Experimental Section

Gerontology

Gerontology 2001;47:9–14

Received: April 18, 2000
Accepted: July 24, 2000

Prenatal Exposure to a Maternal Low Protein Diet Shortens Life Span in Rats

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Key Words
Diet restriction · Low protein diet, prenatal

Abstract
Background: Postweaning diet restriction is associated with prolongation of life span, reduced age-related disease and slower ageing. The effects of diet restriction imposed prior to weaning have not been so well characterised, but studies suggest an opposite effect with increased age-related diseases occurring in offspring exposed to undernutrition in prenatal life. It remains unclear whether life span is similarly adversely affected by early diet restriction. ***Objective:*** The present study in rats aimed to evaluate the impact of a maternal low protein diet upon the life span of the resulting offspring. ***Methods:*** Rat dams were fed either a 180-gram casein/kg control diet or a 90-gram casein/kg low protein diet from conception until the end of pregnancy. The offspring were then maintained with minimal handling until death from natural causes or distress-necessitated euthanasia. ***Results:*** The average life span of female rats exposed to low protein diets in utero was reduced by 11% ($p = 0.044$, Kaplan-Meier analysis). There was a similar but non-significant trend in the male offspring (control 76 ± 3 weeks, low protein 73 ± 3 weeks). In addition the rats exposed to a prenatal low protein diet had significantly higher systolic blood pressure at 4 weeks of age and tended to be smaller than control animals in postnatal life. ***Conclusion:*** The results suggest that intrauterine diet restriction reduces life span in rats and contrasts with the well-recognised increase in life span produced by postweaning diet restriction. The timing of the nutritional intervention appears to be critical and recognition of this is relevant to understanding the mechanisms underlying the effects of diet restriction on ageing and life span.

Introduction

The well-documented beneficial effects of postweaning diet restriction on ageing include prolongation of life span, reduced age-related disease and attenuation of structural and functional changes associated with age [1]. Since the earliest studies in rodents, the work has been replicated in many animal species, most recently in primates [2]. However the effects on ageing of nutritional manipulation earlier in life are not well characterised [3].

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0304–324X/01/0471–0009$17.50/0

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Interest in this area has grown with the results of large scale epidemiological studies in man demonstrating that indices of fetal and infant growth retardation are associated with increased ageing processes [4] and a number of age-related diseases including coronary heart disease [5], hypertension [6], non-insulin-dependent diabetes [7] and osteoporosis [8] in later life. These findings have given rise to the hypothesis that such diseases may be programmed in utero [9]. Programming is defined as the process whereby a stimulus or insult acting at a critical period of development in early life has lasting effects of lifelong importance to the health and well-being of the organism [10]. The major determinant of prenatal growth is nutrition and suboptimal maternal nutrition in pregnancy has been proposed as the major programming influence upon the developing fetus. Further studies have shown associations of high carbohydrate intake in early pregnancy or low intakes of protein in late pregnancy with both impaired fetal growth [11] and raised blood pressure in adult life [12].

Studies with rats have largely reproduced the epidemiological findings and have allowed programming mechanisms to be investigated experimentally [13]. The feeding of a low protein diet, rich in starch and sucrose, to pregnant rats generates offspring which are growth-retarded in late pregnancy and develop increased age-related changes in postnatal life. These include raised blood pressure [14], progressive deterioration of renal function [15] and impaired immune function [16]. This recent work is consistent with the few earlier studies in the area showing that a reduction in maternal diet produced rat offspring with increased effects of ageing, including reduced resistance to hypothermia [17], an earlier age-related decline in haemoglobin [18] and increased age-associated enzymes in the liver and kidney [19].

The effect of prenatal diet restriction on life span has been little addressed. It was suggested as early as 1920 that intrauterine undernutrition may be associated with reduced life span in rodents [20] and findings from two more recent studies support the idea [21, 22] but there appears to be little other work in the area. This study in rats addresses whether the programming effects of maternal protein restriction during pregnancy impact upon the natural life span of the resulting offspring.

