Meal-Timing, Circadian Rhythms and Life Span of Mice[1]

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ABSTRACT The possibility that circadian rhythm alteration may contribute to the life-prolonging effect of food restriction was investigated in female CD2F$_1$ mice housed in a room with a 12-h span of fluorescent lighting daily. A control group was allowed to feed ad libitum throughout life while three other groups began lifelong restriction to about 75% of ad libitum intake when 6 wk old. The daily schedule of food accessibility differed among these three groups: *1*) a single meal during early darkness; *2*) a single meal during early light; *3*) six smaller meals at about 2-h intervals during darkness. Food restriction as such clearly prolonged life, but there were no statistically significant differences in overall mean life span or in 10th-decile life span among the three restricted groups. Telemetered body temperature data confirmed marked differences in the effects of these different restricted feeding schedules on circadian rhythms. The effect of food restriction on survival is probably not due to altered relations among circadian rhythmic variables. Possible contributing factors suggested by the results are a lower body temperature, a reduced overall metabolic rate and an increased circadian amplitude. J. Nutr. *116*: 2244–2253, 1986.

INDEXING KEY WORDS meal-timing • rhythms • life span

Dietary restriction can delay the onset of degenerative diseases and prolong the life of rats and mice (1–4). In most studies on this subject the animals were provided a reduced daily food allotment and probably consumed it within a few hours (5). In effect they were meal-fed, a term applied to animals permitted to feed only during a short time span each day. Consistent with this interpretation is the observation that meal-fed rats eat less and live longer than do ad libitum-fed controls (6).

Meal-feeding is known to alter aspects of carbohydrate and lipid metabolism (7, 8); however, it also changes many circadian rhythms, the kind and extent of change depending on the particular rhythm and on the timing of the meal in relation to the daily lighting regimen (9–14). As a result, time-varying relations among a number of variables, including body temperature, serum corticosterone concentration, liver glycogen content, and measures of cellular proliferation are modified. Circadian rhythm alteration is also observed when food restriction results from alternate-day feeding (15, 16) or from feeding a low protein diet ad libitum (17).

The study described herein sought to determine whether the effect of food restriction on survival could be at least partly attributed to effects on circadian rhythms. The life span and core temperature rhythm of mice feeding ad libitum were compared with those of mice in three other groups, all with food intake restricted to the same extent but on different feeding schedules.

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MATERIALS AND METHODS

Female CD2F$_1$ mice produced in our colony were weaned at about 4 wk of age and housed three per plastic cage (25 × 15 × 13 cm), with San-I-Cel bedding (Paxton Processing Co., Paxton, IL) in a room maintained at about 24 °C, with about 50% relative humidity, and on a daily schedule of fluorescent lighting from 0600 to 1800, darkness from 1800 to 0600. A nonpurified diet (Purina Laboratory Chow, a constant-formula product, Ralston Purina Co., St. Louis, MO) was fed throughout the study, always within 6 mo of manufacture. Water was continuously available. When the mice were about 6 wk old the cages were distributed by an essentially random weaving procedure among four groups, and any necessary exchanges were made so that body weight means and variances of the groups were similar. The groups were then assigned to four different feeding regimens, the largest group remaining on ad libitum feeding (AL) and the other three restricted to ~75% of ad libitum intake but on different daily schedules: *1*) a single meal beginning at the onset of darkness (meal-fed in darkness, MFD); 2) a single meal beginning at the onset of light (meal-fed in light, MFL); and 3) six smaller meals at intervals of ~2 h beginning at the onset of darkness and ending near the onset of light, a regimen intended to approximate the pattern of ad libitum feeding (pattern-fed, PF). These schedules of food restriction were implemented with an automatic feeding apparatus (18), which controlled the timing and duration of food accessibility. For each schedule, the duration of the meal or meals was adjusted, on the basis of regular monitoring of food intake, to achieve the desired average level of restriction. Spans of food accessibility at different stages of restriction are presented in table 1.

The above procedure was performed on four separate cohorts (A–D) of mice produced from BALB/cANN females and DBA/2N males (Charles River Laboratories, Wilmington, MA) at about 3-mo intervals. This method of accumulating animals was adopted for several reasons: *1*) it allowed the production of the desired large total number of mice from a relatively small breeding colony; 2) it could provide evidence that infectious disease was not a factor in determining life span if there were no sudden mortality increases in all cohorts simultaneously; 3) if the effect of feeding regimen varied systematically among cohorts, a seasonal influence could be indicated. The overall total number of mice involved in the study was 446, with 168, 92, 92 and 94 in the AL, MFD, MFL and PF groups, respectively.[2]

TABLE 1

Spans of food accessibility for restricted groups at different stages of study

Week of restriction	Group[1]		
	MFD	MFL	PF
	h		*min*
1	4	3	6 × 20
12	4	3	6 × 11
52	3.25	2.75	6 × 9
143 to end	3.25	2.75	6 × 12

[1]MFD, meal-fed early darkness; MFL, meal-fed early light; PF, pattern-fed (6 times at ~2-h intervals during darkness).

