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The association between birthweight and longevity in the rat is complex and modulated by maternal protein intake during fetal life

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Abstract **Maternal protein restriction in rat pregnancy has been suggested to reduce lifespan of the resulting offspring by inducing fetal growth retardation, followed by postnatal catch-up growth. We tested the hypothesis that lifespan could be programmed in both males and females by exposure to undernutrition at specific stages of fetal development. Protein restriction throughout gestation significantly reduced lifespan in both males and females. Low birthweight increased longevity, whilst rapid postnatal growth had a detrimental effect. There was no evidence that undernutrition programmed lifespan through oxidative processes in the major organs. Fetal programming is an important contributor to the ageing process.**

Keywords: Fetal programming; Longevity; Protein restriction; Rat

1. Introduction

Caloric restriction (CR) of rodents and other species has long been known to prolong lifespan [1]. Studies of rodents indicate that post-weaning dietary restrictions can prolong lifespan, reduce age-related disease and limit the functional and metabolic deficits associated with normal ageing. Some of these findings have been replicated in primates, suggesting that the quality of the diet may also be an important modulator of the ageing mechanism in humans [2]. Rodents subjected to CR exhibit attenuated generation of mitochondrially-derived free radical species, with reduced oxidative damage. *In vitro*, tissues from these animals show greater resistance to oxidative stress. The interaction of dietary components with antioxidant defences represents a key mechanism through which CR modulates ageing processes [3].

An expanding body of epidemiological evidence suggests that the nutritional environment experienced in fetal life programmes risk of chronic degenerative diseases associated with human ageing. Maternal undernutrition in pregnancy results in babies of lower birth weight or exhibiting evidence of disproportionate growth [4]. Such individuals develop hypertension as adults and are significantly more likely to suffer from coronary heart disease, non-insulin dependent diabetes and osteoporosis [4]. Low birth weight and shorter birth length were associated with increased mortality risk between 39 and 49 years of age in Danish men [5]. A large-scale cohort study has indicated that in men and women aged 64–74 years, nuclear lens opacities, reduced grip strength and impaired auditory ability were more common in individuals of lower weight at age 1 year, suggesting associations between early life events and the ageing process in humans [6,7].

The feeding of maternal low protein diets, without caloric restriction, in rat pregnancy has previously been shown to significantly reduce the lifespan of the offspring [8]. We have shown that the feeding of a 9% casein diet in pregnancy resulted in female offspring that lived for approximately 11 weeks less than the offspring of control animals. No significant effect of prenatal diet upon lifespan of prenatally undernourished male rats was noted in this small pilot study. In contrast Jennings et al. [9], found that prenatal protein restriction reduced lifespan in male but not female animals, also in a small and probably underpowered study. These studies of rats and further work with mice [10] have suggested that impaired fetal growth followed by rapid catch-up in early postnatal life may reduce lifespan. The aims of the present study were to determine the impact of prenatal protein restriction using a much larger and more robust study design. As oxidative processes clearly play a role in ageing, programming effects upon oxidative tissue damage were evaluated as a potential mechanism for the early life origins of lifespan.

2. Materials and methods

2.1. Animals

The experiments in this paper were performed in accordance with the Animals (Scientific Procedures) Act 1986 and were licensed by the Home Office. Animals were held under temperature controlled conditions on a 12-h light:dark cycle. The animals had ad libitum access to food and water at all times. 57 virgin female Wistar rats (Harlan Ltd., Belton, UK) were mated at weights between 200 and 250 g. Upon confirmation of mating the rats were allocated to be fed either a control diet (18% casein), or a low protein diet (9% casein, LP diet), as described previously [11]. The diets were isocaloric, the difference in energy between the control and LP diets being made up with additional carbohydrate in a ratio of 2:1 starch:sucrose (w/w). LP feeding was targeted at single weeks in gestation d0-7 (LP(0-7)), d8-14 (LP(8-14)) and d15-22 (LP(15-22)) and also fed throughout gestation (d0-22, LP(0-22)).

At delivery of litters, all mothers were transferred to standard laboratory chow diet (B&K Universal rat and mouse diet, 20% protein, 3% fat) and the litters were culled to a maximum of 8 pups to minimise variation in the nutrition of the pups during suckling. The offspring of the control and LP fed dams thus differed only in terms of their prenatal nutritional exposures. We have previously shown that the protein

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content of milk produced by rats fed LP in pregnancy is similar to that of rats fed control diet [12]. Birthweight was recorded for all litters, along with the total number of pups born (including pups that were dead at delivery). As there is no humane method for marking rat pups at birth, the average birthweight for the males and females in each litter was recorded and used as the basis of the statistical analyses in this paper.

At 4 weeks of age the offspring were weaned onto chow diet. One randomly selected male and one randomly selected female from each litter were killed using a rising concentration of carbon dioxide at ages each of 1, 9 and 18 months. The liver, heart and brain were dissected from each animal, were weighed to the nearest 0.1 mg, and then snap-frozen in liquid nitrogen. Organs were stored at −80 °C until used for further analyses. Prior to cull all animals were housed in metabolic cages for 24 h to allow the collection of urine. In order to determine early life influences on longevity, remaining animals in each litter were left undisturbed and were maintained, housed in groups of two or three, with minimal handling until death from natural causes or distress-necessitated euthanasia, as described previously [8]. In total, 57 males and 47 females were included in this longevity study, representing the offspring of 49 successful mothers.

