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The Effect of Dietary Restriction of Varying Duration on Survival, Tumor Patterns, Immune Function, and Body Temperature in B10C3F$_1$ Female Mice[1]

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Seven groups of mice were maintained on different dietary programs varying with respect to restriction at various stages of life. Restriction was associated with less age-related decline in T-dependent immunological function and a slight but significant lowering of body temperature. The best mean and maximum survival and the lowest late-life mortality rate was found in the group restricted throughout life, but restriction during any part of the lifespan enhanced survival to some degree. The mean life spans of tumor-bearing animals tended to be greater in restricted than in nonrestricted groups, corresponding to an age-decelerating effect. Tumor frequency varied with the period of life during which restriction took place and was not always decreased in restricted animals. These latter results suggest that the mechanisms whereby dietary restriction influences the aging rate and tumor susceptibility may not be entirely identical.

Key Words: Undernutrition, Life span, Hepatomas, Lymphomas, Splenic parameters, T-dependent immunity.

THE early work of McCay and associates (McCay et al., 1939a, 1939b), and Ross and Bras (Ross, 1961, 1972; Ross & Bras, 1965, 1971) demonstrated dramatic increases in maximum survival, decreases in disease incidence, and a shift to later ages of the age-specific incidences of various diseases in rats maintained on long-term dietary restriction. In similar studies in C57BL/6J mice we found that a diet restricted in calories since weaning, but supplemented with salts and vitamins to avoid malnutrition, extended maximum survival and significantly decreased the incidence of lymphoma, the most prevalent tumor in normally fed mice of this strain (Cheney et al., 1980). Immunological function in mice restricted since weaning appeared retarded in its rate of maturation and decline with age, compared with normally fed controls (Gerbase-DeLima et al., 1975; Walford et al., 1973/74, 1977). The current study was designed to assess survival, tumor patterns, body weight and splenic parameters, immune function, and body temperature in a long-lived hybrid mouse strain subjected to undernutrition, including survival and tumor patterns in mice restricted during only part of their life spans (i.e., from before weaning or from weaning until midlife, or from midlife until death).

Materials and Methods

Mice and dietary groups. — Seven dietary groups of (C57BL/10Sn × C3H/HeDiSn)F$_1$ female mice, hereafter referred to as B10C3F$_1$, were created within a single cohort as described in Table 1. Thirty of each group were set aside and held until natural death to obtain data on life span, tumor incidence and body weight. In the N/N, R/N, N/R, and R/R groups additional mice were sacrificed for immune functional and other assays. All deaths,

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Table 1. Dietary Regimens for Seven Groups of B10C3F$_1$ Female Mice

Symbol	Preweaning diet (< 24 days)	Midlife diet (24 days to 61 weeks)	Later-life diet (> 61 weeks)
N/N	5/litter; with mothers continuously	~15 cal. MTuWTh; ~45 cal. F	~15 cal. MTuWTh; ~45 cal. F
R/N	9/litter; separated from mothers every other day after 1 week	Same as N/N	Same as N/N
N/R	Same as N/N	~15 cal. MW; ~30 cal. F	~15 cal. MW; ~30 cal. F
R/R	Same as R/N	Same as N/R	Same as N/R
N/N/R	Same as N/N	Same as N/N	Same as N/R
N/R/N	Same as N/N	Same as N/R	Same as N/N
R/R/N	Same as R/N	Same as N/R	Same as N/N

Note. The days of the week are abbreviated as follows: Monday (M), Tuesday (T), Wednesday (W), Thursday (Th), Friday (F)

whether natural, sacrificial, or accidental, were included in the survival analysis, with appropriate correction factors (see next section).

Mice were weaned at 24 days of age and caged singly thereafter. Only one group (N/N) was fed normally throughout life. Dietary restriction was introduced in the other groups either before weaning, at weaning, or at 61 weeks of age, and continued for specified periods thereafter. Mice restricted prior to weaning were suckled nine per mother and separated from the mothers every other day after 1 week of age. Other mice were suckled five per mother with continuous access to the mother until weaning (Table 1). Post-weaning restriction consisted of feeding the mice a single portion on Monday and Wednesday with a double portion on Friday compared with a single portion Monday through Thursday and a triple portion on Friday for normally fed mice (Table 1). The size of a single portion approximated that which would have been consumed on an ad libitum basis. The postweaning normal diet (Gerbase-DeLima et al., 1975) consisted primarily of 21.6% casein, 15.0% cornstarch, 39.1% sucrose, and 13.5% corn oil. The restricted diet was similar except that salts and vitamins were doubled at the expense of cellulose and cornstarch to avoid malnutrition (Gerbase-DeLima et al., 1975). Both diets were purchased from Nutritional Biological Company and stored at 4°C.

Survival. — Survival curves were plotted using product-limit estimates whereby a point is given for every natural death corrected for any accidental or sacrificial deaths that may have preceded it (Dixon & Brown, 1977; Kaplan & Meier, 1958). The product-limit estimates were generated by the Biomedical Computer Program BMDP1L (Dixon & Brown, 1977), which also generated estimates of mean and median survival. Mean survival estimates were compared using two-tailed, two-sample t tests. In addition, survival distributions were compared by fitting them to $f(t')$, a special form of the Gompertz equation, using a computer program to estimate the Gompertzian parameters and their standard errors by the method of maximum likelihood (Garg et al., 1970). Product-limit estimates (Kaplan & Meier, 1958) were employed to allow for censored data, including accidental and sacrificial deaths, and goodness-of-fit was tested using the Cramer-von Mises w^2 statistic (Mickey et al., 1963). Comparisons between groups of one of the fitted Gompertzian parameters, $\hat{\alpha}'$, were made using two-tailed, two-sample t tests.