To monitor effects of different feeding schedules on a representative circadian rhythm, 21 mice on each regimen (6, 6, 6 and 3 from cohorts A, B, C and D, respectively) were implanted with temperature transensors (Model M Mini-Mitter, Mini-Mitter Co., Sunriver, OR). These transensors provided temperature data from each animal at 10-min intervals for as long as 9 mo. The resulting large amount of data was processed by first computing hourly mean temperatures for each animal; these were then respectively averaged across the members of a given group and across all days of a time span under examination (e.g., one week) to provide an average 24-h pattern of hourly means for that time span. The age of the mice at the time of transensor implantation varied progressively among cohorts so that successive life stages (and so increasing spans

[2]Groups in this study also served as controls for a concurrent investigation of the effects of various shift-schedules on rhythms and life span. A report on the latter research, involving an additional 412 female CD2F$_1$ mice, is in press (Physiol. Behav.).

of food restriction) were subjected to examination of circadian temperature rhythms.

Mortality, environmental conditions and feeder operation were checked daily. Clean cages, bedding and water bottles were provided weekly, at which times food was replenished and the mice were examined for tumors. For each cohort, body weight and food intake were determined for a set of animals on each feeding regimen, initially at weekly intervals but less frequently as the study progressed. As mice died, the number per cage was maintained at two or three, by moving survivors among cages of the same cohort and regimen; this was done because huddling was considered an important factor in energy balance and so could affect survival, especially for mice on a restricted feeding schedule.

RESULTS

Data from the four cohorts were pooled to summarize food intake and body weight changes on the four feeding regimens (fig. 1). Results are based on the same mice through the first year of study for each cohort, at which time there had been no deaths or tumors observed. As the figure indicates, data from animals not previously monitored were included thereafter, to compensate for attrition in the originally monitored sets.

Food intake was very similar for the three restricted groups: When expressed as percentage of AL intake, the across-cohort means of weekly values covering the entire first 46 wk of restriction (after which weekly weighings were discontinued) were 75.8, 75.1 and 76.8% for MFD, MFL and PF

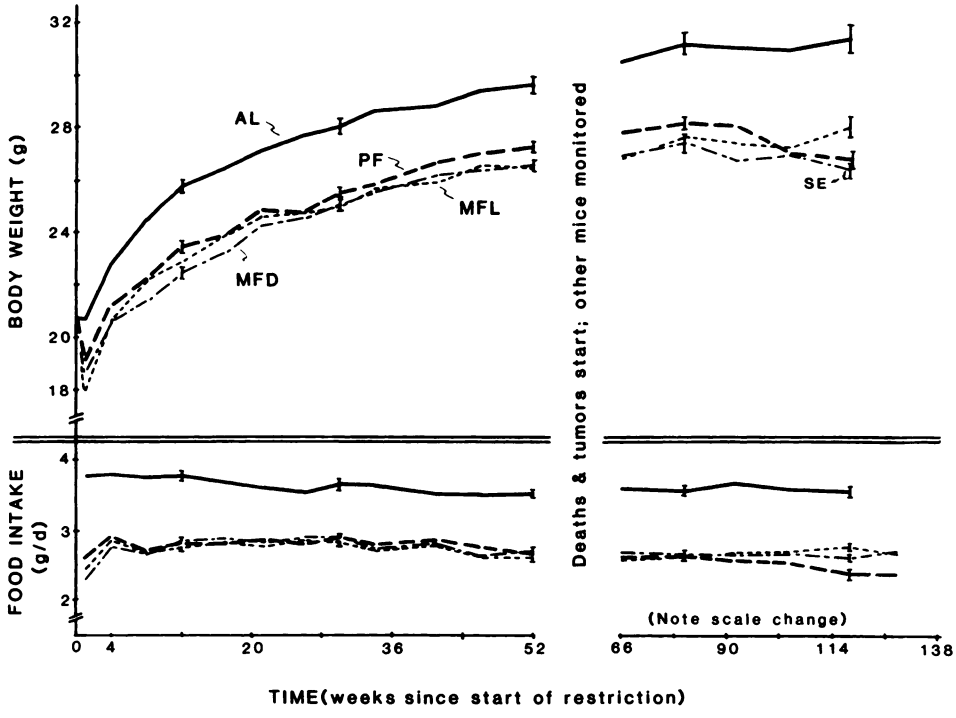

Fig. 1 Food intake and body weight of female CD2F$_1$ mice on 4 different regimens of food accessibility: ——, ad libitum (AL); —·—, early darkness only (MFD); ----, early light only (MFL); — —, 2-hourly during darkness (PF). Data pooled across cohorts. Each curve connects results plotted at ~4-wk intervals during the first year and at ~12-wk intervals thereafter . Body weight determined ~8.5 h after last food access for restricted groups, ~8.5 h after end of dark span for AL group; data from 68–70 mice on each regimen during first year and from variable number thereafter. Food intake determined as average per mouse during week, based on change in weight of food hopper and number of mice per cage; data from 25–26 cages on each regimen during first year and from variable number thereafter.

