PERINATAL PROTEIN RESTRICTION: EFFECTS ON BODY WEIGHT, GLUCOSE TOLERANCE AND INSULIN SENSITIVITY IN MALE RATS' OFFSPRING

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Abstract: Background: Pregnancy and fetal development are periods of rapid growth and cell differentiation when mother and offspring are vulnerable to changes. Adverse events during development can be linked to an increased risk in developing metabolic diseases. Here we investigated if perinatal protein restriction could predispose the offspring to altered food intake, weight gain and glucose control. **Materials and Methods**: Pregnant Sprague-Dawley rats (n=24) were used. They were fed either a control diet (PRC) containing 20% protein or protein-restricted diet (PRR) with 8% protein. The dams were given PRR up to parturition (in-utero group), or from birth to post-natal day 21 (lactation group) or for a period covering both groups (combined group). Control dams with PRC diet was run in parallel for comparison. Feed intake was determined daily and body weight measured at weekly intervals. At 7-8 weeks of age, glucose tolerance and insulin sensitivity were assessed in the offspring. **Results**: At birth, protein-restricted offspring weighed significantly lower than protein-control offspring, ($p<0.05$). Body weight at weaning (postnatal day 21) and postnatal day 49, was significantly higher in protein control offspring than offspring exposed to protein restricted diets in-utero, and both in-utero and lactation, ($p<0.05$). Rats exposed in-utero to PRR diet consumed less food postnatally compared with control rats. In addition, rats exposed in-utero to PRR diet displayed impairment in glucose control during 180-min glucose tolerance test and delayed insulin response in the 120-min insulin tolerance test. **Conclusion**: These results suggest in-utero and lactational exposure to PRR diet negatively affect glucose homeostasis in the young adult rats. Depending on the period of exposure, PRR may have different effects on glucose metabolism.

Keywords: Fetal programming; Thrifty phenotype; Low protein diet; metabolic syndrome.

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Introduction:

An adverse intra-uterine environment is associated with long-term metabolic consequences, in particular obesity, insulin resistance and type 2 diabetes mellitus[1]. Data from epidemiological and animal studies have given rise to the concept of developmental programming, which proposes that challenges during an organism's intrauterine development evoke a persistent physiological response in adult life[2, 3].

Fetal intrauterine growth restriction (IUGR) occurs in humans as a consequence of poor maternal nutrition. IUGR has been associated with the development of adult diseases; this phenomenon is called 'fetal programming'. The association of maladaptive programming with adult disease has been termed the 'Barker hypothesis. In general, the Barker hypothesis contends that the malnourished fetus is programmed to exhibit a 'thrifty phenotype' with increased food intake and fat deposition and possibly decreased energy output[4].

The concept of 'developmental programming' proposes that challenges during an organism's development evoke a persistent physiological response in the offspring[5]. Epidemiological investigations such as those conducted on the children conceived during the Dutch famine of 1944–1945 have highlighted the association between poor maternal nutrition, lowered birth weight and subsequent adult disease[6, 7].

Several different experimental animal protocols have been used for the evaluation of developmental programming of metabolism:

global nutrient restriction[8, 9] or maternal exposure to an isocaloric low protein diet[10, 11].

Given the difficulty and complexity of the regulation of maternal and fetal physiology the physiologist's researchers uses experimental models. These experimental models used are trying to reproduce the form of supply of certain disadvantaged sectors of developing countries, whose own lack protein in their diet. Epidemiological and experimental studies demonstrated that the consequences of inadequate nutrition in utero may extend to adulthood and could lead to glucose intolerance or insulin resistance. Faced with ample available calories, such individuals are at risk of developing obesity, glucose intolerance and other manifestations. Thus, the need for this preliminary study on the effect of perinatal protein restriction on food intake, body weight and glucose metabolism in adult life.

Materials and Methods:

Virgin female Sprague- Dawley rats (n=24) weighing 120- 150 grams were obtained from the animal house of the College of Medicine of the University of Lagos. Experimental animals were kept in well-ventilated, hygienic compartments maintained under standard environmental conditions, acclimatized for three weeks before the experiment. The animals were well fed with standard rodent diet and water ad libitum. The experimental procedures adopted were in accordance with the provisions of the Experimentation Ethics Committee on Animals Use of the College of Medicine of the University of Lagos, Lagos State and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.

