THE EFFECTS OF MATERNAL DIETARY PROTEIN RESTRICTION DURING GESTATION IN RATS ON POSTNATAL GROWTH OF THE BODY AND INTERNAL ORGANS OF THE OFFSPRING

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ABSTRACT

Effects of maternal dietary protein restriction during gestation on postnatal growth of the body and internal organs of the offspring were investigated using 32 pregnant rats randomly assigned to two groups (A and B). Pregnant rats in group A (control group) and group B (treatment group) received experimental diets containing 18 % and 8 % crude protein respectively, throughout the gestation period. Growth of the body and internal organs of the offspring of these rats was studied at various postnatal periods. The results showed that at the adult age of 84 days, the body weight and relative weights of the heart, liver, spleen, kidneys and testes were significantly (p<0.05) lower in rats in treatment B than the control. Histological findings revealed that the testes and kidneys were most adversely affected by prenatal protein restriction. Spermatids and spermatogenic cells were scanty in the seminiferous epithelium, while the renal cortex showed distorted and irregularly shaped renal corpuscles and shrunken glomeruli. These results suggest that maternal dietary protein restriction during pregnancy produced long-term adverse effects on postnatal growth and development of the body and internal organs of the offspring. This supports the idea that postnatal growth of adult structures may be 'programmed' during the foetal stages of development.

Keywords: Gestation in rats, Protein restriction, Postnatal growth, Foetal programming, Internal organs

INTRODUCTION

Maternal nutrition during pregnancy plays an essential role in proper placental and foetal development (Vonnahme, 2007). Clinical, epidemiological and experimental studies have demonstrated that different nutrients are capable of influencing the normal course of pregnancy and embryo/foetal development in different animal species (Whyte *et al.*, 2007; Minkin, 2009; Siemieniuch, 2010). It is thought that a maternal stimulus or insult at a critical period during foetal development may have long-term impacts on the offspring, and this is termed 'foetal programming' (Godfrey and Barker, 2000).

Observations from experimental studies reveal that the embryo is most susceptible to nutritional and other exogenous and/or endogenous factors during the pre-implantation phase of pregnancy (Whyte *et al.*, 2007; Minkin, 2009; Siemieniuch *et al.*, 2010), and these have been associated with blastocyst death and pregnancy blocks (Williams *et al.*, 2014), impaired somatic development at birth (Triunfo and Lanzone, 2015), alterations of endocrine

and metabolic functions (Reusens *et al*., 2007) and impaired maturation of the reproductive system during postnatal life (Imbesi and Castrogiovanni, 2008).

Maternal undernutrition often occurs during gestation in our livestock, particularly during the first and second trimesters of pregnancy (Sletmoen-Olson *et al*., 2000), but information on the impact of such maternal undernutrition during gestation on postnatal development of the body and internal organs of the progeny is very scanty. However, it has been reported that undernutrition during pregnancy significantly reduced the number of infants in mice (Gabory *et al*., 2011), deferred intra-uterine development of the foetus in sheep (Ford and Long, 2011) and resulted in development of significant diseases later in life (Godfrey and Barker, 2000). The present study evaluated the effects of maternal dietary protein restriction during gestation in rats on the growth of the body and internal organs of the progeny.

MATERIALS AND METHODS

Feed Preparation: Feed ingredients used in compounding the experimental diets for the study were maize, cassava meal, soya bean meal, fish meal, bone meal, lysine, methionine, premix and salt. Samples of the experimental diets were subjected to proximate analysis and their crude protein levels were determined using the sulphuric acid digestion technique of Johan Kjeldahl (Labconco, 1998).

Animals: All protocols involving animal experimentation and disposal of animal carcasses were ethically reviewed and approved by the ethics committee for Animal experimentation of the University of Nigeria. The experiment was conducted in compliance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). Forty adult white rats (*Rattus norvegicus var. albinus*), made up of 32 females (13 weeks old) and eight males (15 weeks old) were used as the breeding stock in this study. The rats were obtained from a Veterinary Research Farm in Nsukka, Enugu State, Nigeria. The female rats were mated with males and confirmation of successful mating was by detection of a copulatory plug in the vagina (Baker, 1979) and/or presence of spermatozoa in the vaginal smear as described by Berthelot (1981).