groups, respectively. Analysis of variance indicated that the differences among these means were not statistically significant ($F = 0.86$, df = 2,9, $P > 0.05$). Despite the similarity of food intake among the three restricted groups, the PF group was consistently slightly heavier than the MFD or the MFL group, at least until near the end of the second year of restriction. At that time food consumption by the remaining animals in the PF group began to decline, possibly due to an inability of the old animals to feed vigorously during the allotted short time spans. Increases in these spans stopped the decline after 118 wk and returned food intake close to that of the meal-fed groups. Thus, after about 3 yr of restriction (5 mo after the end of the plot in fig. 1), average food intakes by survivors in the MFD, MFL and PF groups on the final schedules shown in table 1 were 2.58, 2.63 and 2.54 g/d, respectively.

In assessing the effect of feeding schedule on the circadian temperature rhythm monitored by telemetry, it was necessary to consider the fact that the temperature calibration of the transensors tended to drift gradually over the several-month-long span in situ. Therefore, only data obtained soon after transensor calibration and implantation and data obtained just before transensor removal and recalibration are here considered. Figure 2 portrays 24-h patterns of core temperature variation in mice on the different feeding regimens. Differences in form and in time points of high and low hourly means are clearly evident. The data summarized in this figure were obtained over a 4-d span from mice of cohort C when they were about 71 wk old. Similar results were obtained from other cohorts and at other ages ranging from 14 to 96 wk. Any age trend in the characteristics of such patterns of temperature variation was contraindicated by the application of linear-regression analysis and tests of zero-slope to the 24-h overall mean, lowest hourly mean and range of hourly means. Analysis of variance then demonstrated a statistically significant effect of feeding regimen on these endpoints (table 2). Tukey's ω-procedure (19) indicated a higher value for the overall mean and for the lowest hourly mean and a shorter range of hourly means for the AL group compared with any one of the restricted groups. Among the latter, there were differences in range (PF shorter than MFD or MFL) but not in overall mean or in lowest hourly mean.

An earlier study on the effects of MFD and MFL schedules on circadian rhythms in rectal temperature, serum corticosterone and liver glycogen of female BALB/c mice (12) revealed effects similar to those described above for telemetered intraperitoneal temperature, although in the case of serum corticosterone and liver glycogen the 24-h mean was *higher* in the restricted groups than in the AL group. Subsequent work comparing effects of PF and MFD schedules on these same rhythms indicated that the distribution of a restricted amount of food among six to eight small meals, concentrated during the daily span of darkness, acted to reduce the circadian amplitude as compared to that seen when the same amount of food was consumed as a single meal (20). The responses of telemetered intraperitoneal temperature to the MFD, MFL and PF schedules in the present study may thus be considered representative of responses by at least some other variables.

Table 3 summarizes data on life span as a function of feeding regimen and cohort. The overall mean ± SE for the AL group was 116.8 ± 1.6 wk, consistent with a literature value of 121.7 ± 5.2 wk for the life span of $CD2F_1$ females (21). As expected, food restriction prolonged survival time, the overall mean ± SE of the combined restricted groups being 138.4 ± 1.7 wk. Application of analysis of variance to data summarized by the 4 × 4 layout in table 3 was questionable because of the heterogeneity of variances: Bartlett's test (22) yielded $\chi^2 = 37.9$, df = 15, $P < 0.01$. Because of this, the significance of the difference between the unweighted means of the AL and combined restricted groups was tested using separate variance estimates from each of the 16 cells to estimate the standard error (23): $t = 8.91$, estimated df = 338, $P < 0.001$.

It seemed appropriate to consider the data on survival time of the restricted groups separately, since the main intent of this investigation was to look for possible differences in life span among mice on different restricted feeding schedules. In