Autopsy data. — All animals were autopsied, and tissue abnormalities examined microscopically. Tumors were classified according to criteria detailed by Smith et al. (1973). Tumor frequencies were compared between groups using chi-square tests that employed the Yates correction for small sample sizes (Dixon & Brown, 1977) and within groups using the McNemar test (Dixon & Brown, 1977), a within-group chi-square test. Mean ages at death for tumor-bearing animals were compared both between and within groups using two-tailed, two-sample t tests, and in one instance, the sign test, a nonparametric test (Dixon & Massey, 1969).

Body weight, spleen weight, spleen index, lymphocytes/spleen and lymphocytes/mg spleen weight. — Thirty animals of each dietary group listed in Table 1 were weighed monthly throughout life. Body and spleen weights were measured at the time of sacrifice for animals in the N/N, R/N, N/R, and R/R groups used in the functional assays, and spleen indices (spleen weight [mg]/body weight [g]) calculated. Lymphocytes/spleen and lymphocytes/mg spleen weight were determined for animals sacrificed for the PFC (plaque-forming cell)

assay. All parameters were compared within and between age groups using two-tailed, two-sample *t* tests.

Mitogenic assays and the mixed lymphocyte culture (MLC). — Lymphocyte responses to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A) and purified protein derivative (PPD), and in the mixed lymphocyte culture, were determined on mice 19 to 21, 43 to 46, and 141 to 161 weeks of age and on four different diets: N/N, R/N, N/R, and R/R. Techniques were as previously described (Gerbase-DeLima et al., 1975; Meredith & Walford, 1977). Animals showing gross pathology were excluded from these or other functional tests. Results were expressed as decays/minute (DPM), transformed to a log scale, and the means of triplicates or quadruplicates for each animal were averaged after subtraction of the mean background count to obtain group means for each diet at each age group. The responses were reported as the antilogs of these averages, except for the PHA/Con A ratios, which were expressed as the antilogs of the averages of the log PHA/Con A ratios, and were compared within and between age groups using two-tailed, two-sample *t*-tests.

Plaque-forming cell (PFC) responses to sheep red blood cells (SRBC). — Mice 21 to 25, 46 to 50, and 164 to 167 weeks of age were assayed for the diets R/N, N/R and R/R, whereas mice of only two ages (21 to 25 and 46 to 50 weeks) were assayed for the N/N diet. Assays were run in quadruplicate using techniques described elsewhere (Walford et al., 1973/74), and the means of the quadruplicates for each animal were transformed to a logarithmic scale and averaged to give group means for each diet at each age group. Results were expressed as the antilogs of direct and indirect PFC per spleen and per 10^6 spleen cells. Comparisons between groups were made by means of two-tailed, two-sample *t* tests.

Body temperature. — Body temperatures were measured with a Yellow Springs thermistor probe inserted rectally to a uniform depth in N/N and R/R mice 16, 87, and 145 weeks of age. Readings were taken on four consecutive days, Monday through Thursday, at 10 A.M. and at 4 P.M. For this purpose only, the R/R mice were fed .5 portion/day, rather than a full portion on alternate days (Table 1), since preliminary data suggested that the latter feeding mode increased day-to-day variations in body temperature. Inasmuch as there were no significant daily variations or differences between morning and afternoon readings in either group, the readings over 4 days were averaged to give a single value for each mouse. The group means were then compared using two-tailed, two-sample *t* tests.

Statistical analysis. — Biomedical Computer Programs BMDP1V and BMDP1F (Dixon & Brown, 1977) were employed to generate the *t* test and chi-square (including McNemar) statistics, respectively. The *t* test comparisons employed pooled variance estimates that included all groups under consideration (e.g., seven dietary groups in the case of mean survival). Degrees of freedom for significance tests involving product-limit estimates were conservatively estimated as $N_1 + N_2 - 2$, where *N*s represent natural deaths. When the *t* and chi-square tests involved multiple comparisons (e.g., 21 for seven dietary groups in the case of mean survival and tumor frequency), *p* values were adjusted by multiplying the individual *p* values by the number of comparisons to produce an error rate per family of comparisons.

RESULTS

Survival. — In the N/R/N, R/R/N, and N/R groups maximum survival was not ascertainable and mean survival was underestimated because the last survivors died accidentally from a missed feeding(s) (Figure 1). When only natural deaths were considered, maximum survival ranked as R/R > N/N/R > R/N > N/N (Table 2), decreasing as the period of restriction decreased. A similar ranking held for estimates of mean and median survival that were adjusted for censored data (Table 2), except that the N/R group displayed a slightly shorter mean survival than the N/R/N and R/R/N groups and a slightly longer median survival than the R/R group. Mean survival was significantly greater for the R/R group than for the N/N ($p < .0002$) and R/N ($p < .0002$) groups (Table 2).