Methods

All animal experimentation was performed under licence from the Home Office in accordance with the Animals Act (1986). A total of 10 adult female Wistar rats were used to generate the 93 offspring studied in this experiment. All rats were bred within the University of Southampton Animal Facility and came from stock inbred for 15–20 years. They were housed in plastic boxes either singly or in pairs, in rooms maintained at 22 °C, with a 12-hour light cycle. Holding rooms were cleaned with disinfectant on a daily basis. The animals were not maintained in barrier conditions. Interventions were minimised and the animals did not undergo microbiological screening. Rats had free access to food and water at all times.

Virgin female rats weighing 220–250 g were mated and on confirmation of conception, through the observation of a semen plug on the floor of the mating cage, were singly housed and fed diets containing 180 g casein/kg (control diet) or 90 g casein/kg (low protein diet), as has been previously described [23]. The diets were manufactured from purified ingredients within the University of Southampton facility and were balanced in energy content through the addition of carbohydrate to the low protein diet. The full dietary composition is published elsewhere [23]. Feeding of the diets continued throughout the 22 days of pregnancy and during this time, food intake and maternal body weight were measured daily. At delivery all rats were transferred to a non-purified chow diet (CRME, Special Diet Services, UK). All offspring were weighed and the litters were culled to a maximum of 8 pups per litter. One rat fed the low protein diet died during delivery of her litter and data for the food intake and body weight gain of this animal are therefore excluded from all analyses.

The offspring of the rats were maintained on CRME diet throughout life, from weaning at 4 weeks of age. These animals were housed in pairs until 20 weeks of age, when individual housing was introduced. All rats were weighed every 4 weeks until 68 weeks of age. Weighing ceased at this point as several animals had already died and the growth of all of the survivors was complete. The animals were otherwise handled only when cages were cleaned on a weekly basis.

Systolic blood pressure and heart rate were determined using an indirect tail cuff method. This involved locating an inflatable tail cuff on the animals. The cuff contained a light sensor which detected the caudal artery pulse [24]. Systolic blood pressure was determined uneventfully at 4 weeks of age. A further attempt to determine blood pressure was made at 29 weeks but the older animals did not tolerate the procedure and to minimise agitation the measurement was abandoned.

Animals were allowed to die without intervention if there was no evidence of pain, distress or discomfort. The trained animal technicians in the Southampton University Animal Facility monitored the rats on a twice daily basis to avoid animal suffering. Animals that were rapidly losing weight or otherwise exhibiting distress were culled. At this stage they would have been unlikely to survive more than a few days and to compensate for the inaccuracy introduced, the study recorded life span in weeks rather than days. There was only one case where a rat was culled with a condition that was not life-threatening (an injured and ulcerated tail). This rat was however 130 weeks of age and one of the oldest animals to go through the study.

The same criteria were applied to both the control and the low protein groups. This process resulted in 50% of control animals and 72% of the low protein-exposed animals being culled.

Kaplan-Meier plots of survival were analysed using a log-rank test. As life span was measured in weeks, the culled animals were treated identically to the non-culled animals in the survival analysis and censoring was not used. Data for maternal food intake, weight gain, offspring birth weight and blood pressure are presented as mean ± SEM and analysed using Student's t test. A probability of 5% or less was accepted as statistically significant.

Results

Table 1 shows maternal food intake and weight gain during pregnancy. Prior to conception all females were of similar weight (control 227 ± 9 g, low protein 233 ± 6 g). Pregnancy-associated weight gain was similar in control and low protein-fed animals over the first 14 days of pregnancy. Between day 15 and day 22 of gestation the weight gain of rats fed low protein diets was significantly less than that of the control rats. Food intake tended to be greater in the low protein group throughout pregnancy but the difference was not statistically significant. Litter size and birth weight did not differ significantly between the two groups (table 1).

Blood pressure was determined at 4 weeks of age, after the animals had been weaned. Male and female animals exposed to a low protein diet in utero had significantly higher systolic blood pressures than control rats (control 105 ± 2 mm Hg, low protein 121 ± 4 mm Hg; $p = 0.001$) and lower heart rates (control 430 ± 8 beats/min, low protein 401 ± 12 beats/min; $p < 0.05$).