Animal Grouping and Feeding: The 32 pregnant rats were randomly assigned to two groups (A and B) of 16 rats per group. The pregnant rats in each group were housed singly in cages and fed the experimental diet *ad libitum* throughout the period of pregnancy. Pregnant rats in Group A (control group) received the diet containing 18 % crude protein, while those in Group B (treatment group) received the diet containing 8 % crude protein. At parturition, ten male rat pups from each group were randomly selected, weighed and humanely euthanized for the study. During lactation, rats of the two groups received standard rat chow (diet containing 18% crude protein). At weaning (21 days of age), ten male rat pups from each group were randomly selected, weighed and humanely euthanized for the study. Thereafter, all rat pups in the two groups were maintained on standard rat chow and water *ad libitum*. At days 42, 63 and 84 of age, ten male rats from each group were randomly selected, weighed individually and humanely euthanized. Euthanasia was achieved by intravenous injection of ketamine hydrochloride at 300 mg/kg body weight. Internal organs (heart, liver, spleen, kidneys and testes) were dissected from all euthanized rats and weighed. Organo-somatic indices were determined for each organ as organ weight x 100/body weight.

Histological Preparation: Samples of the heart, liver, spleen, kidneys and testes of all the euthanized rats in groups A and B were fixed by immersion in 10 % neutral-buffered formalin, dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. The 5 µm thick sections were cut, mounted on glass slides and stained routinely with haematoxylin and eosin (H and E) (Igwebuike *et al*., 2013). Photomicrographs were captured using a Moticam Images Plus 2.0

digital camera (Motic China Group Limited) attached to a Leica Binocular Light Microscope.

Statistical Analysis: Data obtained in the study were presented as Mean ± SEM. The data were subjected to statistical analysis using student's t-test to determine if significant differences exist between the observed means. Probability values less than 0.05 were considered statistically significant.

RESULTS

Body Weights and Organo-Somatic Indices of Internal Organs: Data on the live body weights of rats in the control and treatment groups are presented in Table 1. Prenatal dietary protein restriction did not produce any significant difference ($p>0.05$) in the body weights of rat pups at birth. However, at 21, 42, 63 and 84 days of age, the body weights of rats of the diet restricted group were always significantly ($p<0.05$) lower than those of rats of the control group. Table 2 shows information on the organo-somatic indices of internal organs of rats of control and treatment groups. The relative weights of the internal organs did not differ significantly ($p>0.05$) at birth and at days 21, 42 and 63, with exception of the liver that was significantly ($p<0.05$) smaller in the treatment group than the control at birth and 42 days of age. The testes and kidneys were significantly ($p<0.05$) greater in the treatment group at 21 and 63 days of age respectively. Conversely, at the adult age of 84 days, the relative weights of all the internal organs (heart, liver, spleen, kidneys and testes) were significantly ($p<0.05$) smaller in the treatment group than the control group.

Histology of Organs: The kidneys of rats of both control and treatment groups showed presence of juvenile, incompletely differentiated and irregularly shaped renal corpuscles in the renal cortex at birth (Figure 1). At days 21, 42, 63 and 84, the kidneys of control rats revealed the normal renal histology, while those of the treatment group exhibited distorted and irregularly shaped renal corpuscles, shrunken glomeruli and very large capsular spaces (Figure 2). The testes of rats in the control group possessed normal seminiferous epithelium, containing spermatogenic cells and sustentacular (Sertoli) cells (Figure 3), but testes of the treatment group showed scanty cells of the germinal epithelium, with apparent reduction in population of elongated spermatids at 84 days of age (Figure 4). The histology of the liver, spleen and heart were normal and did not differ between rats of the control and treatment groups.

DISCUSSION

Dietary protein restriction during pregnancy did not result in reduced birth weight of the offspring in this study. This shows that prenatal exposure to the protein-deficient diet was not associated with intra-uterine growth retardation in the rats. However, a consistently smaller body weight was observed in these rats during the postnatal period from 21 to 84 days of age, indicating that the protein-deficient intra-uterine environment produced life-long retardation of postnatal growth in the rats. Changes in maternal nutritional status during pregnancy often results in permanent structural and functional deficits in foetal and postnatal growth of animals (Osgerby *et al.*, 2002; Bieswal *et al.*, 2006; Caron *et al.*, 2012). It has been postulated that maternal nutritional status can alter the epigenetic state of the foetal genome and imprint gene expression, and such epigenetic alterations in early embryos may be carried forward to subsequent stages of development (Waterland and Jirtle, 2004).

Results of the present study demonstrated that the response of various internal organs to prenatal protein insufficiency varied at different stages of postnatal growth and development, but at the adult age (84 days), all the organs studied were invariably smaller in the treatment group than the control.

