19	14
Mean LS ($M \pm m$), days	638 ± 80.5	692 ± 40.6	479 ± 42.0	442 ± 20.3	507 ± 33.0	542 ± 27.7	547 ± 22.2	615 ± 18.6*	312 ± 12.5	313 ± 14.3
Maximum LS	709	735	739	772	730	766	673	749	393	431
Mortality rate, α, days^{-2}	1.8 (1.3; 2.4)	1.8 (1.7; 2.1)	6.6 (4.2; 10.3)	4.7 (3.3; 6.3)	7.9 (6.0; 12.8)	9.1 (7.3; 16.8)	14.1 (10.8;20.0)	15.8 (11.3; 20.7)	21.4 (17.3;31.5)	17.7 (13.8; 34.4)
MRDT, days	39 (29; 54)	38 (33; 41)	105 (67; 165)	148 (110; 211)	87 (54; 115)	76. (41; 95.3)	49 (35; 64)	44 (34; 61)	32 (22; 40)	39 (20; 50)
Mean BW ($M \pm m$), g	26 ± 0.8	33 ± 1.3*	28 ± 0.95	39 ± 0.9*	30 ± 0.26	34 ± 0.51*	30 ± 0.37	35 ± 0.55*	23 ± 0.42	27 ± 0.37*
No.of total tumor-bearing mice	2 (33.3%)	7 (38.9%)	11 (61.1%)	17 (39.96%)	13 (72.2%)	12 (70.6%)	14 (82.4%)	17 (85%)	16 (84.2%)	11 (78.6%)
	Body weight gain between the 3rd and 12th months of the age								BW gain 2–6 months	
	<36%	>36%	<26%	>26%	<28%	>29%	<29%	>30%	<14%	>14%
Number of mice	19	24	27	37	18	17	18	20	12	12
Mean LS ($M \pm m$), days	662 ± 17.0	716 ± 2.7*	390 ± 39.9	498 ± 29.4*	526 ± 33.6	521 ± 27.7	549 ± 21.5	615 ± 18.6*	305 ± 11.1	330 ± 17.1
Maximum LS	740	739	739	772	730	766	695	749	375	431
Mortality rate, α, days^{-2}	1.9 (1.4; 2.1)	1.9 (1.7; 2.8)	3.8 (2.5; 6.0)	6.4* (4.8; 8.7)	8.6 (6.4; 14.2)	8.4 (6.9; 18.3)	13.3 (10.3;19.4)	15.8 (11.3; 20.7)	2.7 (2.3; 4.3)	1.9 (1.4; 3.2)
MRDT, days	37 (33; 49)	37 (25; 41)	180 (117; 282)	108* (80; 145)	81 (49; 108)	83 (38; 100)	42 (36; 67)	44 (34; 61)	26 (16; 31)	37 (22; 50)
Mean BW gain, %	23.3 ± 2.3	54.8 ± 3.6*	13.1 ± 2.4	46 ± 3.3*	21 ± 1.38	42 ± 2.05*	20 ± 1.68	39 ± 2.02*	8.9 ± 0.82	19.7 ± 1.57*
No.of total tumor-bearing mice	4 (21.1%)	8 (33.3%)	11 (40.7%)	17 (45.95%)	15 (83.3%)	10 (58.8%)	13 (72.2%)	18 (94.7%)	11 (91.7%)	9 (75.0%)

*The difference with the relevant parameter in the same strain of mice is significant, $p < 0.05$.