Diet: The low protein diet was 8% casein isocaloric diet and animals were grouped into four, protein control group (PrC; 20% protein diet), in-utero protein restriction, IUPR (exposed to protein restriction only during gestation), lactational protein restriction, LPR (exposed to protein restriction only during lactation) and combined protein restriction (exposed to protein restriction both during gestation and lactation) (PrR; 8% isocaloric low protein diet).

Mating: After ensuring this, they were all mated with proven male breeders overnight maintained in their respective diet throughout gestation. The day on which spermatozoa were present in a vaginal smear was designated as day of conception – day 0 of pregnancy. Only rats that were pregnant within 5 days of introduction of the male rats were retained in the study. Pregnant rats were transferred to individual metabolism cages and allocated at random to one of four groups to be fed either a 20% casein (control diet) or 8% casein isocaloric (restricted) diet. Food and water were available *ad libitum* for all animals.

Determination of body weight: At parturition, birth weight was measured and recorded, at day 21, the animals were weaned and their body weights were taken with period using a Duet top loading weighing scale *(Salter, England)*

Food intake: Food intake was measured on a daily basis throughout the experimental period using a Duet top loading weighing scale *(Salter, England).*

Glucose tolerance test:

An intraperitoneal glucose tolerance test was performed in six offspring of each of the four groups on postnatal days 70. After an overnight fast, the animals were then administered glucose (250 g l−1) intraperitoneally as a bolus, at a dose of 1 g (kg body weight) −1, and blood samples were collected from the tail vein at 15, 30, 60, 120 and 180 min under light ether anaesthesia. The glucose level was measured with Acu – chek glucose meter.

Intraperitoneal insulin sensitivity test:

The volume of insulin working solution required for an i.p. injection of 0.75 IU/kg in an injection volume of 3.6 µl/g body weight was calculated.1-ml insulin syringes and 25-G × 5/8-in. needles with the calculated volumes for each animal in the experiment was prepared. The blood glucose monitor was calibrated with the standard strip. The basal glucose concentration (T = 0) in each rat was calculated by removing

one rat at a time from its cage, and placed on the top of its cage, a small incision was made over the lateral tail vein (1 to 2 cm from the tail tip) with the aid of a scalpel blade. A small (~3 µl) blood sample directly onto the test strip placed in the blood glucose monitor. Direct pressure was applied to the incision until the blood clots and the rat was returned to its cage. After all rats have been measured for basal glucose concentrations; insulin solution was administered by intraperitoneal injection to each animal at a 30- or 60-sec interval between animals. The timer was started with the first rat injected. At *T*=15 min, the blood glucose was measured again with the first rat injected and the same time interval used for injections was used until all the rats in the cohort were measured. *T* was repeated at 30, 60, 90 and 120 min after insulin injection.

Statistical Analysis

Data were recorded as Mean ± standard error of the Mean. Statistical difference between the means was determined by ANOVA. Any significant difference between means was assessed by the student's T-test and P≤0.05 was accepted as the significant level.

Results:

Effects in female rats (Dams)

Weight gain during pregnancy was significantly lower in protein restricted dams (*P*<0.005) compared with control dams, although there was no abortion and stillbirths in all dams, the litter size was reduced only slightly in protein restricted dams compared with control dams (Table 2).

Parameters in the offspring

Relative food intake

There was significant differences in food intake of the protein restricted offspring from week four up to week seven postnatally, (p<0.05) compared with control offspring, (Table 3).

Growth characteristics.

Body weight at birth (week 0), weaning (week 4) and final body weight (week 7) were significantly (*P*<0.005) lower in IUPR and CPR offspring than in control offspring, (p<0.05) (Table 4).

Glucose tolerance and insulin sensitivity

Offspring exposed in-utero to protein restricted diets displayed impairment in glucose control during 180-min glucose tolerance test and delayed insulin response in the 120-min insulin tolerance test (Graph 1 and 2) respectively.

