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Protein concentration in the rat maternal diet programs appetite and glucose metabolism in the offspring

M.P.G. BARNETT, P.M. HARRIS[1] AND G.J.S. COOPER

School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand.

ABSTRACT

The effect of dietary protein concentration throughout gestation and lactation on maternal appetite, and appetite and glucose metabolism of the offspring, was investigated. Female Sprague-Dawley rats were fed diets containing either 20% whey protein (control; C, n=20) or 5% whey protein (low-protein; LP, n=30) through gestation and lactation. LP mothers gained less weight during gestation (88 *vs*. 136 g, SED=18, P<0.01), and consumed less food during lactation (451 *vs*. 902 g, SED=77, P<0.001). C offspring were heavier at birth (6.5 *vs*. 5.3 g, SED=0.5, P<0.05), consumed more food (273 *vs*. 178 g, SED=15, P<0.05), and gained weight more rapidly (5.9 *vs*. 3.7 g/day, SED=0.4, P<0.001) than LP offspring. LP offspring had lower fasted blood glucose at 40 (2.2 *vs*. 3.5 mmol/L, SED=0.4, P<0.01) and 50 (2.9 *vs*. 4.8 mmol/L, SED=0.2, P<0.001) days of age and were more sensitive (P<0.001) in response to a glucose challenge at 30 (498 *vs*. 615 mmol/L min, SED=24), 40 (557 *vs*. 700 mmol/L min, SED=24) and 50 (535 *vs*. 781 mmol/L min, SED=44) days of age. These results show that maternal dietary protein concentration during gestation and lactation programs appetite and glucose metabolism in the offspring.

Keywords: protein; programming; appetite; glucose tolerance; rat.

INTRODUCTION

Poor nutrition at an early age can lead to permanent changes in an animal. These changes may be structural or metabolic, and may apply to the animal as a whole, and to specific organs. Thus, early adaptations to nutritional stress may permanently alter the physiology and metabolism of an organ such that these changes continue to be expressed in the absence of the original causative events (Patel & Srinivasan, 2002). This phenomenon is referred to as “programming”.

There is evidence that the earlier a nutritional insult occurs, the more likely the effects will persist throughout the life of the organism. Therefore, foetal and early neonatal life represents a developmental stage of particular interest. As the diet of the offspring during this period is determined by that of the mother, the maternal diet likely initiates programming in the offspring.

In this study, the hypothesis was that an inadequate supply of protein in the maternal diet during pregnancy and lactation results in poor nutrition for the offspring during these periods, and that this is a key factor in the programming of appetite, body weight, and glucose metabolism in the offspring. Both rats and mice have been used as a model for monogastric animals (Golovan *et al.*, 2001), and the rat is used in this role in the current study. Because parameters such as liveweight gain and body composition can be important in determining the value of farm animals, appetite, body weight and glucose metabolism were considered of particular interest. Protein was the nutritional variable used due to its importance in foetal growth and pancreatic development, and because it influences appetite. Dietary protein content during gestation and lactation has also been studied in cattle (Carroll *et al.*, 1988; Sasser *et al.*, 1988), thus the results reported here may have some relevance to farmed ruminants, as well as monogastrics.

MATERIALS AND METHODS

Animals

Virgin female Sprague-Dawley rats were housed at 21 ± 1^{o}C and subjected to a standard 12:12-hour light:dark cycle. Diet was standard laboratory chow (NRM Diet 86, 18.5% protein, 5% fat) provided *ad libitum* prior to mating. Females in oestrus were randomly selected, and mated with one of two males. A positive pregnancy was determined by the appearance of a vaginal plug, at which stage the rats were transferred to individual cages and alternately assigned to one of two commercially prepared (Crop and Food Research, Palmerston North, NZ) diets containing either 20% (control; C, n=20) or 5% (low-protein; LP, n=30) whey protein. Composition of these diets is listed in Table 1. Throughout gestation, food intake was restricted to 95% of *ad libitum* feeding. This value was determined from a previous feeding trial (data not shown). All animals had free access to water.

TABLE 1: Composition of experimental diets. Diets were prepared by Crop and Food Research, Palmerston North, according to the specifications listed in the table.

Ingredient	Content of diet (g/100g)	
	Control (20% protein)	Low (5%) protein
Cellulose	5	5
Cornflour	47.8	62.8
DL-methionine	0.2	0.2
Mineral mix	3.9	3.9
Whey protein	20	5
Vegetable oil	7	7
Sucrose	15	15
Vitamin mix	1.1	1.1

Following spontaneous delivery of pups (day 22-23), mothers were maintained on the same diet as they had been supplied during gestation, without restriction of intake.

At 21 days of age, pups from all litters were weaned

[1] AgResearch Limited, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

onto diet C, supplied *ad libitum* for the remainder of the trial.

Food intake and body weight

Food intake of both mothers and offspring was measured daily. For offspring (which were housed as litters), a value of daily food intake per rat for each litter was calculated by dividing total intake by the number of rats in the litter. This data was summarized by calculating the cumulative food intake per rat from weaning to 40 days of age.