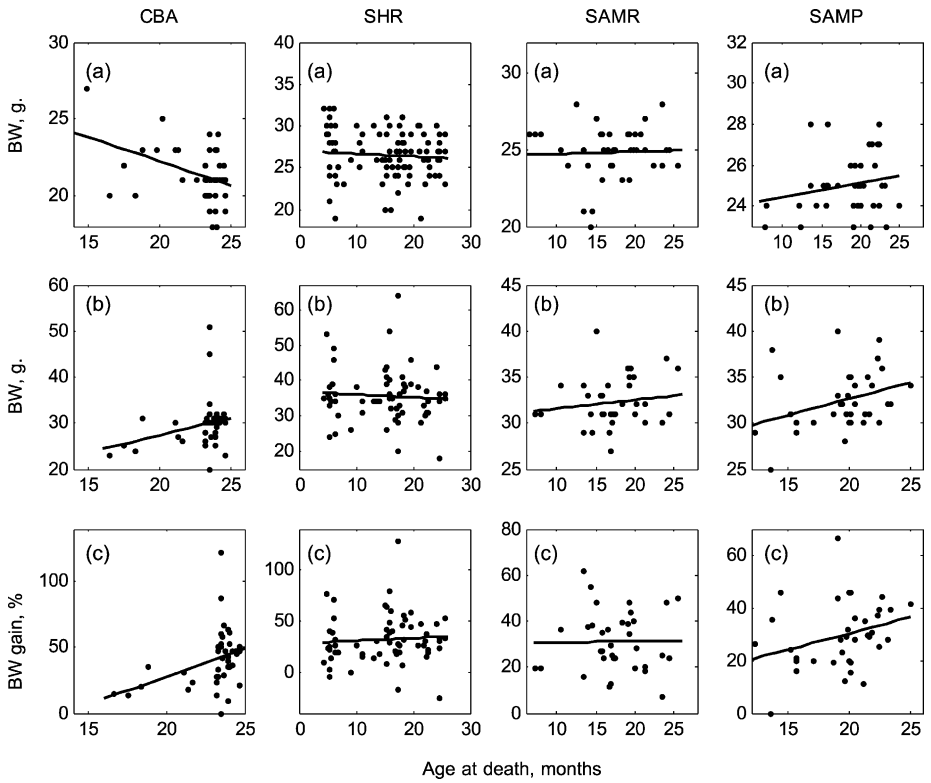

Fig. 2. Association of longevity with body weight in female mice of different strains: (a) regression of body weight at the age of 3 months vs. age at death; (b) regression of body weight at the age of 12 months vs. age at death; (c) regression of body weight gain between 3 and 12 months vs. age at death.

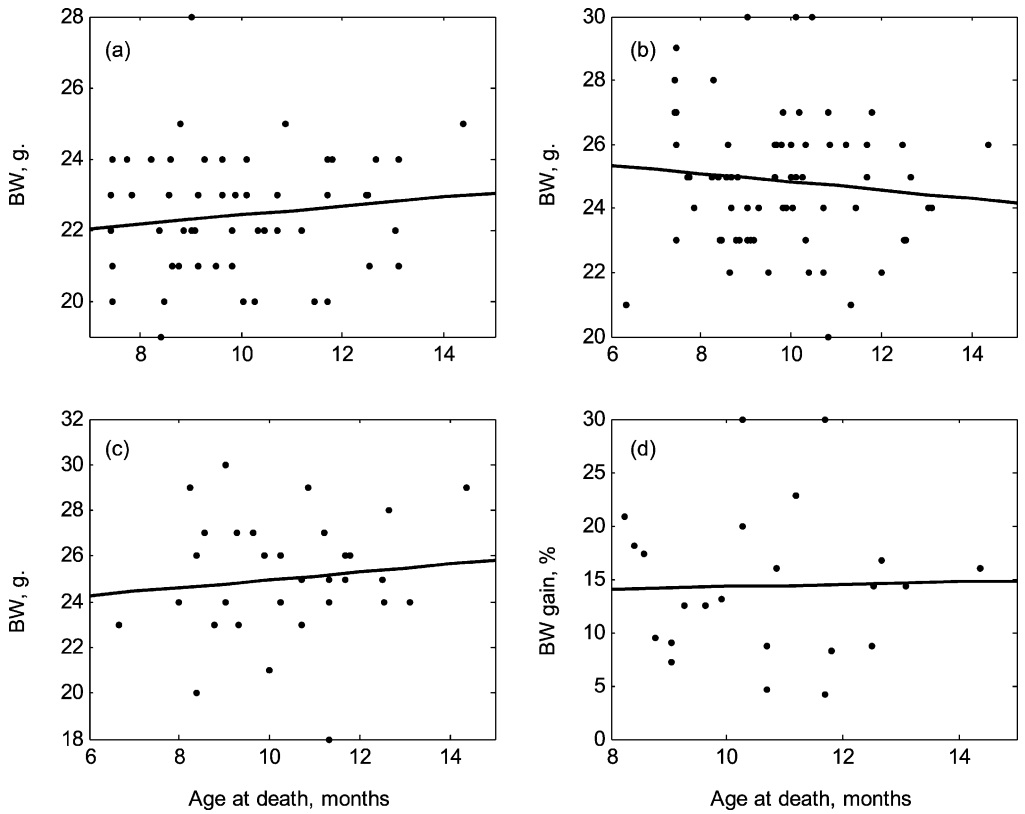

Fig. 3. Association of longevity with body weight in female transgenic HER-2/neu FVB/N mice: (a) regression of body weight at the age of 2 months vs. age at death; (b) regression of body weight at the age of 4 months vs. age at death; (c) regression of body weight at the age of 6 months vs. age at death; (d) regression of body weight gain between 2 and 6 months vs. age at death.