Table: 1 Composition of experimental diet (k/kg) (8% isocaloric diet/low protein diet)

Part Number	Item Details	Qty	Disc
SP156	Cassava flour	30.00	0.00%
SP119	Groundnut cake	10.50	0.00%
SPS02	Pelletizing and Drying	10.00	0.00%
SPS01	Power(Crushing to powder)	0.15	0.00%
SP128	Industrial salt	0.05	0.00%
SP133	Lysine	10.00	10.00%
SP121	Wheat-offal	0.05	0.00%
SP134	Methionine	0.05	0.00%
SPS111	Toxin binder	0.05	0.00%
SPS53	Grower premix	0.05	0.00%

Table: 2 Reproductive performance in control and protein restricted dams

Groups	**Weight gain (g)**	**Still birth (n)**	**Abortion (%)**	**Litter size (n)**
CONT	**198.64**	**0**	**0**	**10**
IUPR	**192.85**	**0**	**0**	**9**
LPR	**185.64**	**0**	**0**	**8**
CPR	**187.38**	**0**	**0**	**7.5**

**=P<0.05 when compared with the control group*

Table: 3 Weekly food intake in PRC and PRR offspring

GROUPS	WEEK 4	WEEK 5	WEEK 6	WEEK 7
CONT	70.10±11.34	110.37±5.72	214.14±8.11	321.20±9.42
IUPR	77.20±9.71*	97.80±6.39*	165.06±6.26*	223.54±12.02*
LPR	91.03±7.43*	132.05±4.23*	229.75±4.32*	325.28±11.80
CPR	83.26±14.30*	110.24±8.12	163.43±4.82*	262.17±15.80*

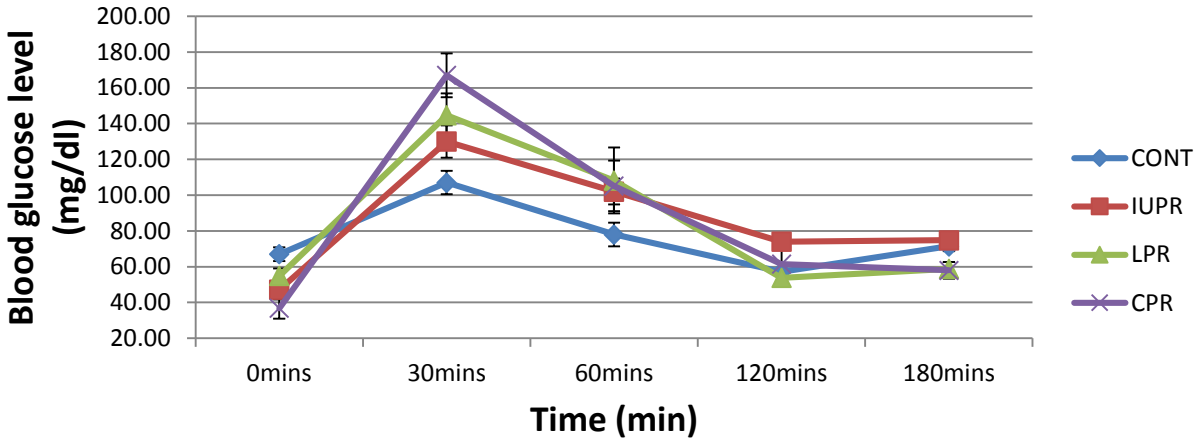
**=P<0.05 when compared with the control group*