Survival distributions were also compared by fitting them to the Gompertz equation, which describes age-specific mortality rates. As previously (Cheney et al., 1980), we obtained a better fit in most dietary groups by excluding earlier deaths (i.e., $t < 120$ weeks). Thus, we fit the following form of the Gompertz equation:

$$\left\{ f(t') = \frac{1}{\alpha'} \exp \left[\frac{t'}{\beta} - \frac{\beta(e^{t'/\beta} - 1)}{\alpha'} \right], \right\}$$

where $f(t')$ describes the frequency distribution of t', the time to death, where $t' = t - t_0$, given $t \geq$

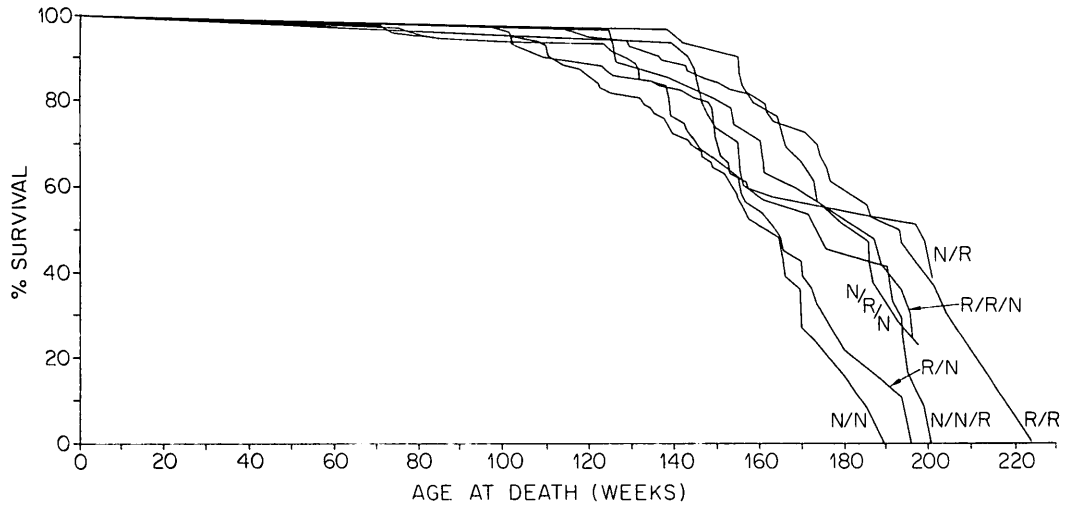

Figure 1. Survival curves for seven dietary groups of B10C3F$_1$ female mice. Points plotted were product-limit estimates with neonatal deaths prior to 8 weeks excluded and correction factors applied for accidental and sacrificial deaths. Group labels are explained in Table 1. Survival curves for the N/R/N, R/R/N, and N/R groups were incomplete due to the accidental deaths of the last survivors.

Table 2. Summary of Survival Data in Seven Dietary Groups of B10C3F$_1$ Female Mice

	Diet Group[a]						
	N/N	R/N	N/R	R/R	N/N/R	N/R/N	R/R/N
N[b]	114	144	114	114	30	30	30
Survival							
M[c]	156.3	157.9	172.2	186.6	170.7	178.7	175.6
SEM[d]	4.1	3.7	4.2	4.5	5.2	5.9	6.2
N[e]	40	49	37	33	24	19	17
Median[c]	164.7	164.6	199.0	193.3	175.6	186.0	187.3
Maximum	189.6	195.9	204.9[k]	224.3	200.6	199.1[k]	200.4[k]
Gompertzian parameters[f]							
N[g]	36	36	27	28	23	19	17
α'[h]	156.5	204.1	549.1	646.0	306.9	397.4	395.9
SEM[d]	66.9	66.9	77.3	75.9	83.7	92.1	97.4
β[i]	25.0	25.0	25.0	25.0	25.0	25.0	25.0
w^2[j]	.0400	.0177	.3371**	.0225	.1442*	.0265	.0923

[a]Group labels are explained in Table 1.

[b]Initial group size.

[c]Estimates, in weeks, are based on product-limit estimation, taking into account censored data (i.e., sacrificial and accidental deaths). Survival times are underestimated in the N/R, N/R/N, and R/R/N groups because the last survivors died accidentally.

[d]The standard errors of the mean (SEM) are based on pooled variance estimates that include all groups.

[e]Number of natural deaths.

[f]Product-limit corrections were made for the censored data (i.e., sacrificial and accidental deaths).

[g]Number of natural deaths past 120 weeks of age.

[h]α' is inversely proportional to the mortality rate past 120 weeks of age.

[i]β, fixed at 25.0, is proportional to the doubling time of the mortality rate past 120 weeks of age.

[j]The Cramer-von Mises w^2 statistic tests goodness-of-fit. Significant p values indicate a poor fit.

[k]Values are not reflective of true maximum survival since last survivors died accidentally.

*$p < .10$

**$p < .01$

$t_0 > 0$, and α' and β are parameters. We also found, as previously (Cheney et al., 1980), that β could effectively be fixed at 25, leaving α' as the only parameter to be estimated. The estimates of α' for all the dietary groups are shown in Table 2, together with the Cramer-von Mises w^2 statistics for goodness-of-fit. The latter reveal that goodness-of-fit was acceptable for all groups except N/R, although only marginally acceptable for the N/N/R group. These difficulties in fitting our data to the model resulted from the N/R and N/N/R survival curves differing somewhat in shape from those of the other groups past 120 weeks of age (Figure 1). Although a good fit was obtained for all groups given $t_0 = 150$ weeks (not shown), we report here the results for $t_0 = 120$ weeks, since this excluded fewer data and allowed for comparison with our earlier results (Cheney et al., 1980). In addition, the relative magnitudes of the differences in $\hat{\alpha}'$ among the groups differed little, whether $t_0 = 120$ or $t_0 = 150$ weeks of age.