Table 5
Results of statistical treatment of differences between survival curves of light and heavy female mice (Log-rank test) and regression of body weight vs. age at death

Strain	Age, months	Log-rank (p-value)		Regression of body weight vs. age at death	
		Survival	Tumor	Slope	Intercept
CBA	3	0.0870	0.6972	−0.314 (−0.320; −0.307)	28.521 (28.366; 28.677)
	12	0.0400[#]	0.4761	0.754 (0.728; 0.779)*	12.305 (11.712; 12.898)*
	3–12[a]	0.0230[#]	0.5263	4.207 (4.102; 4.312)	−56.208 (−58.638; −53.777)
SHR	3	0.3384	0.2060	−0.031 (−0.034; −0.029)	26.995 (26.948; 27.042)
	12	0.9707	0.1798	−0.084 (−0.093; −0.075)*	36.926 (36.776; 37.077)*
	3–12[a]	0.1079	0.4208	0.258 (0.299; 0.287)	28.331 (27.856; 28.806)
SAMR	3	0.2600	0.059	0.014 (0.010; 0.018)	24.583 (24.517; 24.650)
	12	0.5800	0.6296	0.094 (0.088; 0.101)*	30.636 (30.512; 30.760)*
	3–12[a]	0.7530	0.5259	0.050 (0.017; 0.084)	30.259 (29.652; 30.867)
SAMP	3	0.8260	0.8629	0.070 (0.067; 0.074)	23.721 (23.656; 23.785)
	12	0.0140[#]	0.0270[#]	0.356 (03.46; 0.367)*	25.525 (25.317; 25.733)*
	3–12[a]	0.0310[#]	0.1718	1.229 (1.187; 1.270)	5.782 (4.963; 6.602)
HER-2/neu	2	0.2590	0.2592	0.126 (0.118; 0.134)	20.075 (19.968; 20.181)
	6	0.9650	0.9654	0.172 (0.155; 0.189)*	23.239 (23.064; 23.413)*
	2–6[a]	0.1040	0.1040	0.115 (0.059; 0.171)	13.104 (12.506; 13.702)

[#]Significant at the level 0.05. *The difference to the strain-matched mice at the age of 3 months is significant, p, 0.05.
[a] Body weight gain.

In general, these observations are in agreement with the current paradigm that longevity is associated with low calorie intake, lower body weight, decreased metabolism and slower age-related disturbances in estrous function and a low incidence of chromosome aberrations and spontaneous tumors.

In our study, senescence accelerated mice of both strains (prone-SAMP and resistant-SAMR) had a lot of similarities.

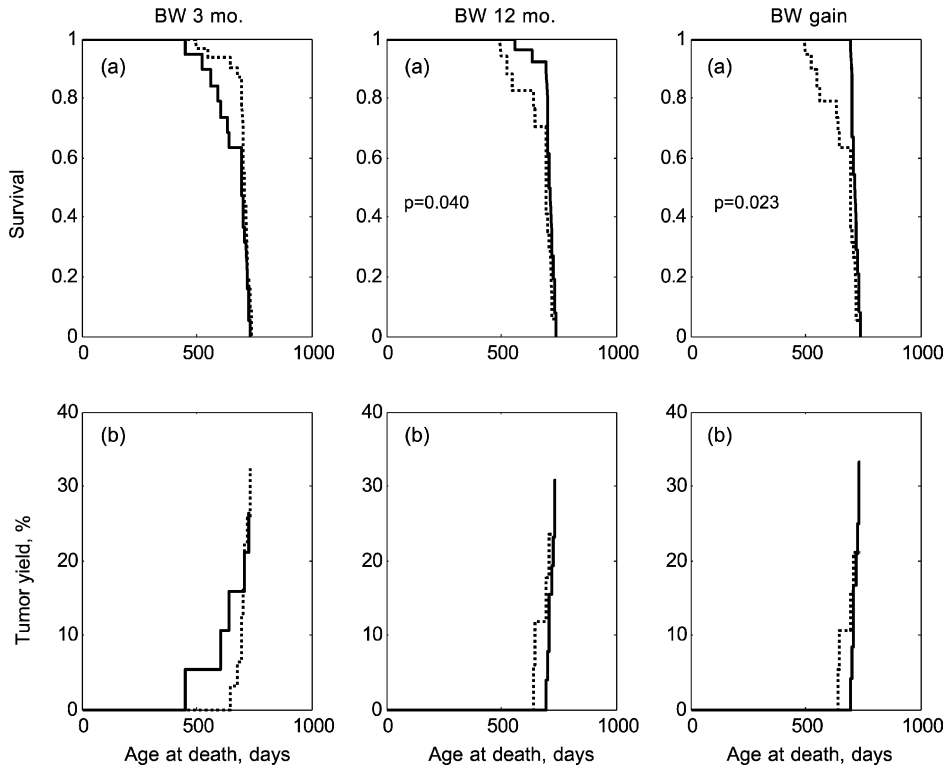

Fig. 4. Survival curves (a) and tumor yield curves (%, b) for female CBA mice. Left graphs: mice with weight at 3 months <21 g (dotted line) vs. >21 g (solid line); middle graphs: mice with weight at 12 months <29 g (dotted line) vs. >29 g (solid line); right graphs: mice with weight gain between 3 and 12 months <36% (dotted line) vs. >36% (solid line).