Table 1: Comparison of the body weights of offspring from dams fed diet containing 18 % crude protein (control group) and those of dams fed diet containing 8 % crude protein (treatment group) using student t-test

Age (days)	N	Body weights of offspring (g)		t-value
		Control group	Treatment group	
1	20	5.72 ± 0.04	5.08 ± 0.35	1.793
21	20	25.77 ± 0.21	20.82 ± 0.68	6.963*
42	20	84.38 ± 2.59	67.03 ± 1.68	5.624*
63	20	123.05 ± 4.54	70.22 ± 10.97	4.450*
84	20	186.75 ± 2.50	155.51 ± 4.80	5.770*

Values represent mean ± standard error of mean for each measurement. Asterisk (*) indicates a significant difference at p<0.05

Table 2: Comparison of the organo-somatic indices of offspring of dams fed diet containing 18 % crude protein (control group) and those of dams fed diet containing 8 % crude protein (treatment group) using student t test

Age (days)	N	Parameters	Organo-somatic indices of offspring (%)		t-value
			Control group	Treatment group	
1	20	Heart	0.64 ± 0.05	0.53 ± 0.03	1.874
		Liver	4.46 ± 0.36	3.20 ± 0.25	2.867*
		Spleen	0.13 ± 0.00	0.23 ± 0.04	-2.524
		Kidney	0.84 ± 0.01	0.90 ± 0.05	-1.322
21	20	Heart	0.56 ± 0.03	0.62 ± 0.05	-1.076
		Liver	4.33 ± 0.16	4.33 ± 0.25	0.013
		Spleen	0.22 ± 0.00	0.27 ± 0.03	-1.340
		Kidney	1.08 ± 0.06	1.10 ± 0.11	-0.155
		Testes	0.51 ± 0.01	0.60 ± 0.02	-4.657*
42	20	Heart	0.54 ± 0.03	0.48 ± 0.04	1.232
		Liver	5.40 ± 0.19	4.54 ± 0.19	3.199*
		Spleen	0.55 ± 0.04	0.52 ± 0.07	0.398
		Kidney	1.12 ± 0.03	0.88 ± 0.09	2.432
		Testes	1.04 ± 0.05	0.98 ± 0.09	0.600
63	20	Heart	0.39 ± 0.01	0.32 ± 0.14	0.547
		Liver	5.45 ± 0.19	5.43 ± 0.20	0.054
		Spleen	0.41 ± 0.01	0.23 ± 0.02	11.389*
		Kidney	0.82 ± 0.01	0.92 ± 0.03	-3.188*
		Testes	1.54 ± 0.04	1.37 ± 0.12	1.293
84	20	Heart	0.40 ± 0.01	0.33 ± 0.01	4.217*
		Liver	4.80 ± 0.07	4.34 ± 0.11	3.509*
		Spleen	0.36 ± 0.02	0.15 ± 0.00	9.782*
		Kidney	0.73 ± 0.02	0.65 ± 0.01	3.431*
		Testes	1.40 ± 0.01	1.07 ± 0.05	6.661*

Values represent mean ± standard error for each measurement. Asterisk indicates a significant difference at p < 0.05

Thus, intra-uterine protein restriction eventually affected the growth of these internal organs later in life. It is known that various organs have different critical phases of development during the foetal period and the foetus adapts to nutrient deficiency by reduction in rate of cell division, especially in organs undergoing their critical phase of development (Barker and Clark, 1997). Beck *et al.* (2010) showed that reduction in cell numbers was associated with decrease in weight of organs of foetuses exposed to nutritional stress.

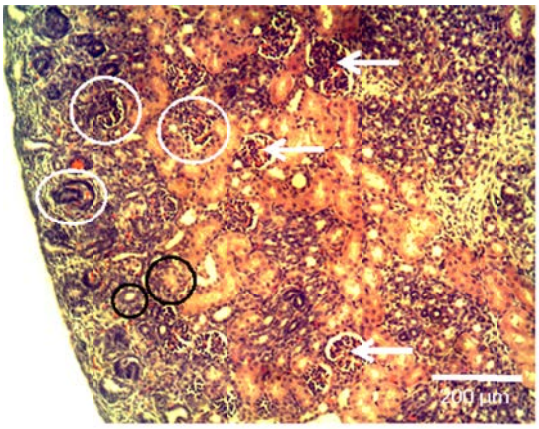

Figure 1: Kidney of control rat at birth showing irregularly shaped renal corpuscles (white circle) and renal tubules (black circle). Note normal renal corpuscles (white arrows). H and E stain, objective x10, scale bar = 200 µm