Comparisons among groups reveal that $\hat{\alpha}'$ (Table 2) was significantly greater for N/R and R/R than for N/N ($p < .0021$ and $< .0002$, respectively) and R/N ($p < .0105$ and $< .0005$, respectively). Because α' is inversely proportional to the age-specific mortality rate past 120 weeks of age, the N/R and R/R groups thus enjoyed significantly lower age-specific mortality rates past 120 weeks of age than the N/N and R/N groups.

Tumors. — Unlike survival, which varied with the length of the restricted period, tumor frequency varied with the period in life during which restriction was imposed (Table 3). Both the N/N/R and N/R groups, which had in common restriction from 61 weeks of age onward, had significantly fewer total tumors than the N/N ($p = .0315$ and .0042, respectively), R/N, N/R/N, and R/R/N ($ps < .0021$) groups. Moreover, the N/R, R/R, N/R/N, and R/R/N groups, which had in common restriction from weaning until 61 weeks of age, displayed significantly greater numbers of hepatomas than lymphomas, the two most prevalent tumor types overall ($ps < .005$, .0184, .0001, and .0124, respectively). Hepatoma frequency was greater in N/R/N than in N/N ($p < .0021$), R/N ($p = .0063$), N/R ($p < .0021$), and N/N/R ($p < .0028$); the frequency also was greater in R/R/N than in N/R ($p = .0168$) and N/N/R ($p = .0063$). Lymphoma frequency was less in N/R than in N/N ($p = .0021$) and R/N ($p = .0147$). The occurrence of two or more different types of tumors in any individual animal was rare.

In contrast with tumor frequency, which was independent of survival time in that longer-lived

Table 3. Summary of Tumor Data in Seven Dietary Groups of B10C3F$_1$ Female Mice

	Diet Group[a]						
Tumor	N/N	R/N	N/R	R/R	N/N/R	N/R/N	R/R/N
Hepatomas							
N[b]	8	12	6	13	1	14	10
Frequency (%)[c,d]	20.0	26.8	15.1	34.7	6.7	72.4	53.9
M age at death (weeks)[b]	151.7	154.2	174.6	182.9	145.4	179.4	178.6
SEM[e]	9.2	7.5	10.6	7.2	26.0	6.9	8.2
Lymphomas							
N[b]	11	11	0	4	3	2	3
Frequency (%)[c,d]	28.9	23.2	0	14.3	10.0	10.3	15.4
M age at death (weeks)[b]	130.8	141.6	–	168.9	129.2	170.9	161.0
SEM[e]	10.9	10.9	–	18.1	20.9	25.6	20.9
All tumors							
N	27	38	11	23	6	23	21
Frequency (%)[c]	60.0	67.9	20.8	46.9	20.0	79.3	80.8
M age at death (weeks)	142.5	151.4	160.3	175.3	142.7	176.8	174.4
SEM[e]	5.3	4.5	8.4	5.8	11.3	5.8	6.1

[a]Group labels are explained in Table 1.

[b]Animals with two tumors are excluded.

[c]Percentages are of those animals autopsied that died either natural or accidental deaths.

[d]Animals with two tumors are included.

[e]The standard errors of the mean (SEM) are based on pooled variance estimates that include all groups.

