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# Is late-life caloric restriction beneficial?

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*ABSTRACT. Caloric restriction initiated in young mice and rats results in increases in mean and median life span. When caloric restriction is implemented in older animals, an increase in life span is still observed; however, the magnitude of the increase is not as great as that observed in animals calorie restricted since they were young. Here we report the results of a pilot study in which caloric restriction was initiated in mature, older rats. Survival rates and terminal pathology were characterized and compared between a cohort of 17 continually* ad libitum *fed Long Evans rats and a cohort of 18 Long Evans rats, which were gradually introduced to 33% restriction in diet consumption at 18 months of age. No difference in the median life span was observed between the two groups. The data suggest there may be a level of maturity, or a stage in the aging process, after which caloric restriction no longer increases longevity.*
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## INTRODUCTION

The restriction of caloric intake without compromising nutritional adequacy, termed caloric restriction (CR), has repeatedly been shown to increase mean and maximum life span in mice and rats (1-3). CR has been demonstrated to delay onset and decrease incidence of a wide variety of pathologies (4), slow progression of many disease processes (2), and retard age-associated changes in a broad spectrum of physiologic parameters (3).

The most dramatic modulation of life span is observed in animals that begin CR as weanlings (5-7). The capacity of this dietary manipulation to increase longevity is statistically robust even when established in animals at one year of age (1). It has been suggested that the effect of CR on life span extension may be dependent upon and proportional to the duration and magnitude of the restriction (8).

Reviewing the early report of Vallejo in humans (9), and work with mice and rats (5-7), there are data to suggest that there may be a maximum age at which caloric restriction can be initiated and still impact favorably on longevity. Although there are many diseases exacerbated by obesity (10), the possibility that generalized weight loss in adult populations may not be of benefit, needs to be considered.

This is a late intervention pilot study designed to examine whether CR instituted in fully mature, old rats modulates aging as measured by changes in median life span.

## MATERIALS AND METHODS

A cohort of 35 male Long Evans rats was obtained from Harlan Sprague Dawley (Indianapolis, IN) at 9 months of age. They were individually caged with *ad libitum* access to Agway 3000 pelleted chow (Agway Country Foods, Syracuse, NY) and purified water. They were maintained in a room at 23°C and 45% humidity with a 12-hour light:dark cycle, and allowed to age without perturbation for an additional 9 months. During this time, their food intake was measured as the difference between presented food weight and remaining food weight. Consumption of chow averaged 30 g/day/rat. The animals were divided into two groups matched for weight; the control group of 17 rats were fed at their previous *ad libitum* consumption rate of 30 g/day (105 kcal/day), while the CR group of 18 rats were fed 20 g/day (70 kcal/day), which was 33% less than the control group. All animals continued to be fed Agway 3000 chow, and have free access to water. This diet was sufficient to provide micronutrients in ex-

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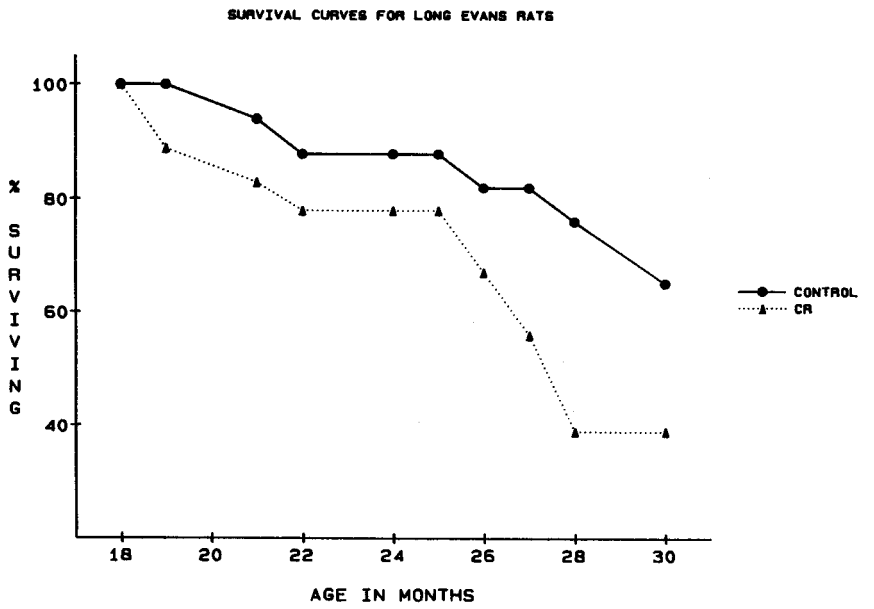


Figure 1 - *Survival curves for control and CR male Long Evans rats.*

cess of the established requirements (11) to both groups (data not shown). The animals were monitored daily for morbidity and mortality, and weighed monthly.

The experiment was terminated when 50% of the animals had died to insure that a sufficient sample size (N ≥7) for each group would be available to confirm the