During gestation, dams were weighed daily. During lactation, dams and offspring were weighed every second day. After weaning, offspring were weighed every second day.

Intra-peritoneal glucose tolerance test

An intra-peritoneal glucose tolerance test (IPGTT) was performed on all surviving offspring at 30, 40, 50 and 60 days of age. Each animal was fasted overnight (16-18 hours) with free access to water. An intra-peritoneal injection of sterile 10% (w/v) glucose was then administered (10μL per g body weight). Blood (20μL) was sampled from the tail vein immediately prior to injection (t=0), and then at 15, 30, 60, 90, and 120 minutes post-injection. Blood glucose was measured using an Advantage II system (Roche, Basel, Switzerland). As a measure of overall glucose sensitivity, blood glucose was plotted against time, and the area under the curve (AUC) was calculated for each animal using the trapezium rule (Pham *et al.*, 1999).

Statistical methods

Because some offspring were used for another trial after 40 days of age, the effective number of observations for parameters in the current trial at 50 and 60 days of age was lower, thus the statistical power of the analysis at these times was less.

All statistical analyses were performed using GenStat® for Windows 5th (Lawes Agricultural Trust, 2001) or 6th (Lawes Agricultural Trust, 2002) edition. Unless otherwise stated, the analysis was a linear mixed model Residual Maximum Likelihood (REML). Diet was the fixed effect, and litter the random effect, with litter size used as a covariate in the analysis of birth weight and offspring growth rates. In all cases, the experimental unit was the mother, thus the n value was determined by the number of litters investigated, rather than the total number of offspring.

Ethical approval

All work was approved by the University of Auckland Animal Ethics Committee, under applications N633 and N857.

RESULTS

In both diet groups, approximately 30% of pregnant females successfully raised litters to weaning, because of a combination of unsuccessful pregnancy and offspring mortality. Females in a concurrent breeding trial using standard rat chow (NRM Diet 86) also showed low rate of pregnancy, but an explanation for this phenomenon was not identified. Offspring mortality was highest in the LP group (60%) compared with 20% in the C group.

TABLE 2: Food intake and body weight data for female Sprague-Dawley rats and their offspring. Mothers were fed commercially prepared isocaloric diets containing either 20% (C) or 5% (LP) whey protein through gestation and lactation. All offspring were weaned onto diet C. Data represent predicted mean values (Residual maximum likelihood, REML), standard error of the difference (SED) and probability (P) for comparisons between C and LP groups. BW = body weight, LWG = liveweight gain. Total number of observations was n = 6 and 8 for the C and LP groups respectively. This applied to offspring as well as dams because the litter was the experimental unit for statistical purposes.

	C	LP	SED	P
Maternal LWG (g)				
Gestation	136	88	18	**
Lactation	-11	-44	25	NS
Maternal food intake (g)				
Gestation	489	498	42	NS
Lactation	902	451	77	***
Live offspring per litter				
3 days (~ birth)	11.2	9.9	2.6	NS
21 days (weaning)	8.5	6.9	2.0	NS
Offspring BW (g)				
3 days (~ birth)	6.5	5.3	0.5	*
Offspring LWG (g/day)				
Pre-weaning	2.3	0.7	0.2	***
Post-weaning	5.9	3.7	0.4	***
Offspring food intake (g)				
Post-weaning	273	178	15	***

Table 2 summarizes food intake and body weight data of mothers and offspring. During gestation, there was no difference in food intake between the C and LP mothers, although weight gain during this period was significantly higher ($P<0.01$) in the C group. During lactation, C mothers consumed significantly ($P<0.001$) more than LP mothers, both in terms of total intake and intake expressed as a function of total supported biomass (i.e., weight of the mother + weight of all offspring; data not shown). Change in weight of C and LP mothers during this period was not significantly different. Offspring of C mothers were heavier at birth ($P<0.05$), consumed more food ($P<0.05$), and gained weight more rapidly ($P<0.001$) than offspring of LP mothers.

The LP offspring had lower fasted blood glucose levels at 40 and 50 days of age (Table 3) and were more sensitive ($P<0.001$) in response to a glucose challenge at 30 (area under curve (AUC) 498 *vs.* 615 mmol/L.min, SED=24), 40 (AUC 557 *vs.* 700 mmol/L.min, SED=24) and 50 (AUC 535 *vs.* 781 mmol/L.min, SED=44) days of age (Figure 1).