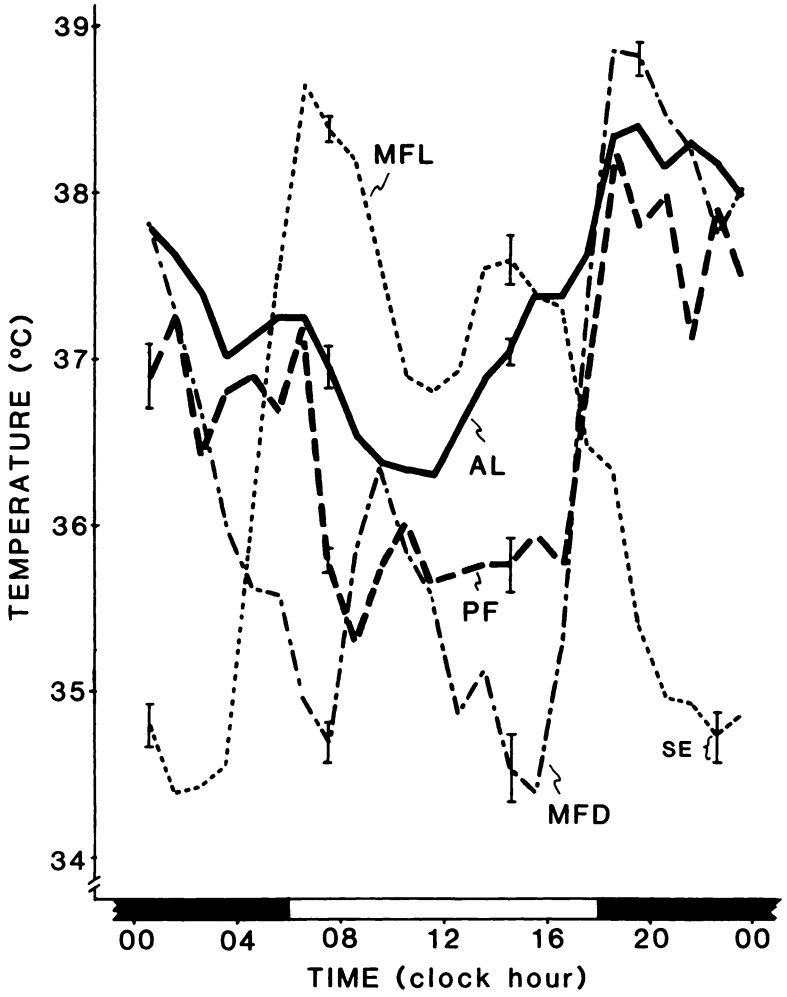

Fig. 2 Twenty-four-hour patterns of telemetered temperature variation in female CD2F$_1$ mice on 4 different feeding regimens. See figure 1 legend for key. Each curve connects 24 hourly means, each representing data from 6 mice over 4-d span. SE plotted only at a few time points to avoid confusion.

that case, the use of analysis of variance was valid because the condition of variance homogeneity among the 12 cells (3 schedules, 4 cohorts) was satisfied (χ^2 = 17.13, df = 11, $P > 0.05$). Results from analysis of variance indicated that differences among the 12 cell means were not statistically significant (F = 0.83, df = 11,266, $P > 0.05$). Thus, neither feeding schedule (MFD, MFL or PF) nor cohort (season of birth) had a demonstrable effect on survival time of the restricted mice. (The absence of a cohort effect was also indicated by applying analysis of variance to data from the AL groups: F = 1.17, df = 3,164, $P > 0.05$).

Survival curves (fig. 3) present data pooled across the four cohorts. Application of the Kolmogorov-Smirnov test (24) to these data indicated no statistically significant departure from a normal distribution on any of the four feeding regimens. Both figure 3 and table 3 include data from mice that bore transensors at some stage of their lives. Application of t-tests to data pooled across cohorts indicated no statistically significant effect of the transensor load on life span for

TABLE 2

Summary of telemetered core temperature data

Endpoint on 24-h scale (°C)	Group[1] AL	MFD	MFL	PF	*F*	*P*
Overall mean	37.65 ± 0.14*	36.39 ± 0.20	36.26 ± 0.14	36.16 ± 0.23	14.94	< 0.01
Lowest hourly mean	36.71 ± 0.13*	34.44 ± 0.28	34.29 ± 0.23	34.56 ± 0.38	17.87	< 0.01
Range of hourly means	1.90 ± 0.09*	4.40 ± 0.23	4.19 ± 0.24	3.32 ± 0.20*	32.83	< 0.01

[1]AL, ad libitum feeding; MFD, meal-fed early darkness; MFL, meal-fed early light; PF, pattern-fed (6 times at ~2-h intervals during darkness). Each value is mean (± SE) of 7 determinations covering age span 14–96 wk and represents total of 175, 184, 176 and 165 mouse-days for AL, MFD, MFL and PF regimens, respectively. *Difference from other means in row significant at $P < 0.05$.

either the AL group or the combined restricted groups ($t = 0.50$ and 0.54, respectively; $P > 0.05$).

The 10th-decile mean life span, an alternative to the overall mean as a measure of longevity (25), was computed from data pooled over cohorts, with results presented in table 4. This end point also clearly distinguishes the AL group from the restricted groups. The Kruskal-Wallis test (22) applied to the 10th-decile survival times of the three restricted groups indicated no statistically significant differences ($H = 1.19$; $P > 0.05$). (Parametric analysis of variance, although inappropriate due to inhomogeneity of variance, also indicated no statistically significant variation among the means of the 3 restricted groups: $F = 2.44$, df = 2,24, $P > 0.05$.)