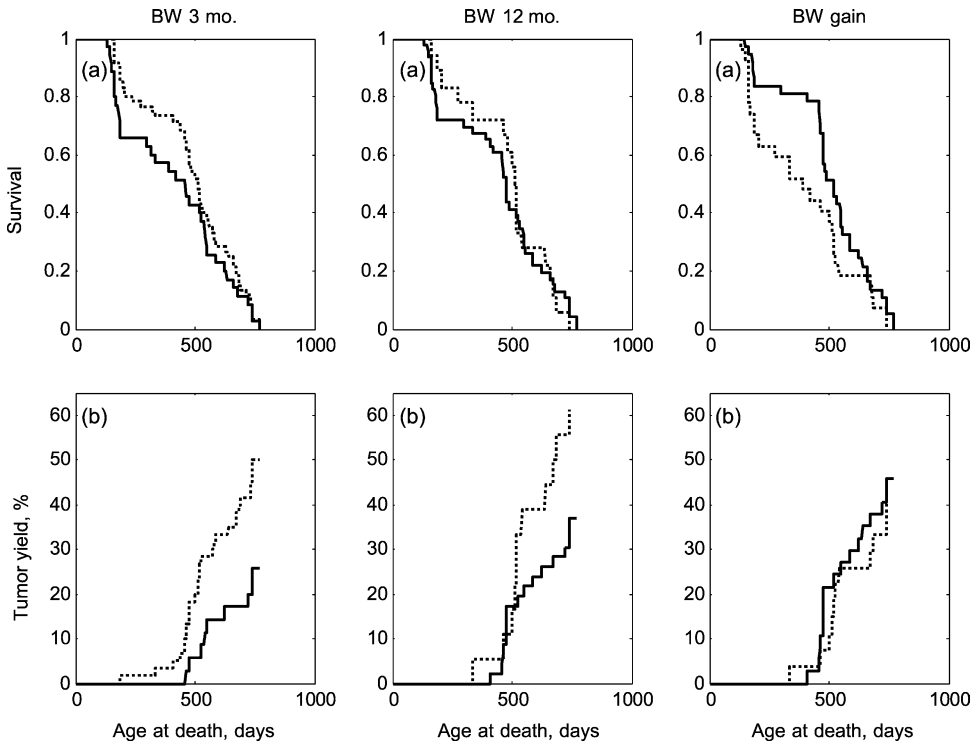

Fig. 5. Survival curves (a) and tumor yield curves (%, b) for female SHR mice. Left graphs: mice with weight at 3 months <27 g (dotted line) vs. >28 g (solid line); middle graphs: mice with weight at 12 months <33 g (dotted line) vs. >33 g (solid line); right graphs: mice with weight gain between 3 and 12 months $<26\%$ (dotted line) vs. $>26\%$ (solid line).