Growth of the rats between 4 and 40 weeks of age is shown in figure 1. No analyses were performed on the data beyond this age as growth was largely complete in both males and females and the number of animals in each group began to vary after the first death at 43 weeks. Female rats were significantly heavier than their male littermates at 4 weeks of age ($p < 0.05$), but were thereafter lighter than the males ($p < 0.001$). No significant differences in the growth of control and low protein-exposed females were noted until week 36, when the growth rate of control females appeared to be more rapid than in the low protein group. By 40 weeks of age, control females were significantly heavier (20 g, $p = 0.04$). Low protein-exposed males tended to be lighter than control males at all time points. This difference was significant at 4, 8, 16 and 40 weeks of age.

The average life span of the female rats was 92 ± 4 weeks and the male rats 74 ± 2 weeks. This difference was significant ($p < 0.001$). Female rats exposed to the low protein diet in utero lived significantly shorter lives than control females (fig. 2). Mean age at death was 11 weeks shorter (control 97 ± 5 weeks, low protein 86 ± 4 weeks, log-rank $p = 0.044$). There was a similar but much smaller effect in the male rats (fig. 3) which did not reach significance (control 76 ± 3 weeks, low protein 73 ± 3 weeks).

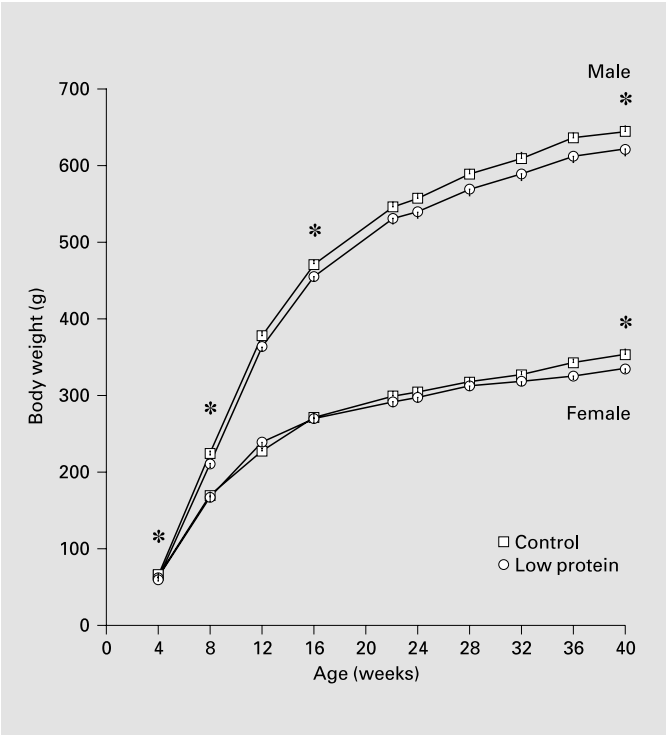

Fig. 1. Body weights of male and female offspring from 4 to 40 weeks. Data are shown as means ± SEM for 12–19 rats per group. * $p < 0.05$ between control and low protein-exposed rats of the same sex. Male and female body weights were significantly different ($p < 0.05$) throughout the experiment.

Table 1. Maternal food intake, weight gain and the outcome of pregnancy

	n	Control	Maternal diet		p
			n	low protein	
Weight gain, g					
Day 0–7	5	36 (5)	4	41 (3)	NS
Day 8–14	5	44 (3)	4	37 (4)	NS
Day 15–22	5	71 (4)	4	52 (8)	<0.05
Food intake, g/day					
Day 0–7	5	27.5 (1.0)	4	30.0 (1.0)	NS
Day 8–14	5	28.0 (1.0)	4	30.0 (2.0)	NS
Day 15–22	5	27.5 (1.0)	4	29.0 (3.0)	NS
Litter size	5	11 (1)	4	9 (1)	NS
Birth weight, g	55	6.01 (0.11)	38	5.76 (0.12)	NS

All data are shown as means (SEM) for n observations. Food intake and weight gain were determined daily throughout pregnancy and are presented as averages for the 3 separate weeks of gestation. NS = Not significant.