Tumors, considered to be mostly mammary on the basis of location, appeared sooner and were more prevalent in the AL group than in the restricted groups (overall incidence: 36% vs. 21%; $\chi^2 = 10.5$, $P < 0.005$). There were no statistically significant differences among the three restricted groups ($\chi^2 = 1.28$, $P > 0.05$). Although histological examination of these tumors was not routinely performed, their occurrence seems an indicator of the quality of life in this study. The shorter average life span of the AL group cannot be attributed to its higher tumor incidence, however. On the contrary, the overall mean survival time of the tumor-bearing AL animals was greater than that of the non-tumor-bearing (123.7 vs. 113.0 wk; $t = 3.27$, df = 166, $P < 0.01$), probably because the tumors developed relatively late in life.

DISCUSSION

The three different restricted feeding schedules employed in this study all increased

TABLE 3

Survival time (weeks) of mice from 4 cohorts, on 4 different feeding regimens

Cohort[1]	Group[2] AL	MFD	MFL	PF
A	112.9 ± 2.7 (48)	128.4 ± 4.7 (29)	137.3 ± 3.9 (30)	143.3 ± 4.4 (30)
B	115.9 ± 3.4 (46)	136.8 ± 7.2 (21)	135.0 ± 8.0 (21)	131.5 ± 5.3 (22)
C	120.7 ± 2.9 (38)	141.7 ± 6.5 (24)	145.1 ± 6.1 (23)	142.3 ± 5.4 (24)
D	119.1 ± 3.8 (36)	136.8 ± 8.6 (18)	143.2 ± 4.9 (18)	140.8 ± 6.4 (18)
Overall	116.8 ± 1.6 (168)	135.4 ± 3.2 (92)	139.9 ± 2.9 (92)	139.8 ± 2.6 (94)

[1]Cohorts A, B, C and D born in February, May, August and November, respectively. [2]AL, ad libitum feeding; MFD, meal-fed early darkness; MFL, meal-fed early light; PF, pattern-fed (6 times at ~2-h intervals during darkness). Each value is mean ± SE for number of mice indicated in parentheses.

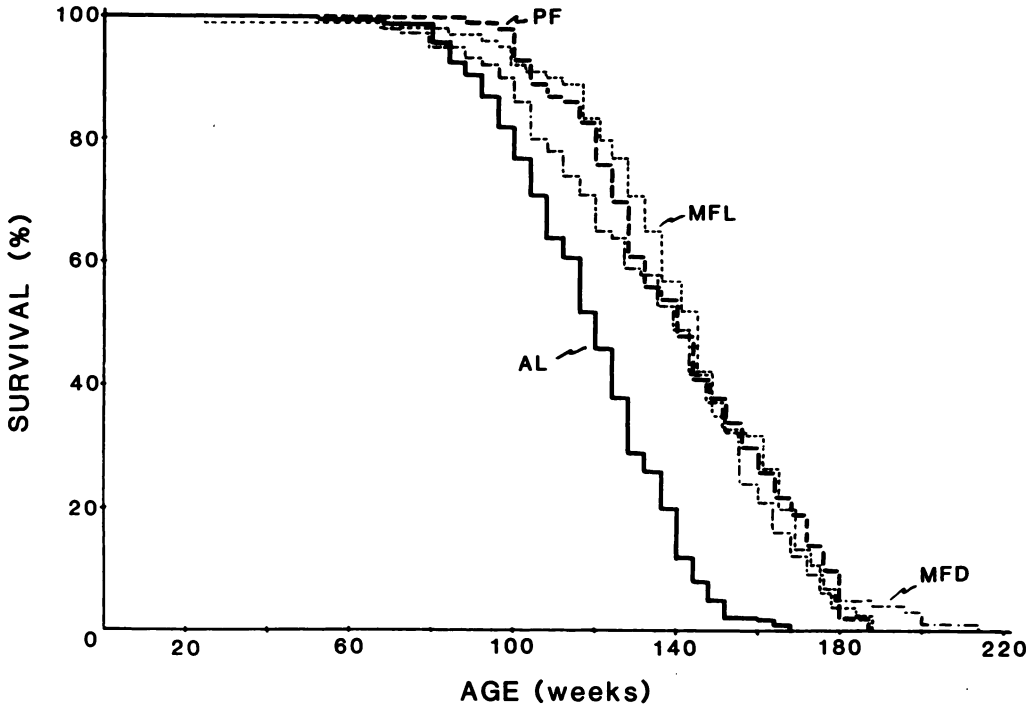

Fig. 3 Survival curves of mice on 4 different feeding regimens. See figure 1 legend for key. Data pooled across cohorts from totals of 168, 92, 92 and 94 mice in AL, MFD, MFL and PF groups, respectively. Plots were stepped at 4-wk intervals rather than at each occurrence of death(s) on weekly basis to enable use of different kinds of lines for different groups.