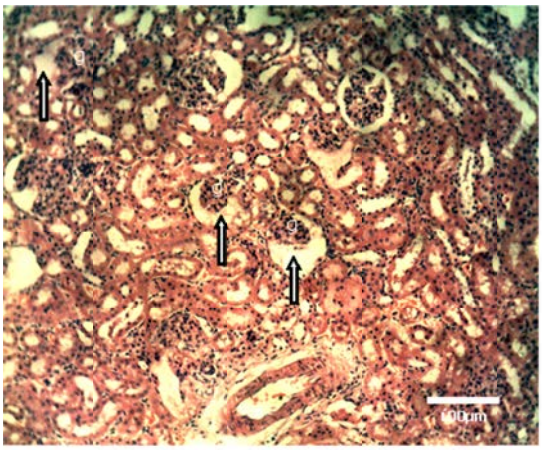

Figure 2: Kidney of treated rats at day 84 of age showing shrunken glomeruli (g) and enlarged capsular spaces (short white arrows) enclosed by Bowman's capsule. H and E stain, objective x10, scale bar = 200 µm

Such reduction in cell division may be carried forward, and give rise to overall decrease in growth of the organs during postnatal life. Impairment of organ growth at adulthood, as observed in this study, may lead to functional defects and development of disease conditions.

Occurrence of juvenile and incompletely differentiated renal corpuscles and tubules in both protein-restricted and control rats at birth supports the idea that less than 20 % of renal nephrons are formed during the prenatal period, while the remaining 80 % are formed during the

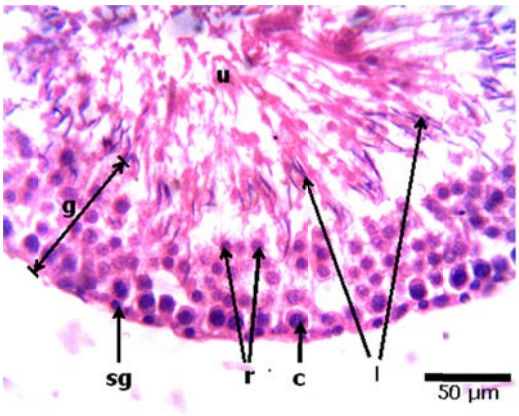

Figure 3: Testes of control rats at day 84 of age showing the germinal epithelium (g) containing spermatogonia (sg), Sertoli cells (c), round spermatids (r) and elongated spermatids (l). Note also the lumen (u) of the tubule. H and E stain, objective x40, scale bar = 50 µm

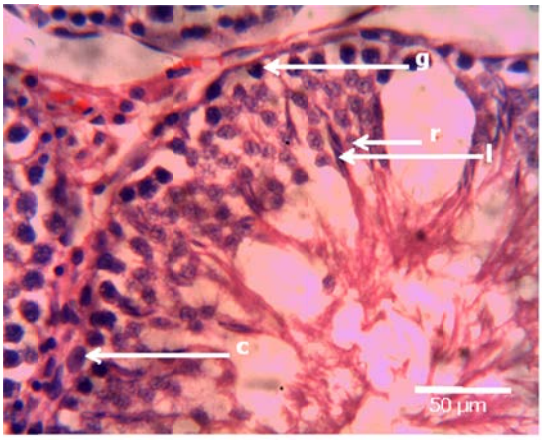

Figure 4: Testes of treated rats at day 84 of age showing scanty spermatogonia (g), Sertoli cells (c), round spermatids (r) and elongated spermatids (l). Note the scarcity of the primary spermatocytes. H and E stain, objective x40, scale bar = 50 µm

postnatal period in normal kidney development (Moritz, 2003). However, persistence of distorted renal corpuscles in the protein-restricted rats during postnatal development suggests long-term adverse effect of prenatal protein restriction on the kidneys. Similarly, scanty presence of cells of the germinal epithelium and elongated spermatids in the testes of the protein-restricted rats may have resulted from disruption of spermatogenic activities and retardation of germ cell

maturation. Protein deficiency has previously been shown to impair testicular cells development and reduce steroidogenic activities (Verhoeven and Van Leeuwe, 2008). The mechanism by which maternal dietary protein restriction during pregnancy affects postnatal growth of internal organs of the offspring is not within the scope of the present study, but it has been reported that maternal nutrition may affect organogenesis through its impact on the foetal insulin-like growth factor system (Igwebuike, 2010).

Conclusion: Maternal dietary protein restriction during gestation in rats had long-term impact on the offspring, giving rise to postnatal growth retardation of the body and internal organs and significant damages to the histologic appearance of testes and kidneys of the rats. This supports the idea that postnatal growth of adult structures may be 'programmed' during the foetal stages of development.

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