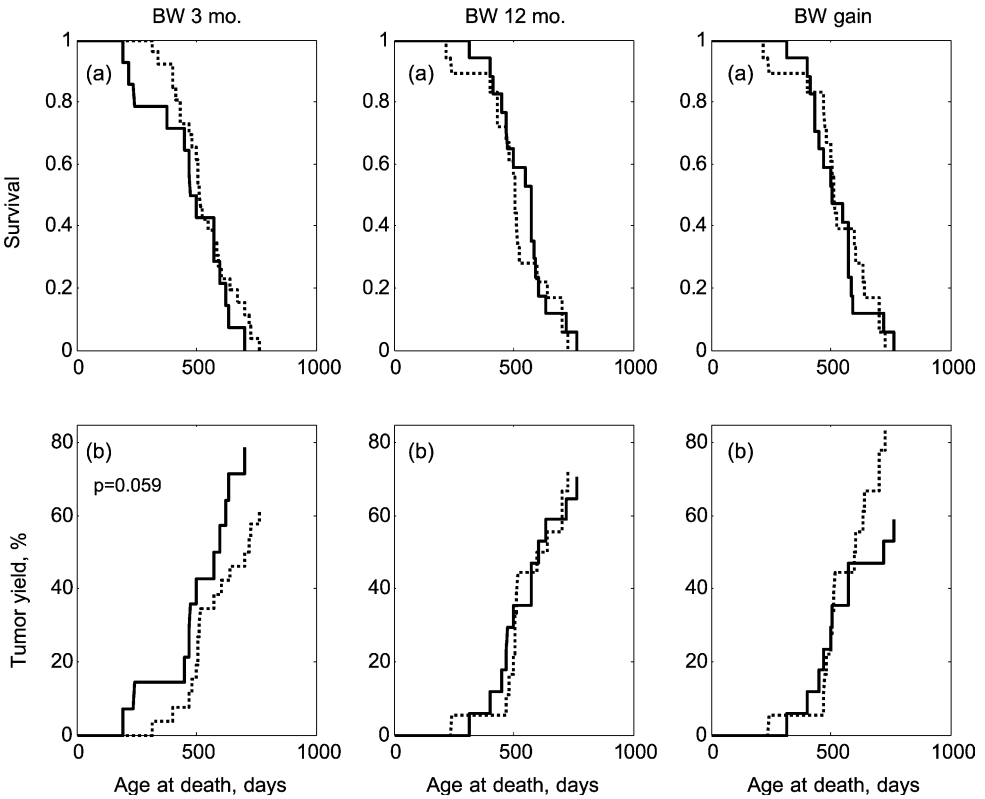

Fig. 6. Survival curves (a) and tumor yield curves (%, b) for female SAMR mice. Left graphs: mice with weight at 3 months <25 g (dotted line) vs. >26 g (solid line); middle graphs: mice with weight at 12 months <31 g (dotted line) vs. >32 g (solid line); right graphs: mice with weight gain between 3 and 12 months $<28\%$ (dotted line) vs. $>29\%$ (solid line).

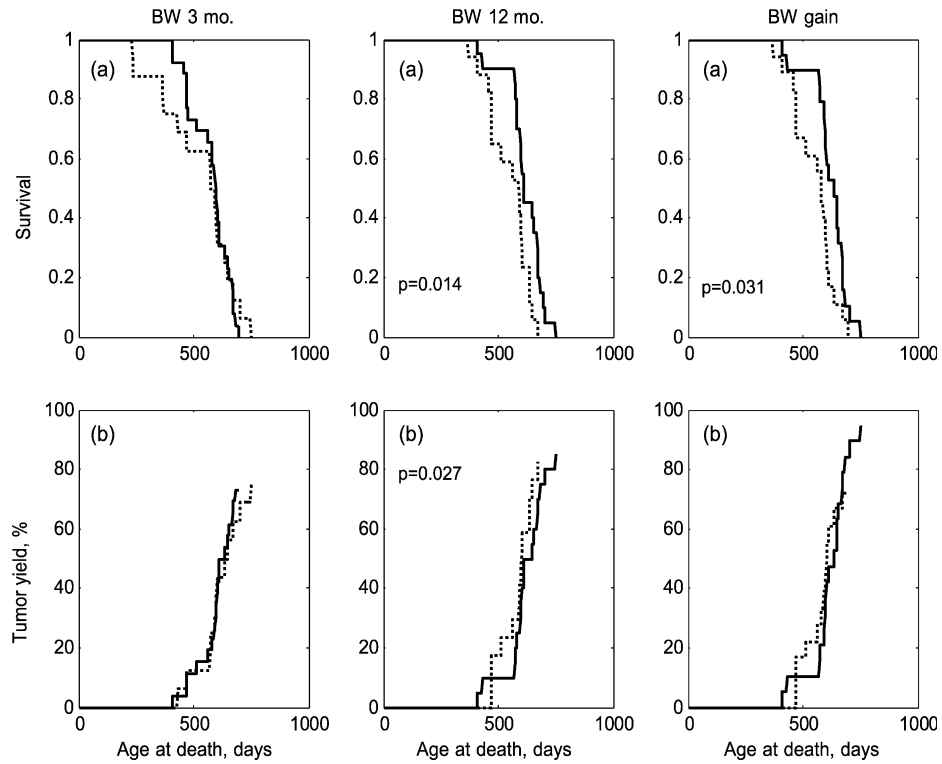

Fig. 7. Survival curves (a) and tumor yield curves (%, b) for female SAMP mice. Left graphs: mice with weight at 3 months <24 g (dotted line) vs. >25 g (solid line); middle graphs: mice with weight at 12 months <31 g (dotted line) vs. >32 g (solid line); right graphs: mice with weight gain between 3 and 12 months $<29\%$ (dotted line) vs. $>30\%$ (solid line).