life span to a similar extent but affected rhythms in core temperature (fig. 2) and other variables (12, 20) in markedly different ways. This may mean that circadian rhythm alteration plays no role in the effect of food restriction on life span. On the other hand, the different kinds of alteration observed on the three schedules may have been equally beneficial. If a change in rhythms away from their usual relations is responsible for improved survival, then similar effects would be expected under other conditions known to produce such a change. Repeated shifting of the daily lighting schedule may either increase or decrease the survival of insects, depending on such factors as the interval between shifts (26, 27). The life span of rodents is not increased by weekly or twice-weekly shifting of the lighting schedule, however (27–29). The lack of a statistically significant effect of repeated schedule shifts on mean survival time was also apparent in an investigation (performed concurrently with the one described herein)

TABLE 4

Tenth-decile survival time (weeks) of mice on 4 different feeding regimens

Group[1]			
AL	MFD	MFL	PF
150.5 ± 2.0 (17)	187.6 ± 4.8 (9)	179.3 ± 1.5 (9)	179.7 ± 1.1 (9)

[1]AL, ad libitum feeding; MFD, meal-fed early darkness; MFL, meal-fed early light; PF, pattern-fed (6 times at ~2-h intervals during darkness). Each value is mean ± SE for number of mice indicated in parentheses.

in which mice were fed either ad libitum or a single daily meal and were subjected throughout postweaning life to weekly 12-h shifts of the daily lighting regimen, with or without shifts of the meal-feeding schedule (report in press, Physiol. Behav.). In a review questioning the "intrinsic significance of circadian order," mention is made of several generations of mice raised in continuous light without apparent ill effect (30). Presumably, the presence of any favorable effect would have been noted as well. Continuous lighting has been found to shorten the life span of an insect (31). The weight of evidence thus seems to be against a beneficial effect of altered rhythm relations, at least in the case of rodents. It remains possible, however, that only certain kinds of altered relations, achieved on all three of the restricted feeding schedules examined in the present study and perhaps by certain conditions of schedule shifting not yet tested in rodents, will result in improved health and extended life span.

Another interpretation of the results herein is that the rhythms examined merely exhibited masking effects (32) or an uncoupling of anticipatory rhythms (33) in response to the restricted feeding schedules, and that the underlying circadian system was unaltered. One might have expected, nevertheless, that schedules producing different relations among metabolic, hormonal and cellular indices (12–14) would have different long-term consequences reflected in survival.

It has been suggested that the strongly periodic nature of feeding by food-restricted animals may be responsible for the observed metabolic changes (7) and also for the extended life span (34). The PF group in the present study was allowed six short spans of feeding daily, the first at the onset of darkness and the last near the onset of light. Adherence to this schedule was confirmed by direct observation and also by remote recording of the activity of transensor-bearing mice, based on changes in field strength at the antennae. Thus, the longest span without food for this group was about 12 h, roughly comparable to that of ad libitum controls, for which feeding is largely nocturnal (35). In any event, the feeding periodicity in the PF group was surely less pronounced than that in the MFD and MFL groups, which received a single daily meal and fasted for 20 h or more. The absence of statistically significant differences in survival among these three restricted groups argues against a major role for feeding periodicity in the effect of food restriction on life span.

Although relations of circadian rhythms were changed in different ways by the different restricted feeding schedules, certain features of the rhythms did exhibit common effects that may relate to the similar extension of life span. Thus, the 24-h mean core temperature and the lowest hourly mean temperature were reduced to a similar extent on all three restricted feeding schedules (table 2). These findings are consistent with suggestions that food restriction exerts its life-prolonging effect by way of a reduced body temperature (34, 36). If one assumes a close correlation between core temperature and total metabolic rate, as has been demonstrated over a 24-h span in mice (37) and rats (38), these results are also consistent with the hypothesis that the increased life span of food-restricted animals is due to a lower rate of energy expenditure (34, 36). Some studies on meal-fed rats have in fact found a low 24-h mean metabolic rate in comparison with controls feeding ad libitum (39, 40). Other results (41, 42) do not support the hypothesis, however.