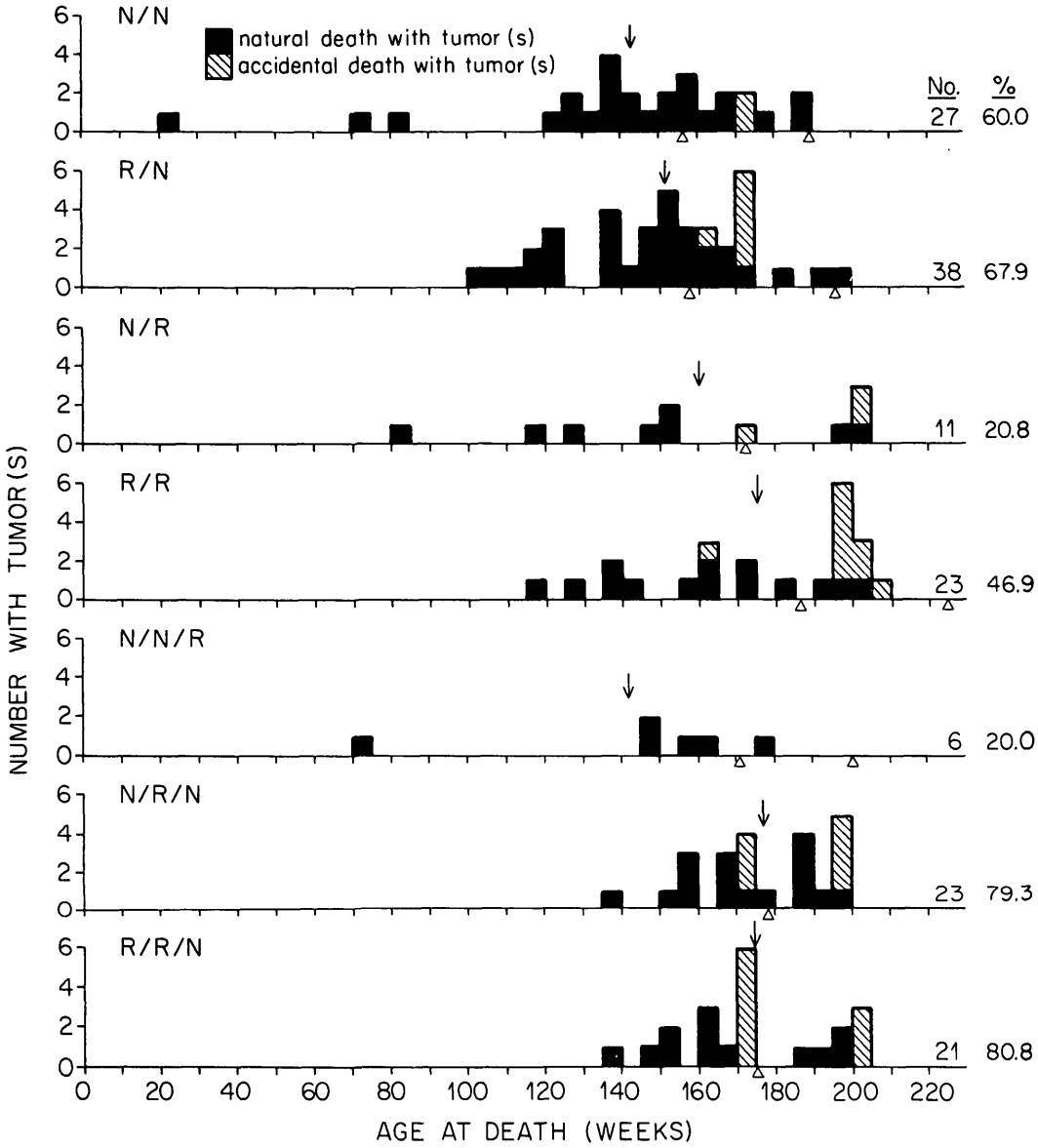

Figure 2. Age-specific distributions of the ages at death for tumor-bearing animals in seven dietary groups of B10C3F$_1$ female mice. Group labels are explained in Table 1. The arrows (↓) above the distributions represent the mean ages at death for the tumor-bearing animals in each group, whereas the arrows (△) below the baselines represent the mean and maximum ages at death for the whole groups. (Arrows representing maximum survival for the N/R, N/R/N and R/R/N groups are not present, as the last survivors in these groups died accidentally.) Percentages are of those mice autopsied.

groups did not necessarily have fewer tumors, actual survival times of tumor-bearing animals followed group survival times. As illustrated in Figure 2, there were significant between-group differences in the mean ages at death for the tumor-bearing populations as a whole, with R/R ($p < .0021$), N/R/N ($p < .0011$), and R/R/N ($p < .0053$) greater than N/N, and R/R and N/R/N greater than R/N ($ps < .0210$) (Table 3). Similar trends were observed for between-group differences in the mean ages at death for hepatoma- and lymphoma-bearing animals as well (Table 3), but the smaller sample sizes rendered the differences not significant.

Body weight, spleen weight, spleen index, lymphocytes/spleen and lymphocytes/mg spleen weight. — Weighings throughout life for all groups are given in Table 4. Weighings at the time of sacrifice in four dietary groups at three ages (Table 5) showed greatest differences between the N/N and R/N and the N/R and R/R groups. Similar differences, with some variations obtained for spleen weight, spleen index, lymphocytes/spleen, and lymphocytes/mg spleen weight, but only at 141 to 167 weeks of age (Table 5).

Mitogenic and MLC responses. — Differences between dietary groups in responses to the mitogens were generally more significant at 141 to 161 weeks of age than at earlier ages, and more significant for PHA and Con A than for PPD (Table 6). At 141 to 161 weeks the ranking was R/R > N/R > R/N > N/N. PPD responsiveness declined significantly with age only in the N/N group, whereas both PHA and Con A responsiveness generally declined with age in all groups, though not always to an equivalent degree, as reflected by the PHA/Con A ratios. For the MLC (Table 6), which measures the T-cell proliferative response to alloantigens, the N/R response was superior to that of other groups at both ages tested. All groups showed a decline in advanced age.

Response to SRBC. — For technical reasons only small sample sizes were available for the T-dependent PFC assay for most dietary groups at 21 to 25 and 46 to 50 weeks of age, and no N/N animals were available at 164 to 167 weeks of age. Although comparisons were not uniformly statistically significant, the N/R and R/R groups, by a wide margin, had direct (IgM) and indirect (IgG) PFC responses below those of the N/N and R/N groups at 21 to 25 weeks of age, but at 46 to 50 weeks of age responses in all the groups were similar (data not shown). At 164 to 167 weeks of age the R/R group showed greater numbers of direct PFC/10^6 spleen cells than the N/R group and, especially, the R/N group. Indirect PFC responses were negligible in all groups tested at this age. Judging by the changing patterns of responsiveness over time, the data, although limited, suggest that the N/R, and particularly the R/R, diet postponed the maturation and, at least to some extent, the decline of the PFC responses relative to that of N/N and R/N.

Body temperature. — Mean body temperature was slightly but significantly lower in the R/R group than in the N/N group at 16 ($p < .0009$), 87 ($p = .0045$), and 145 ($p = .0117$) weeks of age (Table 7).

Discussion

This report documents the effects of various periods of dietary restriction on survival, tumor patterns, immune function, body weight and splenic parameters, and body temperature. On the basis of mean and maximum survival and mortality rate past 120 weeks of age, the R/R diet was superior to all others, with the N/R diet a close second. Survivals of N/N/R, N/R/N, and R/R/N were superior to those of R/N and N/N animals, and R/N was slightly better than N/N. Thus, while life-long restriction yielded best results in terms of most of the parameters measured, restriction during any portion of the life span appeared beneficial to some degree. Survival results reported previously for C57BL/6J mice (Cheney et al., 1980) largely paralleled those reported here, except that life-span extension in the restricted C57BL/6J mice was less pronounced.