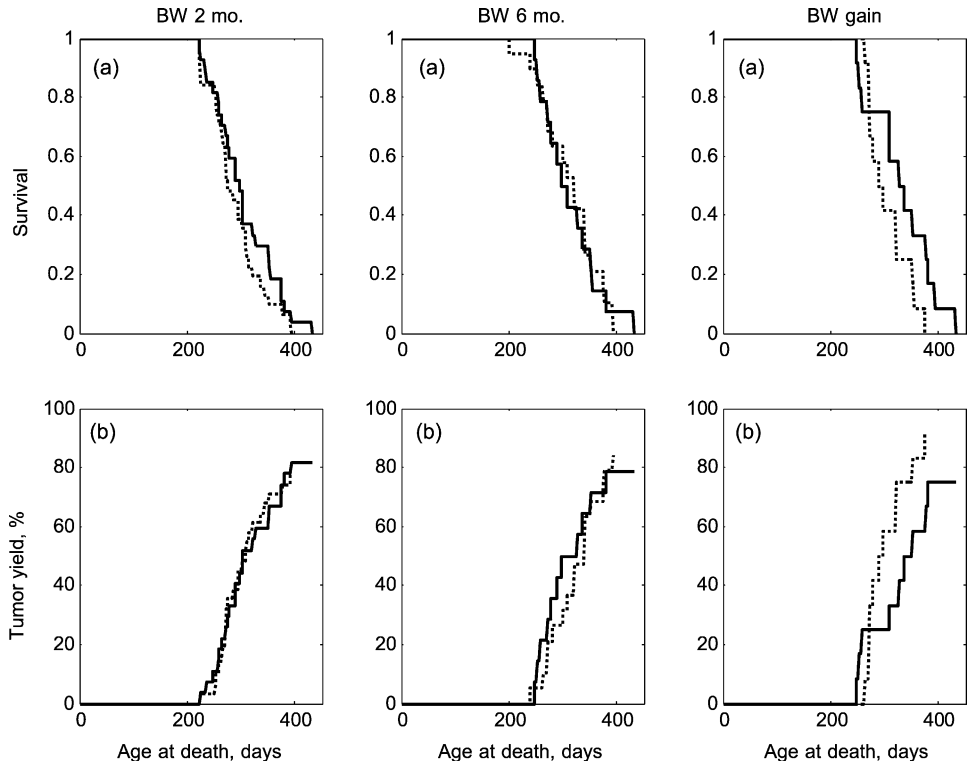

Fig. 8. Survival curves (a) and tumor yield curves (%, b) for female transgenic HER-2/neu FVB/N mice. Left graphs: mice with weight at 2 months <22 g (dotted line) vs. >23 g (solid line); middle graphs: mice with weight at 6 months <25 g (dotted line) vs. >26 g (solid line); right graphs: mice with weight gain between 2 and 6 months $<14\%$ (dotted line) vs. $>14\%$ (solid line).

There was neither a difference in the life span parameters between the SAMP and SAMR strains, nor in BW at 3 or 12 months, BW gain or spontaneous tumor incidence. SAMP mice have a shorter estrous cycle but a higher rate of disturbances in estrus function as compared with SAMR mice, as well as lower food consumption and body temperature. The SAMP strain's survival curve was shifted to the right in comparison to that of SAMR mice.

The SAM strain was generated by selective inbreeding of AKR/J mice (Takeda et al., 1997; Takeda, 1999). There are several senescence-prone strains (SAMP), which live 12–15 months, and several senescence-resistant (SAMR) strains, which are normal controls for accelerated-aging mice and have a life span of 24–30 months. It has been shown that SAMP mice develop normally until the age of 4 months and then they display signs of accelerated aging such as loss of hair, skin ulceration, decrease of locomotor activity, deficiency in learning and memory, emotional disorders, abnormal circadian rhythms, brain atrophy, hearing impairment, cataracts, and increased production of reactive oxidation specimens (ROS) and 8-hydroxyguanine levels in all organs (Takeda et al., 1997; Choi et al., 1999; Takeda, 1999; Yuneva et al., 2000). Our colonies of SAMP and SAMR mice were established from several pairs of breeders of 105th generation SAMP-1 and 96th generation SAMR-1 mice and for this report we observed 110–111th generation SAMP-1 and 101st–102nd generation SAMR-1 animals. We failed to find any difference between the survival parameters of these two strains (Table 1).

The reproductive life span of SAMP is shorter than that of the SAMR and the reproductive senescence of the SAMP strain is more accelerated than that of the SAMR strain (Miyamoto et al., 1995). We also observed earlier reproductive system senescence in our colony of SAMP mice as compared with SAMR mice.