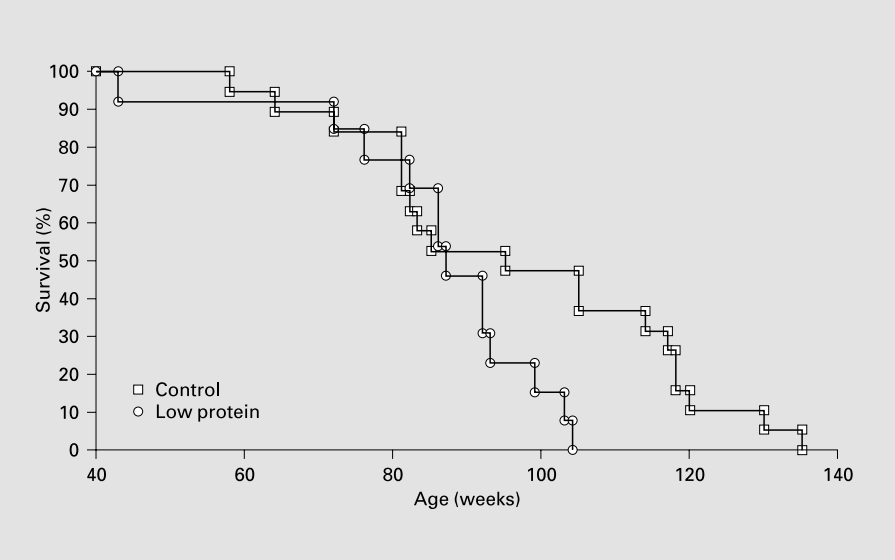

Fig. 2. Kaplan-Meier survival curve for female rats. Data represents percentage survival of 19 control and 13 low protein-exposed rats up to the age of 135 weeks. p = 0.044.

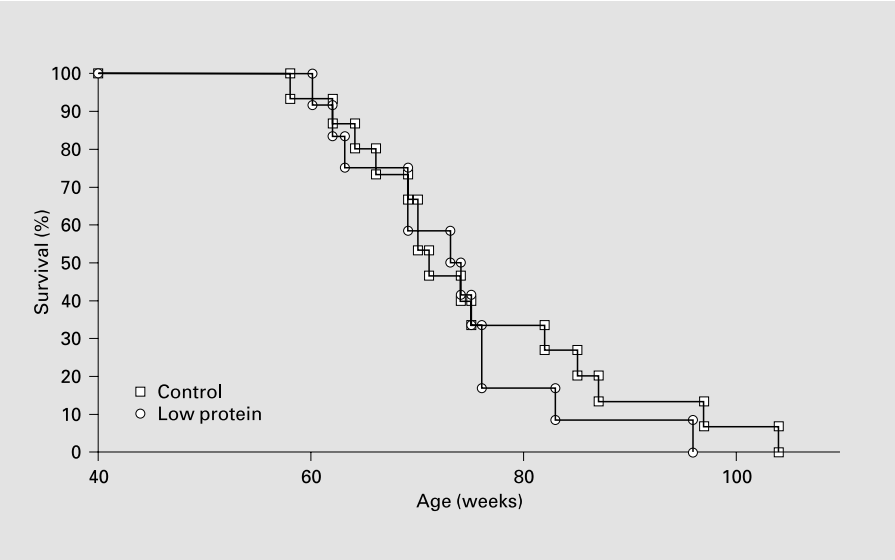

Fig. 3. Kaplan-Meier survival curve for male rats. Data represents percentage survival of 15 control and 12 low protein-exposed rats up to the age of 104 weeks. p = 0.481.

Discussion

This study has demonstrated that a low protein diet in utero is associated with a reduced life span in rats. The effect was seen in both female and male rats but only reached significance in the females. The work is important because the effect of preweaning diet restriction on ageing has been little studied and the results suggest an opposite effect to postweaning diet restriction. Further consideration of the findings is therefore required.

The average life span of the rats was lower than the 24 months usually reported for Wistar rats. This probably reflected the inbred nature of the colony and was similar to that reported in another study using inbred Wistar stock [22]. The animals were not maintained in barrier conditions and may therefore have been exposed to a range of infective agents. However, postmortem examination did not show gross evidence of pulmonary inflammation and was therefore not suggestive of long-term respiratory illness in any of the animals.

The study was a small one and the loss of one pregnant rat fed a low protein diet during delivery reduced the numbers further. This would act to reduce the power of the study to detect a significant difference between the

two groups. The significant difference demonstrated in the female rats is therefore less likely to be a chance finding and the lack of power could explain the non-significant result in the males.