Tumor frequency appeared to depend on the *timing* of the period of restriction. Thus, the N/N/R and N/R groups, which had in common restriction from 61 weeks of age to death, had strikingly fewer tumors than all other groups except R/R; the N/R/N, R/R/N, N/R, and R/R groups, which had in common restriction from weaning until 61 weeks of age, had a greater proportion of hepatomas within their tumor-bearing populations than the other groups.

The increase in life span in the N/N/R group over that of the N/N group may have resulted at least partly from its lower tumor frequency rather than from decelerated aging per se. The shift, however, in the mean ages at death for tumor-bearing animals to later ages, with or without a reduction in tumor frequency, in the N/R/N, R/R/N, and R/R groups, and to a lesser extent in the N/R and R/N groups, compared with the N/N group is consistent with a model of decelerated aging (Walford, 1974) which predicts that the frequencies of the various diseases of aging will not necessarily be altered by methods that decelerate aging but that their age-specific incidences will be shifted to later ages. Within our range of dietary groups, the N/R diet would appear to come closest to the ideal, with relatively few tumors and good longevity. The N/N/R diet, which gave significant reduction in tumor incidence but less extension of survival, would appear most applicable to humans.

Dietary restriction also appears to alter the maturation and/or age-related decline of immune function. In our earlier studies with C57BL/6J mice

Table 4. Body Weight Throughout Life in Seven Dietary Groups of B10C3F$_1$ Female Mice

Age (weeks)	Diet Group						
	N/N	R/N	N/R	R/R	N/N/R	N/R/N	R/R/N
30	29.0 ± .2 (30)	26.7 ± .2 (30)	17.6 ± .2 (30)	17.8 ± .2 (30)			
60	30.9 ± .7 (30)	28.4 ± .5 (30)	20.4 ± .3 (30)	19.5 ± .3 (30)			
90	32.9 ± .4 (27)	29.6 ± .5 (29)	21.3 ± .3 (29)	20.7 ± .3 (30)	21.3 ± .2 (29)	26.2 ± .4 (29)	25.3 ± .3 (27)
120	29.7 ± .6 (26)	28.5 ± .7 (23)	20.8 ± .4 (26)	19.5 ± .2 (29)	19.7 ± .2 (29)	26.8 ± .3 (28)	26.6 ± .4 (27)
150	29.4 ± .5 (17)	27.9 ± .6 (15)	16.7 ± .2 (21)	17.6 ± .1 (28)	17.7 ± .2 (22)	28.4 ± .3 (27)	28.0 ± .5 (20)
180	27.5 ± 2.0 (2)	25.3 ± 2.3 (2)	16.9 ± .3 (15)	16.9 ± .4 (14)	17.3 ± .3 (11)	25.4 ± .6 (11)	25.4 ± 1.1 (10)
210	(0)	(0)	(0)	14.0 (1)	(0)	(0)	(0)

Note. Entries are mean body weight in grams plus or minus the standard error of the mean. Sample sizes are in parentheses.

Table 5. Body Weight, Spleen Weight, Spleen Index, Lymphocytes/Spleen and Lymphocytes/Mg Spleen Weight in Four Dietary Groups of B10C3F$_1$ Female Mice Sacrificed at Three Different Ages

Parameter	Diet group	Age (weeks)		
		21–25	46–50	141–167
Body weight (g)[a]	N/N	23.7 ± 1.0 (6)	26.9 ± .9 (8)	29.9 ± .9 (9)
	R/N	24.5 ± 1.0 (6)	26.3 ± .9 (8)	27.5 ± .6 (18)
	N/R	19.5 ± 1.0 (6)	22.8 ± .9 (8)	20.1 ± .5 (31)
	R/R	18.9 ± 1.0 (6)	23.6 ± .9 (8)	20.3 ± .5 (31)
Spleen weight (mg)[b]	N/N	81.2 ± 6.0 (6)	83.7 ± 5.2 (8)	72.7 ± 4.9 (9)
	R/N	105.6 ± 6.0 (6)	97.6 ± 5.2 (8)	76.0 ± 3.5 (18)
	N/R	89.3 ± 6.0 (6)	85.0 ± 5.2 (8)	27.0 ± 2.7 (31)
	R/R	62.4 ± 6.0 (6)	67.5 ± 5.2 (8)	27.8 ± 2.7 (31)
Spleen index[c]	N/N	3.42 ± .23 (6)	3.13 ± .20 (8)	2.45 ± .19 (9)
	R/N	4.34 ± .23 (6)	3.75 ± .20 (8)	2.78 ± .13 (18)
	N/R	4.57 ± .23 (6)	3.72 ± .20 (8)	1.34 ± .10 (31)
	R/R	3.43 ± .23 (6)	2.88 ± .20 (8)	1.37 ± .10 (31)
Lymphocytes per spleen × 10^{-6}[d]	N/N	187.0 ± 15.4 (5)	195.6 ± 12.1 (8)	not done[f]
	R/N	220.0 ± 14.0 (6)	175.6 ± 12.1 (8)	171.6 ± 13.0 (7)
	N/R	172.1 ± 14.0 (6)	176.3 ± 12.1 (8)	53.6 ± 12.1 (8)
	R/R	137.5 ± 14.0 (6)	175.3 ± 12.1 (8)	47.0 ± 12.1 (8)
Lymphocytes × 10^{-6} per mg spleen weight[e]	N/N	2.28 ± .20 (5)	2.36 ± .16 (8)	not done[f]
	R/N	2.10 ± .18 (6)	1.83 ± .16 (8)	2.09 ± .17 (7)
	N/R	1.97 ± .18 (6)	2.11 ± .16 (8)	1.60 ± .16 (8)
	R/R	2.41 ± .18 (6)	2.61 ± .16 (8)	1.49 ± .16 (8)

Note. The entries are means plus or minus SEM with *ns* in parentheses. The SEMs are based on pooled variance estimates that include all dietary groups at all ages. Labels of dietary groups are explained in Table 1. Significance values for statistical comparisons are given in footnote to each parameter. Abbreviations for ages are Y (21 to 25 weeks), M (46 to 50 weeks), and O (141 to 167 weeks).