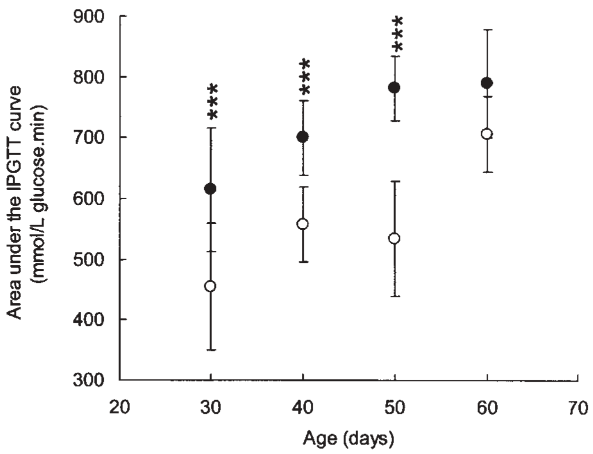
DISCUSSION

The lower maternal food consumption of LP compared to C dams throughout lactation was consistent with the reported loss of appetite observed in lactating rats fed a low-protein diet (Kanarek *et al.*, 1986; Rogers, 1979). Food intake as a function of total supported biomass during lactation (i.e., combined weight of the mother and all offspring) was also significantly lower in the LP group compared to the C group - thus there was no compensation for the lack of protein by greater food consumption during this period.

TABLE 3: Fasted blood glucose measurements (mmol/L) performed on offspring of mothers fed a control (20% whey; C) or low-protein (5% whey; LP) diet during gestation and lactation. Measurements were performed after an overnight (16-18hr) fast. Data represent predicted mean values (REML), standard error of the difference (SED) and probability (P) for comparisons between C and LP groups. The number of observations (litters) for each value is shown in parentheses next to that value.

Age (days)	C	LP	SED	P
30	2.9 (6)	3.2 (5)	0.3	NS
40	3.5 (5)	2.2 (6)	0.4	**
50	4.8 (2)	2.9 (1)	0.2	***
60	4.4 (1)	4.7 (1)	0.9	NS

FIGURE 1: Intra-peritoneal glucose tolerance tests (IPGTT) performed on offspring of mothers fed a control (20% whey; C;●) or low-protein (5% whey; LP;—○) diet during gestation and lactation. Measurements were performed after an overnight (16-18hr) fast. Data represent the predicted mean values (REML) of the area under the glucose tolerance curve (AUC) for each group at the four ages when this value was determined. The standard deviation (SD) and probability (P) for comparisons between C and LP groups are also shown. The number of observations at each point is as described in Table 3.



While there is evidence that a LP maternal diet during gestation and lactation can result in increased offspring mortality (Chugh *et al.*, 1991), 40% survival in the offspring of LP dams was lower than reported values. The pattern of survival in C litters was also unusual - overall survival of individual pups was approximately 80%, but the mean survival rate of litters was only 60%. This was due to 100% mortality in two of the litters. Both of these dams had been fed diet freeze-dried into pellet form, which was the method of preparation used early in the trial. Powdered diet was fed to subsequent dams. Overall survival in C litters of mothers fed powdered diet was 83%, and none of these litters had 100% mortality, indicating that freeze-drying of the diets may have contributed to early neonatal mortality. The unusually low level of successful pregnancies indicated that factor(s) other than a LP diet affect fertility. The factor(s) responsible remain to be elucidated, but as this phenomenon was also observed in dams fed standard rat chow, it suggested either a deficiency in this diet or a problem with the colony, rather than any deficiency in the C diet. There was also no difference between the C group and animals from a previous trial using standard chow with respect to litter number and offspring growth rates and food intake (data not shown). We therefore believe that the C diet was an adequate control, and differences observed between offspring of the C and LP diet groups are valid results representing an effect of maternal protein concentration.

Normal offspring (i.e., of C-fed mothers) each consumed consistently more food than offspring of LP-fed mothers, a pattern reflected in consistently lower (40-60%) body weights of LP offspring compared to control animals at the same age. Following adjustment for differences in body weight (i.e., g food intake / g body weight), there was no difference in food intake between age-matched C and LP groups. Thus, there was no evidence of compensatory growth in the LP offspring, although this behaviour has been observed in similar trials (Latorraca *et al.*, 1998). In addition, these observations indicate that insufficient protein during gestation and lactation can reduce appetite and liveweight gain in offspring, and, therefore, reduce the potential value of an animal. This has been observed previously in pigs (Schoknecht *et al.*, 1993).

The LP offspring in these studies showed better glucose tolerance than the C offspring at ages 30, 40 and 50 but not at age 60. The result at day 60 is consistent with a previous study(Petry *et al.*, 2000) at 63 days of age, by which time any observed differences in the current studies lacked statistical significance. The lack of difference at 60 days observed in the current study may be due to a more rapid deterioration of glucose tolerance in LP offspring compared to C offspring, or as a result of the much lower number of animals tested at this age. If the latter is the case, the difference in glucose tolerance between the C and LP animals may persist into adulthood. Investigations using older rats are required to address this question.

These effects of a low-protein diet during gestation and lactation in the rat suggest that providing an appropriate concentration of maternal dietary protein is important for health and well-being in the offspring.

Overall, these studies in rats may indicate that in other monogastric animals, appetite, body weight and glucose metabolism can be programmed by a LP maternal diet, and that the protein concentration in the maternal diet during gestation and lactation may be a key factor determining the later economic value of the offspring. This may be particularly true in such circumstances as out-of-season pregnancy. Programming effects of maternal diet during critical periods of foetal growth comparable to those described here may also be significant during later development of ruminant farm animals. Further studies using appropriate ruminant models are required to confirm this.

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