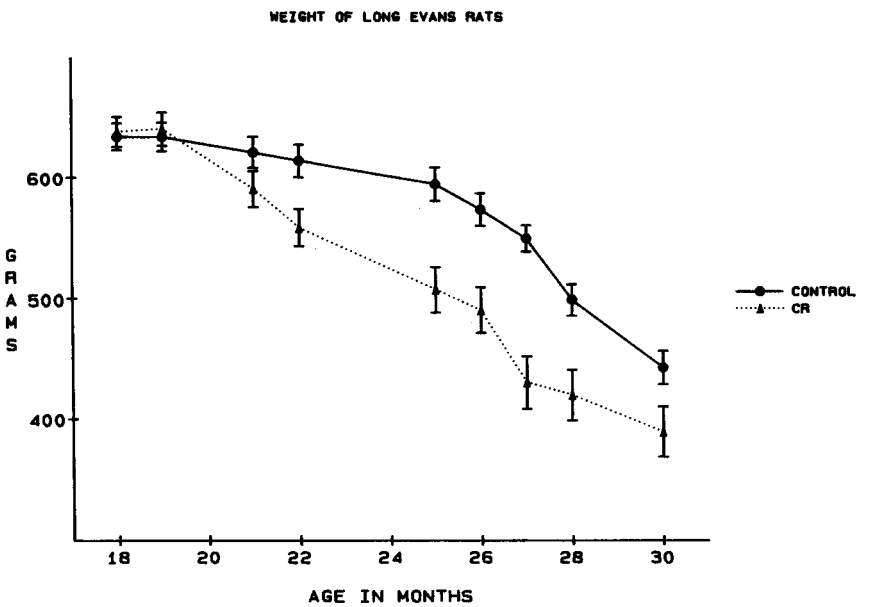


*Figure 2 - Mean weights for control and CR male Long Evans rats. Results are given as mean±SEM.*

Table 1 - *Serum analysis of control and CR rats.*

| Analyte | Units | Controls | Restricted |
|---|---|---|---|
| sodium | mmol/L | 140.0 ± 3.9 | 143.71 ± 6.1 |
| potassium | mmol/L | 6.52 ± 1.3 | 6.29 ± 1 |
| cholesterol | mg/dL | 164.17 ± 14.1 | 136.29 ± 9.7 |
| bilirubin | mg/dL | 0.2 ± 0 | 0.17 ± 0.1 |
| alanine aminotransferase | U/L | 81.32 ± 7.2 | 95.69 ± 8.7 |
| aspartate aminotransferase | U/L | 31.95 ± 2.2 | 41.53 ± 7 |
| lactate dehydrogenase | U/L | 175.6 ± 22.5 | 400.86 ± 108.9** |
| alkaline phosphatase | U/L | 56.73 ± 4.8 | 75.6 ± 11.9 |
| triglycerides | mg/dL | 67 ± 11.1 | 53.57 ± 3.2 |
| uric acid | mg/dL | 2.52 ± 1.3 | 2.33 ± 1 |
| chloride | mmol/L | 100.5 ± 2.0 | 100.29 ± 7.9 |
| creatine phosphokinase | mg/dL | 131.5 ± 29.2 | 275.74 ± 69.1 |
| glucose | mg/dL | 140.49 ± 8.5 | 101.66 ± 10.2* |
| blood urea nitrogen | mg/dL | 15.75 ± 4.1 | 16.74 ± 4.3 |
| calcium | mg/dL | 10.55 ± 0.5 | 9.41 ± 0.6* |
| total protein | g/dL | 6.43 ± 0.6 | 6.01 ± 0.6 |
| albumin | g/dL | 2.77 ± 0.3 | 2.77 ± 0.5 |
| phosphorus | mg/dL | 6.8 ± 0.6 | 6.8 ± 1.4 |
| magnesium | MEQ/L | 2.25 ± 0.3 | 2.2 ± 0.2 |

Values presented as mean±SEM.
*$p<0.05$, **$p<0.05$ after log transformation.

calorie restricted status of the restricted group based on glucose homeostasis.

Rats surviving past the median life span were fasted for 8 hours, and blood was then taken *via* cardiac puncture after $CO_2$ asphyxiation. The serum was separated by centrifugation and stored at -80°C prior to automated analysis. Necropsies were performed, and gross lesions documented.

Weight and mortality data were compared using Pearson's $X^2$ analysis. Analysis of the blood chemistry was conducted with a Student's *t* test using a significance level of $p<0.05$.

## RESULTS

Survival and weight data are shown in Figures 1 and 2. Rats reached 50% mortality at 28 months which is comparable to that observed by others (12, 13). During the 10 months of study, 11 of 18 CR rats and 8 of 17 control rats died, or 61% and 47% mortality, respectively; this difference was not significant ($p=0.6$). Using two-dimensional contingency tables, it was estimated that 700 rats (350/ diet group) would have been needed for the difference observed between groups to reach statistical significance.

Results of blood chemistry analysis obtained from rats surviving beyond the mean are presented in Table 1. The significant differences between the two groups were for circulating levels of glucose, as predicted, lactate dehydrogenase (LDH), and calcium. Fasting levels of glucose and calcium in CR rats were 27% and 11% lower, respectively, than controls. After correction for the large difference of within group standard deviations, LDH for CR rats was more than twice that of controls ($p<0.05$).

There was no significant difference between the diet groups in terms of percentages of animals with no significant lesions, nor in the incidence of pathologies observed (Table 2). The lesions observed are among those common to multiple rat genotypes (14).

## DISCUSSION

The literature suggests that the beneficial impact of CR may be titrated by the age at which it is initiated. Our hypothesis is that there is an age at which initiation of CR would not be beneficial. Our finding

Table 2

| Lesion | CR | AL |
|---|---|---|
| No significant lesions | 6 | 11 |
| Liver nodules | 39 | 53 |
| Adrenal gland hyperplasia | 26 | 29 |
| Hydronephrosis | 11 | 18 |

that there was no observable increase in mean life span when caloric restriction was initiated in rats 18 months of age is consistent with the results from every-other-day feeding studies with mice in which a difference was observed in mean life span when dietary intervention was initiated at 1.5 and 6 months of age, but not at 10 months of age (15).

Further detailed study of the impact of CR on older animals is prudent prior to human application. The possible inability of late-life CR to affect longevity in rats may provide some novel insight into the mechanisms by which this dietary manipulation alters the aging process.

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