[a]N/N Y, R/N Y > R/R Y (p = .0360, .0060); R/N Y > N/R Y (p = .0330); N/N O, R/N O > N/R O, R/R O (ps < .0030); R/R M > R/R Y (p = .0224); R/R M > R/R O (p = .0390); N/N O > N/N Y (p < .0030).

[b]R/N Y, N/R Y > R/R Y (p < .0030, .0090); R/N Y > N/N Y (p = .0270); R/N M > R/R M (p < .0030); N/N O, R/N O >N/R O, R/R O (ps < .0030); R/N O, N/R O, R/R O < R/N M, N/R M, R/R M, respectively (p = .0030, < .0030, < .0030); R/N O, N/R O, R/R O < R/N Y, N/R Y, R/R Y, respectively (ps < .0030).

[c]The spleen index is the spleen weight (mg) divided by body weight (g). N/R Y > N/N Y, R/R Y (p = .0180, .0210); N/N O, R/N O > N/R O, R/R O (ps < .0030); R/N O, N/R O, R/R O < R/N M, N/R M, R/R M, respectively (p = .0030, < .0030, < .0030); N/N O, R/N O, N/R O, R/R O < N/N Y, R/N Y, N/R Y, R/R Y, respectively (p = .0450, <.0030, < .0030, < .0030).

[d]R/N Y > R/R Y (p = .0030); R/N O > N/R O, R/R O (ps < .0030); N/R O, R/R O < N/R M, R/R M, respectively (ps < .0030); N/R O, R/R O < N/R Y, R/R Y, respectively (ps < .0030); N/R O, R/R O < N/R Y, R/R Y, respectively (ps < .0030).

[e]R/R M > R/N M (p = .0200); R/R O < R/R M, R/R Y (p < .0025, .0075).

[f]No animals were available.

Table 6. Mitogenic and MLC Responses in Four Dietary Groups of B10C3F$_1$ Female Mice

Response	Diet group	Age (weeks) 21–25	43–46	141–161
PHA[a]	N/N	384,768 ± 28.8% (5)	268,349 ± 18.6% (11)	38,045 ± 28.8% (5)
	R/N	362,660 ± 28.8% (5)	283,595 ± 28.8% (5)	110,739 ± 23.9% (7)
	N/R	512,743 ± 28.8% (5)	524,203 ± 28.8% (5)	247,343 ± 19.6% (10)
	R/R	396,551 ± 28.8% (5)	407,005 ± 28.8% (5)	287,011 ± 23.9% (7)
Con A[b]	N/N	1,388,353 ± 21.5% (5)	822,242 ± 14.0% (11)	206,680 ± 21.5% (5)
	R/N	1,592,575 ± 21.5% (5)	1,212,271 ± 21.5% (5)	478,409 ± 17.9% (7)
	N/R	1,771,740 ± 21.5% (5)	1,912,493 ± 21.5% (5)	544,628 ± 16.6% (8)
	R/R	2,285,598 ± 21.5% (5)	1,591,109 ± 21.5% (5)	700,164 ± 19.4% (6)
PHA/Con A ratio[c]	N/N	.2771 ± 17.6% (5)	.3264 ± 11.6% (11)	.1841 ± 17.6% (5)
	R/N	.2277 ± 17.6% (5)	.2340 ± 17.6% (5)	.2315 ± 14.7% (7)
	N/R	.2894 ± 17.6% (5)	.2741 ± 17.6% (5)	.3092 ± 16.0% (6)
	R/R	.1735 ± 17.6% (5)	.2558 ± 17.6% (5)	.4248 ± 19.9% (4)
PPD[d]	N/N	234,530 ± 26.5% (5)	121,283 ± 20.4% (8)	75,910 ± 26.5% (5)
	R/N	186,552 ± 26.5% (5)	247,970 ± 26.5% (5)	157,217 ± 23.9% (7)
	N/R	174,220 ± 26.5% (5)	194,177 ± 26.5% (5)	208,881 ± 30.0% (4)
	R/R	179,556 ± 26.5% (5)	145,344 ± 26.5% (5)	246,263 ± 35.4% (3)
MLC[e]	N/N	277,971 ± 16.1% (5)	247,286 ± 14.6% (6)	86,977 ± 21.2% (3)
	R/N	412,762 ± 16.1% (5)	186,594 ± 16.1% (5)	54,613 ± 21.2% (3)
	N/R	526,865 ± 16.1% (5)	380,189 ± 16.1% (5)	not done[f]
	R/R	419,082 ± 16.1% (5)	338,064 ± 16.1% (5)	not done[f]

Note. The entries are means of the antilogarithms of decays/minute (except the entries for the PHA/Con A ratios, which are the means of the antilogarithms of the PHA/Con A ratios) plus or minus SEM with *ns* in paretheses. The SEMs are based on pooled variance estimates that include all dietary groups at all ages. Labels of dietary groups are explained in Table 1. Significance values for statistical comparisons are given in footnote to each parameter. Abbreviations for ages are Y (21 to 25 weeks), M (43 to 46 weeks), and O (141 to 161 weeks).