The 24-h range of hourly mean temperatures was increased on all three schedules, although to a smaller extent on the PF schedule than on the MFD or MFL schedules. This observation is similar to that described for other variables (12, 20).[3] It is therefore possible that life span is maximally extended if the extent of change within a 24-h span is maintained above a certain threshold, exceeded by all three restricted groups in the present study. If so, food restriction could counteract an age-related

[3] In previous reports on the subject from this laboratory, results concerning the extent of change within a 24-h span were expressed in terms of circadian amplitude, assessed by the fit of a 24-h cosine curve. In the case of telemetered temperature data from restricted mice in the present study, reliance on a simple cosine model could obscure points of possible interest such as secondary peaks, the low value and the range; estimates of the latter in figure 2 and table 2 are considered reliable because of the large data base. On the other hand, the use of a cosine model has the distinct advantage of providing quantitative estimates of a rhythm's amplitude, timing and overall average in one inferential statistical package. When applied to the data summarized in figure 2 and table 2, this method indicated an increased amplitude in all three restricted groups while taking into consideration changes in the other rhythm parameters.

decline in circadian amplitude, reported for core temperature (43) and for energy metabolism (37).

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LITERATURE CITED

1. McCay, C. M., Crowell, M. F. & Maynard, L. A. (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. J. Nutr. *10*, 63–79.
2. Berg, B. N. & Simms, H. S. (1960) Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. J. Nutr. *71*, 255–263.
3. Ball, Z. B., Barnes, R. H. & Visscher, M. B. (1947) The effects of dietary caloric restriction on maturity and senescence with particular reference to fertility and longevity. Am. J. Physiol. *150*, 511–519.
4. Lee, Y., Visscher, M. & King, J. (1956) Life span and cause of death in inbred mice in relation to diet. J. Gerontol. *11*, 364–371.
5. Berg, B. N. (1960) Nutrition and longevity in the rat. I. Food intake in relation to size, health and fertility. J. Nutr. *71*, 242–254.
6. Leveille, G. A. (1972) The long-term effects of meal-eating on lipogenesis, enzyme activity, and longevity in the rat. J. Nutr. *102*, 549–556.
7. Fabry, P. (1967) Metabolic consequences of the pattern of food intake. In: Handbook of Physiology, sect. 6, Alimentary Canal, vol. 1, Control of Food and Water Intake (Code, C. F., ed.), pp. 31–49, Am. Physiol. Soc., Washington, DC.
8. Leveille, G. (1970) Adipose tissue metabolism: influence of periodicity of eating and diet composition. Fed. Proc. *29*, 1294–1301.
9. Halberg, F. & Visscher, M. B. (1952) A difference between the effects of dietary caloric restriction on the estrus cycle and on the 24-hour adrenal cortical cycle in rodents. Endocrinology *51*, 329–335.
10. Halberg, F., Visscher, M. B. & Bittner, J. J. (1953) Eosinophil rhythm in mice: range of occurrence; effects of illumination, feeding and adrenalectomy. Am. J. Physiol. *174*, 109–122.
11. Hopkins, H. A., Bonney, R. J., Walker, P. R., Yager, J. D. & Potter, V. R. (1973) Food and light as separate entrainment signals for rat liver enzymes. Adv. Enzyme Reg. *11*, 169–191.
12. Nelson, W., Scheving, L. & Halberg, F. (1975) Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. J. Nutr. *105*, 171–184.
13. Philippens, K., Mayersbach, H. von & Scheving, L. (1977) Effects of the scheduling of meal-feeding at different phases of the circadian system in rats. J. Nutr. *107*, 176–193.
14. Lakatua, D., White, M., Sackett-Lundeen, L. & Haus, E. (1983) Change in phase relations of circadian rhythms in cell proliferation induced by time-limited feeding in BALB/c × DBA/2F_1 mice bearing a transplantable Harding-Passey tumor. Cancer Res. *43*, 4068–4072.
15. Potter, V. R., Gebert, R. A. & Pitot, H. C. (1966) Enzyme levels in rats adapted to 36-hour fasting. Adv. Enzyme Reg. *4*, 247–265.
16. Shankaraiah, K., Halberg, F., Yunis, E. & Watson, A. L. M. (1986) Alternate-day feeding alters the circadian system, reduces breast cancer incidence and prolongs life. Proc. 2nd Int. Conf. Medico-Social Aspects of Chronobiology, Florence, Oct. 2, 1984, (Halberg, F., Reale, L., Tarquini, B., eds.), Istituto Italiano di Medicina Sociale, Rome, pp. 345–366.
17. Leto, S., Kokkonen, G. C. & Barrows, C. H. (1976) Dietary protein, life-span, and physiological variables in female mice. J. Gerontol. *31*, 149–154.
18. Nelson, W., Fundakowski, R., Baer, J., Cadotte, L. & Halberg, F. (1982) An apparatus for automatically timing access to food by mice. Lab. Anim. Sci. *32*, 66–69.
19. Steel, R. & Torrie, J. (1960) Principles and Procedures of Statistics, pp. 109–110, McGraw-Hill.
20. Halberg, F., Nelson, W., Lakatua, D., Cadotte, L. & Haus, E. (1984) Circadian amplitude increase associated with caloric restriction of mice by single daily meal reduced by 'pattern feeding.' In: Chronobiology 1982-1983 (Haus, E. & Kabat, H., eds.), pp. 490–492, S. Karger, Basel.
21. Goodrick, C. L. (1975) Life-span and the inheritance of longevity of inbred mice. J. Gerontol. *30*, 257–263.
22. Sokal, R. & Rohlf, F. (1969) Biometry. W. H. Freeman & Co., San Francisco.
23. Nie, N., Hill, C., Jenkins, J. Steinbrenner, K. & Bent, D. (1975) Statistical package for the social sciences. McGraw-Hill, New York.
24. Lilliefors, H. W. (1967) On the Kolmogorov-Smirnov test for normality with mean and variance unknown. J. Am. Statist. Ass. *62*, 399–402.
25. Smith, G. S., Walford, R. L. & Mickey, M. R. (1973) Lifespan and incidence of cancer and other diseases in selected long-lived inbred mice and their F_1 hybrids. J. Natl. Cancer Inst. *50*, 1195–1213.
26. Aschoff, J., Saint Paul, U. v. & Wever, R. (1971) Die Lebensdauer von Fliegen unter dem Einfluss von Zeit-Verschiebungen. Naturwissenschaften *58*, 574.
27. Halberg, J., Halberg, E., Hayes, D., Smith, R., Halberg, F., Delea, C., Danielson, R. & Bartter, F. (1979) Schedule shifts, life quality and quantity-modeled by murine blood pressure elevation and arthropod life span. Int. J. Chronobiol. *7*, 17–64.
28. Halberg, F., Nelson, W. & Cadotte, L. (1977)