Table: 4 Body weight changes in PRC and PRR offspring

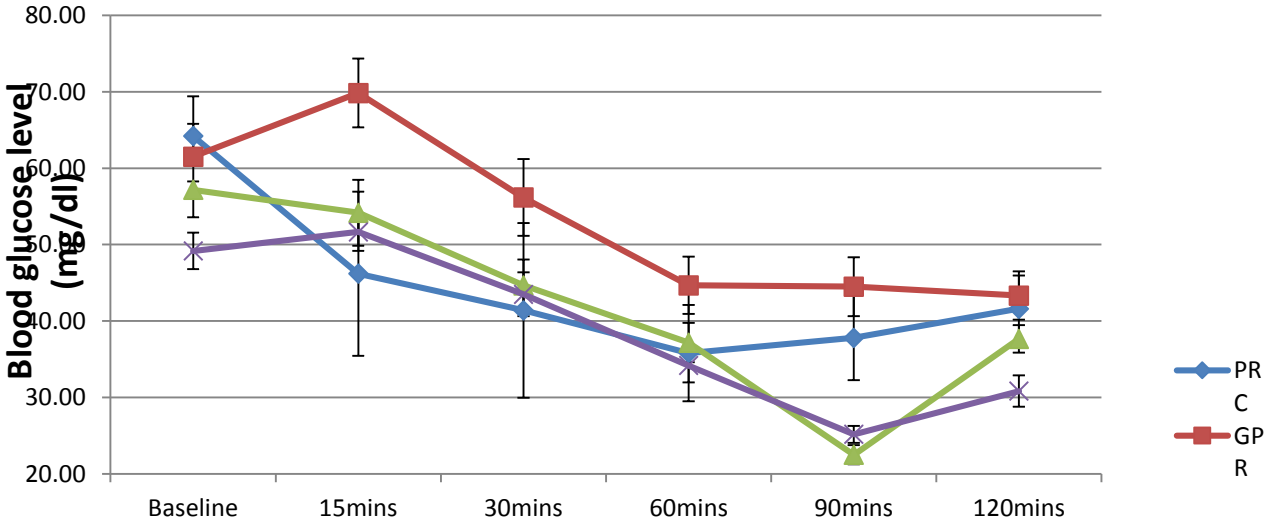
GROUP	Initial body weight (week 0)	Weaning weight (week 4)	Final body weight (week 7)	Weight change	% Weight change
CONT	4.86±0.26	42.57±2.56	88.00±5.88	83.14.33±6.00	1712±99.25
IUPR	2.43±0.20*	38.00±0.72*	67.33±5.03*	64.90±5.16*	2671±124.19*
LPR	4.71±0.29	40.57±2.92	76.00±5.90	71.29±5.88	1514.00±120.67*
CPR	2.14±0.26*	32.43±0.90*	66.71±4.33*	64.57±4.26*	3017.50±85.20*

**=P<0.05 when compared with the control group*

Graph: 1 Blood glucose level during oral glucose tolerance test (OGTT) in control and PRR offspring



Graph: 2 Blood glucose level during intraperitoneal insulin sensitivity test, IPIST in PRC and PRR offspring



Discussion:

Beside calorie and global nutrition restrictions, the use of an isocaloric low protein diet is one of the most extensively utilized manipulations to induce programming effects on the offspring in animal models[12, 13]. Results from the present study showed decreased birth weight in protein restricted offspring compared with control offspring. This agrees with previous study by[14] which stated that low protein diet resulted in decreased birth weight. Rat offspring born to dams fed low protein diet had approximately 15% to 20% lower body weight at birth[15], this strongly agrees with the present findings.

Low protein diets fed during gestation and lactation to rats also have been reported to lead to higher food intake[16] in adulthood. This is contradictory to the present study although there was hyperphagia in early postnatal life. Literature has shown that food intake in the offspring of the under-nourished rats was increased early in postnatal life[16]. Both low and high protein maternal diets have detrimental effects on body weight and body composition of offspring. Although maternal low protein diets have been reported to increase body weight of rat offspring, their effect on birth weight is not consistent. Where low protein diets during pregnancy have led to low birth weight in most studies, high protein diets have been reported to result in lower, no effect or higher birth weight.

Results from the present study showed delayed glucose tolerance and impaired insulin sensitivity in protein restricted offspring compared with control, although this is more marked in IUPR and CPR offspring. Male but Wistar rats born to low protein fed dams (8% of total calories) were relatively insulin resistant[17]. The present study disagree with[18] who reported that fasting plasma glucose and insulin levels were normal in young offspring born to the dams fed a low-protein diet during pregnancy. As would be expected from the effects of feeding dams low protein diets on body composition of the offspring, metabolic consequences may also result. This study therefore agrees with the Barker hypothesis which contends that the malnourished fetus is programmed to exhibit a 'thrifty phenotype' with increased food intake and possibly decreased energy output[19].

Conclusion:

These results suggest in-utero and lactational exposure to PRR diet negatively affect glucose homeostasis in the young adult rats. Depending on the period of exposure, PRR may have different effects on glucose metabolism.

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