[a]N/R O, R/R O > N/N O (*ps* < .0030); N/N O < N/N M (p < .0030); N/N O, R/N O < N/N Y, R/N Y, respectively (p < .0030, .0210).

[b]N/R M > N/N M (p = .0210); N/R O, R/R O > N/N O (p = .0060, < .0030); N/N O, R/N O, N/R O < N/N M, R/N M, N/R M, respectively (p < .0030, .0180, < .0030); N/N O, R/N O, N/R O, R/R O < N/N Y, R/N Y, N/R Y, R/R Y, respectively (*ps* < .0030).

[c]R/R O ≥ N/N O (p = .0330); R/R O > R/R Y (p = .0150).

[d]N/N O < N/N Y (p < .0390).

[e]N/R M > R/N M (p = .0378); R/N M < R/N Y (p = .0126); R/N O < R/N M, R/N Y (*ps* < .0021).

[f]Insufficient spleen cells were available in these animals to perform both the MLC and the mitogenic assays.

Table 7. Body Temperature in Two Dietary Groups of B10C3F$_1$ Female Mice at Three Ages

Diet group	Age (weeks) 16	87	145
N/N	38.7 ± .10 (10)	37.3 ± .10 (10)	37.9 ± .15 (5)
R/R	38.0 ± .10 (10)	36.6 ± .19 (3)	37.4 ± .11 (9)

Note. Entries are means plus or minus SEM with *ns* in parentheses. The SEMs are based on pooled variance estimates that include all groups. Labels of dietary groups are explained in Table 1. Abbreviations for ages are Y (16 weeks), M (87 weeks), and O (145 weeks). Significance values for statistical comparisons are: N/N Y, N/N M, N/N O > R/R Y, R/R M, R/R O, respectively (p < .0009, .0045, .0117); N/N M, R/R M < N/N Y, R/R Y, respectively (*ps* < .0009); N/N O, R/R O > N/N M, R/R M, respectively (p = .0018, .0009); N/N O, R/R O < N/N Y, R/R Y, respectively (*ps* < .0009).

(Gerbase-DeLima et al., 1975), both T- and B-cell responses appeared to peak later in life in N/R and R/R mice than in N/N mice. We termed this delayed peaking a reversal effect (Walford et al., 1977) to signify that whereas the N/N responses were higher than the N/R and R/R responses at earlier ages, the opposite was true at later ages. In the B10C3F$_1$ mice tested here only PFC responses were lower early in life and generally similar or higher later in life in N/R and R/R compared with N/N and R/N mice. In contrast, mitogenic and MLC responses were similar or higher at all ages tested in N/R and R/R mice compared with N/N and R/N mice. Thus, if a reversal occurred in the mitogenic and MLC responses, it was probably prior to the first age group assayed.

Differences in PHA/Con A ratios noted here with respect to age and diet may reflect differences in T-cell subclasses (Meredith et al., 1975). The question arises whether actual numbers of cells and/or the proportion of responding cells differ within each subclass according to age and diet. Recent results (Weindruch et al., 1982b) suggest that PHA responsiveness in restricted mice may be

increased at least in part by a greater proportion of responding cells. Our determinations of splenic weight, splenic index, lymphocytes/spleen, and lymphocytes/mg spleen weight disclosed rather profound effects of dietary restriction in some instances, but none of these would appear to explain the differences in immunological function with age and diet, particularly the perceived differences in T-cell function.

Because of the limited number of animals, it was not possible to assess the effects of midlife dietary changes on immune function in the N/N/R, N/R/N, and R/R/N groups. Other studies (Mann, 1978), including those from our laboratory (Weindruch et al., 1982a), reported that dietary restriction begun at midlife can retard the age-related decline of T-dependent immune responses.

Body temperature was also altered by dietary restriction in our mouse populations. At each of three ages from youth to old age, R/R mice showed slightly but significantly lower mean body temperatures than N/N mice. During the weeks in which body temperature measurements were taken, the R/R mice were fed .5 portion/day, Monday through Thursday, rather than full portions on alternate days; preliminary measurements suggested that differences in body temperature between the N/N and R/R mice were lessened on days when both were fed, but were magnified on days when only the N/N mice were fed. Thus, while measurements reported here do not address the very real possibility that daily fluctuations in body temperature may have occurred in the restricted mice due to alternate-day feeding, they do document the genuine lowering of body temperature due to food restriction. We note that even intermittent body temperature lowering may be useful in extending survival. Mammals and birds that display certain degrees of heterothermicity (i.e., undergo seasonal or diurnal periods of torpor during which time body temperature falls) enjoy unusually long life spans compared with similar species that are stricter homeotherms (Liu & Walford, 1972). In a study reported by Leto et al. (1976), body temperature was about .63°C lower throughout life and mean and maximum survival were extended in C57BL/6J female mice fed a 4% protein diet compared with those fed a 26% protein diet. The mice on the 4% protein diet consumed about 90% of the calories of those on the 26% protein diet. It is well established that life span can be increased in poikilotherms such as annual fish (Liu & Walford, 1972) and *Drosophila* (Maynard-Smith, 1962) by lowering environmental temperature. Because lowering body temperature, at least in poikilotherms, is immunosuppressive (Liu & Walford, 1972), it seems unlikely that the preservation of immune function with age in restricted animals is a temperature-induced effect. It is difficult to evaluate from existing data what role, if any, the mild lowering of body temperature in restricted animals may play in their prolonged survival, altered tumor frequency, and age-specific incidence of tumors.

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