2.2. Markers of oxidative damage

To investigate whether programmed differences in longevity might be explained by oxidative senescence, two different markers of oxidative injury were assessed in the animals culled at 18 months of age (siblings of the animals in the survival experiment). The urinary excretion of 8-deoxy-2-hydroxyguanosine (8-OHDG) was determined using a commercially available kit (Japan Institute for the Control of Ageing) according to the manufacturers instructions. Whilst 8-OHDG provided a marker of damage at the whole body level, tissue specific damage was assessed by determination of the total protein carbonyl content of the brain, liver and heart, as previously described [13].

2.3. Statistical analysis

Data were analysed using one or two way analysis of variance using SPSS version 11.5. Where ANOVA indicated a significant effect of treatments, posthoc tests were performed using the LSD test. Correlations between variables were determined using a partial correlations test to correct for the influence of sex. Survival distributions in males and females exposed to differing dietary protocols were analysed using Cox's regression analysis.

3. Results

Weight at birth was not significantly altered by the feeding of maternal low protein diet at any stage of gestation. Males gained more weight than females between birth and 6 weeks of age ($P < 0.001$). Among males, weight gain in the first 6 weeks of life was lower ($P < 0.05$) in the LP(0-7) (223 ± 11 g) and LP(15-22) (220 ± 8 g) groups than in controls (255 ± 8). Weight gain was similar in all groups of female offspring.

Female animals lived for significantly longer than male animals (female mean 94.3 ± 3.2 weeks, males 74.2 ± 3.2 weeks, $P < 0.01$). Comparing the survival curves for male and female animals exposed to varying maternal diets during differing phases of fetal life revealed no clear differences (Fig. 1). Animals of the LP(0-22) group tended to have shorter lives than controls, whilst offspring from LP(0-7) (females) and LP(15-22) (males and females) groups had longer lifespan. Lower birthweight was predictive of longer life (partial correlation between lifespan and birthweight, adjusted for sex, $r = -0.223$ $P = 0.023$). The number of offspring in the litter at birth was not predictive of lifespan. A significant association between lifespan and weight gain between birth and 6 weeks of age was also noted ($r = -0.364$, $P = 0.001$), indicating that rapid growth in the early postnatal period was related to reduced longevity. Cox's regression analysis, adjusting for sex, birthweight and postnatal growth, showed that protein restriction throughout gestation (LP(0-22)) significantly increased risk of earlier death compared to controls (Table 1).

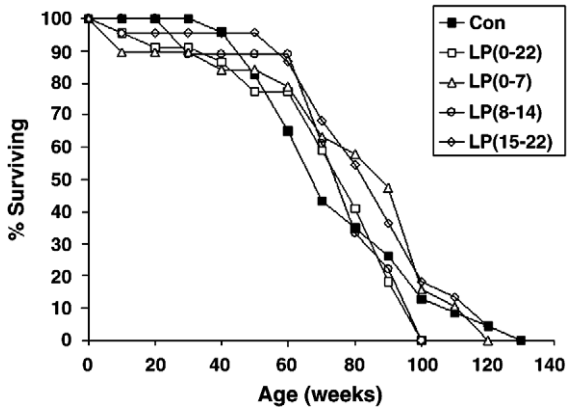

Fig. 1. Survival distribution curves for rats exposed to maternal diets of varying composition during fetal development (male and female offspring combined). Mean ages at death in males were: 74.1 ± 4.9 weeks (Control); 69.0 ± 8.1 (LP(0-22)); 69.7 ± 11.8 (LP(0-7)); 71.3 ± 7.5 (LP(8-14)); 82.9 ± 6.1 (LP(15-22)). Mean ages at death in females were: 90.3 ± 8.6 (Control); 88.6 ± 5.0 (LP(0-22)); 99.9 ± 7.7 (LP(0-7)); 92.2 ± 3.9 (LP(8-14)); 103.4 ± 10.3 (LP(15-22)).

Table 1
Cox's regression analysis of survival data

Maternal diet	*n*	Relative risk	95% Confidence intervals	*P*
Control	23	1.0	–	–
LP(0-22)	22	2.03	1.012– 4.071	0.045
LP(0-7)	19	0.93	0.455–1.884	0.831
LP(8-14)	18	1.54	0.740–3.218	0.248
LP(15-22)	22	0.75	0.385–1.456	0.394

Cox linear regression indicated a significant effect of maternal diet upon lifespan after adjustment for sex, birthweight and postnatal growth (birth to 6 weeks). Overall model χ^2 34.64 df7, $P < 0.001$.

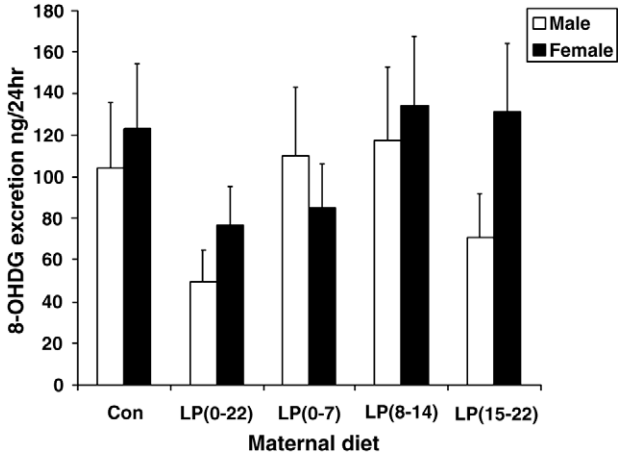

Fig. 2. Excretion of 8-OHDG by 18-month-old rats exposed to maternal diets of varying composition during fetal development. Data are shown as means ± S.E.M. for 5–8 observations per group. Analysis of variance indicated that there was no significant effect of either sex or maternal diet upon this marker of oxidative injury.