The accelerated senescent-prone strain, SAMP-1, shows a striking increase in the frequency of chromosome aberrations from the age of 3–8 months, whereas the SAMR-1 strain shows only a slight increase of chromosome aberrations at the same age (Nisitani et al., 1990). Our observations are in agreement with these data. Uryvaeva et al. (1999) have shown an accelerated accumulation of micronuclear aberrations with age in the liver cells of SAMP mice as compared to the SAMR strain. The age-associated incidence of somatic *Hprt* mutations in splenic lymphocytes, as well as DNA damage (mainly DNA single strand breaks) in six organs, are also accelerated in SAMP-1 mice as compared to the SAMR-1 (Odagiri et al., 1998; Hosokawa et al., 2000).

The incidence of spontaneous lymphomas is 17.5% in SAMP strains (from 0% in SAMP-11 and SAMP-6 to 60.2% in SAMP-7) and 13.7% in SAMR strains (from 2.7% in SAMR-5 and 23.1% in SAMR-4). The incidence of other malignancies varies from 0 to 4.8% in SAMP and from 3.8 to 4.1% in SAMR strains (Takeda et al., 1997). In our colony of SAMP and SAMR mice, the incidence of lymphomas was 73.8 and 67.5%, respectively (Table 1)—higher than reported by Takeda et al. (1997). In general, only two parameters were statistically different between our SAMP and SAMR mice: food intake at the age of 3 months (SAMR > SAMP) and the rate of age-related disturbances in the estrus function (SAMP earlier than SAMR). Genetic drift could be the cause of the divergence in these parameters in SAMP and SAMR strains.

In Table 6, we summarize the correlation between some parameters and heavy BW in mice of the strains studied. There was no uniform pattern of correlation for all strains. Longevity was negatively correlated with heavy BW at the age of 3 months in CBA and SHR mice, but positively correlated with greater BW gain in both of these strains. At the same time, tumor incidence negatively correlated with heavy BW at 3 or 12 months in SHR, but not in CBA mice. Both longevity and tumor incidence negatively correlated with BW gain in transgenic HER-2/neu mice.

Heavy BW at 3 months negatively correlated with longevity in CBA and SHR mice but there is no correlation between these parameters in cancer-prone SAMR, SAMP or HER-2/neu mice. Excessive body weight at the age of 12 months positively correlates with longevity in CBA and SAMP mice.

In our study mice were fed ad libitum. In wild life mice practically never have food ad libitum. At the same time, calorie restriction is the most effective and reproducible intervention for increasing life span in a variety of animal species, including mammals (Weindruch and Walford, 1988; Mattson et al., 2003). It is also a potent cancer-prevention regimen in experimental carcinogenesis models (Weindruch and Walford, 1988; Hursting and Kari, 1999). The important question arising: is the restricted feeding the normal one in the life of organisms or is it the ad libitum feeding? Logically speaking, we can also say that ad libitum feeding, therefore low lean mass and high fat content, is the not normal situation and accelerated aging. Nevertheless, in the absolute majority of long-term experiments both in cancer research or in experimental gerontology commonly accepted ad libitum protocol for feeding. It is one of the difficulties in the discussions on effects of caloric restriction (Masoro, 2000, 2003; Bozhkov, 2001; Anisimov, 2003).

The decrease in insulin-like growth factor-1 (IGF-1) signaling pathway activity also plays a key role in the control of aging and life span in invertebrates as well as in mammals (Longo and Finch, 2003). Body weight decline is one of the main characteristics of animals maintained on a calorie-restricted diet. Genetic manipulations leading to dwarfism in mice were followed by a significant increase in LS and tumor latency or by a decrease in tumor incidence (Bartke et al., 2003). Miller et al. (2000, 2002) stressed that low BW, at as low an age as two months, is a predictor of longer life span. The authors noted that this association is seen in both male and female mice, although males are substantially heavier throughout life. An inverse correlation between BW and survival is described by Roe et al. (1995)

Table 6
Dynamics of some parameters in heavy mice in comparison with light mice of different strains*

Strain	Age	Mean LS	Maximum LS	Mortality rate, α	MRDT	Tumor incidence	Survival curves	Regression slope
CBA	3	↓	=	=	=	=	=	↓
	12	↑	↑	=	=	=	→	↑
	3–12[a]	↑	=	=	=	=	→	↑
SHR	3	↓	=	=	=	↓	=	↓
	12	=	↑	=	=	↓	=	↓
	3–12	↑	↑	=	=	=	=	↑
SAMR	3	=	↓	=	=	=	=	↑
	12	=	↑	=	=	=	=	↑
	3–12	=	↑	=	=	↓	=	↑
SAMP	3	=	↓	=	=	=	=	↑
	12	↑	↑	=	=	=	→	↑
	3–12	↑	↑	=	=	=	→	↑
HER-2/neu	2	=	↑	=	=	=	=	↑
	6	=	↑	=	=	=	=	↑
	2–6	=	↑	=	=	=		↑