The necessary constraints of the experiment required culling of a high proportion of the animals. This was unlikely to bias the findings as euthanasia was only employed when it was clear that the animals were close to death and life span was measured in weeks to compensate for the inaccuracy introduced. Those responsible for the process were blinded to their original in utero diet so avoiding the bias associated with, for example, a greater readiness to cull low protein diet rats at an earlier stage.

The male and female rats exposed to a low protein diet in utero had significantly higher blood pressure at 4 weeks. It was not possible to confirm that this effect was sustained into later life because measurement of blood pressure at 29 weeks had to be abandoned for methodological reasons. However, previous work suggests that the effect is permanent [25].

The demonstrated association between intrauterine undernutrition and reduced life span may be causal but other explanations need to be considered. Early exposure to a low protein diet affected postnatal growth and was associated with lower weight in adult life. The effect was more marked and occurred at an earlier stage in the male rats. We have previously noted that in females exposed to maternal low protein diets, the cessation of growth which accompanies full maturity appears to occur at a lower weight [25]. This is the first such observation in males. Whilst overall body weights were lower in the low protein group, differences in body composition were not assessed. There is some evidence that rats undernourished in utero are more prone to develop obesity, particularly if introduced to cafeteria diets [26]. The reduced survival in the protein-restricted group could therefore have been a result of adult adiposity. However the CRME diet used post-weaning in this study was low in fat (2.9 g/100 g) and was designed to minimise obesity in breeding rat colonies.

The effects demonstrated on life span are consistent with the previously reported studies [20–22] although the latter studies demonstrated the effect on male rather than female rats and used a slightly different dietary manipulation to achieve protein restriction. The outcomes of intrauterine protein restriction other than life span, observed in this study, are also comparable with results from previous experiments [13]. Maternal food intake was not significantly altered by protein restriction although there was a tendency for intake to be above that of control animals. Weight gain in pregnancy was similar in the two groups of rats until the final week of gestation. As previously reported, maternal weight gain at the time of most rapid fetal growth was attenuated by low protein feeding [27]. Despite this, reproductive performance was not significantly impaired and litter sizes were similar in the two groups. Control and low protein-exposed pups were of similar weight at birth. This contrasts with earlier studies which have demonstrated that maternal protein restriction generates offspring that are of low to normal birth weight [27, 28]. The mean difference in systolic blood pressure between the groups (16 mm Hg) at 4 weeks was consistent with previous reports [13].

The major finding that intrauterine exposure to a maternal low protein diet shortens life span in rats appears to be robust and is consistent with the limited existing literature. It could be a specific effect of reduced maternal protein intake or be ascribable to other associated changes in the maternal diet. The mechanism for the effect remains speculative. It may reflect programming of age-related diseases by early undernutrition. Systolic blood pressure was elevated at 4 weeks of age in the diet-restricted rats and hypertension may be relevant. However, in many cases the cause of death or terminal illness was unclear. Frequently end stage disease in the rats was manifest by non-specific signs such as rapid weight loss and cessation of feeding. At postmortem examination it was possible to identify putative causes of death in less than half of the rats.

In males a common feature was renal disease, although this was largely absent in females. Jennings et al. [22] reported reduced life span in male rats exposed to intrauterine protein restriction, associated with renal telomere shortening. We have previously reported that the kidneys of low protein-exposed rats are structurally different and lose function more rapidly than those of control animals [15].

It has also been proposed that early undernutrition adversely programmes processes fundamental to ageing such as molecular and cellular repair capacity. Evidence for this has come from work in man demonstrating that poor early growth, essentially determined by insufficient nutrient supply, is associated with increased markers of ageing in certain body systems including the lens, ear, muscle and skin. These systems have particularly high proportions of long-lived molecules and are therefore most dependent on the ability to repair molecular damage [29]. Impaired repair processes may be associated with faster ageing and shorter life span.

The findings of this study support the concept that undernutrition in the fetal period is associated with

reduced life span. This contrasts with the prolongation of life seen with postweaning diet restriction. It appears that the timing of the nutritional intervention is critical and recognition of this is relevant to understanding the mechanisms underlying the effects of diet restriction on ageing and life span.

Acknowledgment

The expert technical assistance of Mr Colin Bunce is gratefully acknowledged. This work was funded by the Wellcome Trust.

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