Living routine shifts simulated on mice by weekly or twice-weekly manipulation of light-dark cycle. Proc. XII Int. Conf. Int. Soc. Chronobiol., pp. 133–138.
29. Finger, F. (1983) Does repeated internal desynchronization shorten life? Chronobiologia *10*, 127.
30. Daan, S. & Aschoff, J. (1982) Circadian contributions to survival. In: Vertebrate Circadian Systems: Structure and Physiology (Aschoff, J., Daan, S. & Groos, G. A., eds.), pp. 305–320, Springer-Verlag, Berlin.
31. Pittendrigh, C. & Minis, D. (1972) Circadian systems: longevity as a function of circadian resonance in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *69*, 1537–1539.
32. Aschoff, J. (1960) Exogenous and endogenous components in circadian rhythms. Cold Spring Harbor Symp. Quant. Biol. *25*, 11–28.
33. Boulos, Z., Rosenwasser, A. M. & Terman, M. (1980) Feeding schedules and the circadian organization of behavior in the rat. Behav. Brain Res. *1*, 39–65.
34. Sacher, G. A. (1977) Life table modification and life prolongation. In: Handbook of the Biology of Aging (Finch, C. & Hayflick, L., eds.), pp. 582–638, Van Nostrand-Reinhold Co., New York.
35. Sigdestad, C., Connor, A., Sharp, J. & Ackerman, M. (1974) Evaluating feeding cycles of small rodents. Lab. Anim. Sci. *24*, 919–921.
36. Cutler, R. G. (1981) Life-span extension. In: Aging: Biology and Behavior (McGaugh, J. & Kiesler, S., eds.), pp. 31–76, Academic Press, Inc.
37. Sacher, G. A. & Duffy, P. H. (1978) Age changes in rhythms of energy metabolism, activity, and body temperature in *Mus* and *Peromyscus*. In: Aging and Biological Rhythms (Samis, H. V. & Capobianco, S., eds.), pp. 105–124, Plenum Press, New York.
38. Heusner, A. (1963) Analysis of the 24-hour variation of energy metabolism in the white rat. D.Sc. Thesis, University of Strasbourg, France.
39. Lackey, W., Broome, L., Goetting, J. & Vaughan, D. (1970) Diurnal pattern of rats determined by calorimetry under controlled conditions. J. Appl. Physiol. *29*, 824–829.
40. Sugano, Y. (1983) Heat balance of rats acclimated to diurnal 2-hour feeding. Physiol. Behav. *30*, 289–293.
41. Masaro, E. J., Yu, B. P. & Bertrand, H. A. (1982) Action of food restriction in delaying the aging process. Proc. Natl. Acad. Sci. USA *79*, 4239–4241.
42. McCarter, R., Masoro, E. J. & Yu, B. P. (1985) Does food restriction retard aging by reducing the metabolic rate? Am. J. Physiol. *248*, E488–E490.
43. Halberg, F. & Nelson, W. (1978) Chronobiologic optimization of aging. In: Aging and Biological Rhythms (Samis, H. V. & Capobianco, S., eds.), pp. 5–56, Plenum Press, New York.