*Only statistically significant results are given. (↑) The increase in the parameter in heavier animals; (↓) the decrease in the parameter in heavier animals; (=) no difference between heavy and light animals; (→) significant shift of survival curve for heavy animals to the right; (←) significant shift of survival curve for heavy animals to the left.
[a] Body weight gain.

and Turnbull et al. (1985) for Wistar and Sprague-Dawley rats. There are numerous reports on the positive correlation between higher BW and tumor incidence (Gries and Young, 1982; Gillettep-Guyonnet and Vellas, 2003; Haseman et al., 1992, 1977; Rao, 1995; Rao et al., 1990; Roderick and Storer, 1960; Roe et al., 1995; Ross et al., 1970, 1982, 1985; Selkop, 1995). However, the relationship between BW and longevity was not entirely consistent with LS effects. Ingram and Reynolds (1987) have shown that the reduction of dietary protein from a normal level of 20–8% was associated with reduced BW, but not with a significant increase in LS. Reviewing the available data on the intra-group correlation between BW and LS, Ingram and Reynolds (1987) concluded that, in general, the existence and direction of the significant correlation between BW and LS was dependent upon age and genotype. The authors found positive correlation between BW in the middle of life and LS in Wistar rats. Everitt and Webb (1977) found a significant positive correlation between LS and BW at the age of 400 days. BW at other ages was not significantly correlated with LS. Weindruch et al. (1986) showed correlation between LS and BW at weaning, 5, 10, 15 and 22 months of age within groups of female C3B10RF$_1$ mice on ad libitum and calorie-restricted regimens. The only significant correlation to emerge were positive in direction and they all occurred beyond the age of 5 months, with at least one occurring at 22 months. Ingram and Reynolds (1987) stressed that the most prevalent significant correlation between BW and LS appeared to be positive in direction within male rodent species. Our findings show that heavier BW at the age of 1 year is also a significant predictor of longevity in female mice of the CBA and SAMP strains (but not in SHR, SAMR and HER-2/neu). Wirth-Dzieciolowska and Czuminska (2000) analyzed the differences between longevity and aging process in two lines of mice selected divergently for body weight for over 90 generations. They showed that heavy females live longer than light ones and light male mice lived 30 days longer than heavy ones. Our results are in good agreement with these observations.

Excess in BW at the age of 2 or 12 months is not a predictor of increased tumor risk in the strains studied.

There is abundant evidence that obesity is associated with excessive morbidity and mortality in humans. At the same time, extreme leanness is also associated with excessive mortality (Samaras and Elrick, 1999; Samaras et al., 2002). Instead of a linear correlation between BW and LS, some investigators of human populations have emphasized the curvilinear nature of the relationship (Waaler, 1988; Ingram and Reynolds, 1987). Andres et al. (1985) studied the correlation between ideal BW (in terms of the lowest risk of mortality) and age. It was found that ideal BW increases linearly with adult age. Ingram and Reynolds (1987) have discussed two possible explanations of these observations. One deduction might be that at higher ages, relatively heavier persons face a lower mortality risk than lighter counterparts. The other deduction is that gaining weight with age might reduce mortality risk. Based on several literature reviews, Andres et al. (1985) concluded that little evidence exists to support an inverse relationship between adult BW and mortality risk.

In a comprehensive review on the relationship of BW with longevity within laboratory rodent species, Ingram and Reynolds (1987) suggested that the relationship between

BW and LS is curvilinear. This model attempts to accommodate the existence of genotypes. The authors suggested that for genotypes prone to leanness, heavier BW should be positively correlated with LS, but for the genotypes prone to obesity, BW should be negatively correlated with LS. Recently, Fontaine et al. (2003) estimated the expected number of years of life lost due to overweight and obesity across the life span of an adult. Among whites, a J- and U-shaped association was found between overweight or obesity and the number of years of life lost. For any given degree of overweight, younger adults generally had greater loss of years than did older adults. The maximum number of years of life lost for whites aged 20–30 years with obesity was 13 for men and 8 for women. Among black men and black women older than 60 years, overweight and moderate obesity were generally not associated with an increased loss of life years, however, blacks at younger ages with severe levels of obesity had a maximum loss of years of 20 for men and 4 for women. Our results are not contradictory to these observations and are in agreement with the conclusion by Ingram and Reynolds (1987) that there is no evidence that being thinner is necessarily better for survival.

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