Life Sciences 92 (2013) 763–774

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Implications of intrauterine protein malnutrition on prostate growth, maturation and aging

Jaqueline C. Rinaldi [a], Luis A. Justulin Jr. [b], Livia M. Lacorte [a], Carolina Sarobo [a], Patricia A. Boer [a], Wellerson R. Scarano [a], Sergio L. Felisbino [a,*]

[a] Department of Morphology, Institute of Biosciences, Univ Estadual Paulista (UNESP), Brazil
[b] Department of Morphology, School of Odontology, Univ Estadual Paulista (UNESP), Brazil

ARTICLE INFO

Article history:
Received 20 August 2012
Accepted 7 February 2013

Keywords:
Prostatitis
Epithelial dysplasia
Collagen
Protein malnutrition
Fetal programming
Androgen receptor

ABSTRACT

Aims: Maternal malnutrition by low protein diet is associated with an increased incidence of metabolic disorders and decreased male fertility in adult life. This study aimed to assess the impact of maternal protein malnutrition (MPM) on prostate growth, tissue organization and lesion incidence with aging.
Main methods: Wistar rat dams were distributed into two groups, which were control (NP; fed a normal diet containing 17% protein) or a restricted protein diet (RP, fed a diet containing 6% protein) during gestation. After delivery all mothers and offspring received a normal diet. Biometrical parameters, hormonal levels and prostates were harvested at post-natal days (PND) 30, 120 and 360.
Key findings: MPM promoted low birth weight, decreased ano-genital distance (AGD) and reduced androgen plasma levels of male pups. Prostatic lobes from RP groups presented reduced glandular weight, epithelial cell height and alveolar diameter. The epithelial cell proliferation and collagen deposition were increased in RP group. Incidences of epithelial dysplasia and prostatitis were higher in the RP offspring than in the NP offspring at PND360.
Significance: Our findings show that MPM delays prostate development, growth and maturation until adulthood, probably as a result of low testosterone stimuli. The higher incidence of cellular dysplasia and prostatitis suggests that MPM increases prostate susceptibility to diseases with aging.

Introduction

Epidemiological studies have linked malnutrition in pregnancy with many chronic diseases (Langley-Evans et al., 2012). Malnutrition is a medical condition caused by an improper or insufficient diet. It is technically a category of diseases that includes the following: undernutrition, obesity and overweight, and micronutrient deficiency among others. However, it is frequently used to mean just undernutrition from either inadequate calories or inadequate specific dietary components, such as protein (Dimosthenopoulos, 2010).

During gestation, the female rat needs at least 12% of the diet to be protein (Benevenga et al., 1995). Maternal protein malnutrition (MPM) during gestation impairs overall growth and development (Wu et al., 2012). The "Barker hypothesis" (Barker, 1997) proposes that suboptimal intrauterine environment induces compensatory responses in the fetus that may permanently affect the adult phenotype and disease susceptibility (Bateson et al., 2004; Nijland et al., 2008). Rodent models have shown that MPM by low protein diet during pregnancy or during early postnatal life can lead to metabolic and physiological changes in the offspring, even when the animals have free access to a normal diet after weaning (Zambrano et al., 2005, 2006).

Table 1
Composition of the two isocaloric diets.

Ingredients (g/kg)	Control diet 17% of protein	Low protein diet 6% of protein[a]
Cornstarch	397	480
Casein (84%)	202	71.5
Dextrin (90–94%)	130.5	159
Sucrose	100	121
Soybean oil	70	70
Fiber	50	50
Mineral mix (AIN 93%)[b]	35	35[c]
Vitamin mix (AIN 93)[b]	10	10
L-Cystine	3	1
Choline bitartrate	2.5	2.5
Total energy (Kcal g^{-1})	3.76	3.76

[a] The low protein diet was prepared by PragSoluções (PragSoluções, SP, Brazil). Diets were supplemented with L-Cystine as sulfur amino acid.
[b] Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves et al., 1993).
[c] Potassium phosphate, monobasic, was added to the salt mix of this diet to maintain phosphorus at the levels found in the control casein diet (3 g/kg of diet) and the calcium: phosphorus ratio has been kept at 1.3 in both diets.

* Corresponding author at: Sao Paulo State University, Institute of Biosciences, Department of Morphology, Rubiao Jr, District of Botucatu, Zip Code:18618-970, SP, Brazil. Tel./fax: +55 14 38800474.
E-mail address: felisbin@ibb.unesp.br (S.L. Felisbino).

0024-3205
http://dx.doi.org/10.1016/j.lfs.2013.02.007

Table 2
Effects of maternal protein malnutrition on the biometric parameters from dams and litter.

Parameters	Experimental groups	
	NP	RP
Data of pregnant rat (n = 12 each group)		
Body weight (g) — GD1	234.21 ± 14.83	236.32 ± 12.35
Body weight (g) — GD21	358.42 ± 11.61	328.53 ± 11.91[a]
Body weight gain (g) — GD21-GD1	124.24 ± 12.25	93.25 ± 13.12[a]
Maternal food intake (g day^{-1}) at GD2	20.26 ± 2.01	19.95 ± 2.33
Maternal food intake (g day^{-1}) at GD20	22.17 ± 2.32	22.85 ± 3.13
Data of litter (n = 96 each group) at PND1		
Litter male birth weight (g)	6.68 ± 0.38	4.46 ± 0.39[a]
Litter male ano-genital distance (mm)	3.33 ± 0.34	2.68 ± 0.22[a]
Litter male ano-genital distance/birth weight (mm g^{-1})	0.21 ± 0.01	0.16 ± 0.01[a]

NP, normal-protein diet group; RP, restrict-protein diet group; GD, gestational day; PND, post-natal day. Values are expressed as mean ± SD.
[a] Indicates that RP group is significantly different from NP group with $p<0.05$.

MPM could promote structural change in different organs, such as an altered cell number, an imbalanced distribution of different cell types within an organ, and an altered blood supply and receptor numbers (Vicente et al., 2004; Lins et al., 2005). In addition, these changes can modify hormone production and the capacity of cells to respond to hormone signals (Bertram and Hanson, 2001; Langley-Evans and McMullen, 2010; Qasem et al., 2012).

A few reports in sheep and rats indicate that male sexual development and the normal ontogeny of gonadal development and function can be disrupted by maternal malnutrition (Rae et al., 2002; McMillen et al., 2008; Gardner et al., 2009). Zambrano et al. (2005) have shown that intrauterine maternal protein malnutrition alone was sufficient to reduce sperm count and influence their ability to impregnate female rats. MPM also reduces the serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone. In these studies, the weights of the testis, epididymis and prostate were reported to be decreased (Fernandez-Twinn et al., 2003, 2007; Santos et al., 2004; Zambrano et al., 2005, 2006; Guzman et al., 2006; Ramos et al., 2010). However, no previous studies investigated the effects of MPM during pregnancy on the prostate development. The prostate gland plays a fundamental role in reproductive biology. The prostate gland secretes different nutrients that partly compose seminal fluid, which is essential for sperm motility and nutrition (Untergasser et al., 2005). Moreover, early changes in prostate development may permanently alter the prostate morphology and function and influence the onset of late-life diseases, such as prostatitis, benign prostatic hyperplasia and prostate cancer (Risbridger et al., 2005; Prins et al., 2006; Cowin et al., 2008).

Therefore, the aim of the present study is to evaluate the effects of MPM on the prostate gland morphology of rats at three important phases: growth, maturation and aging.

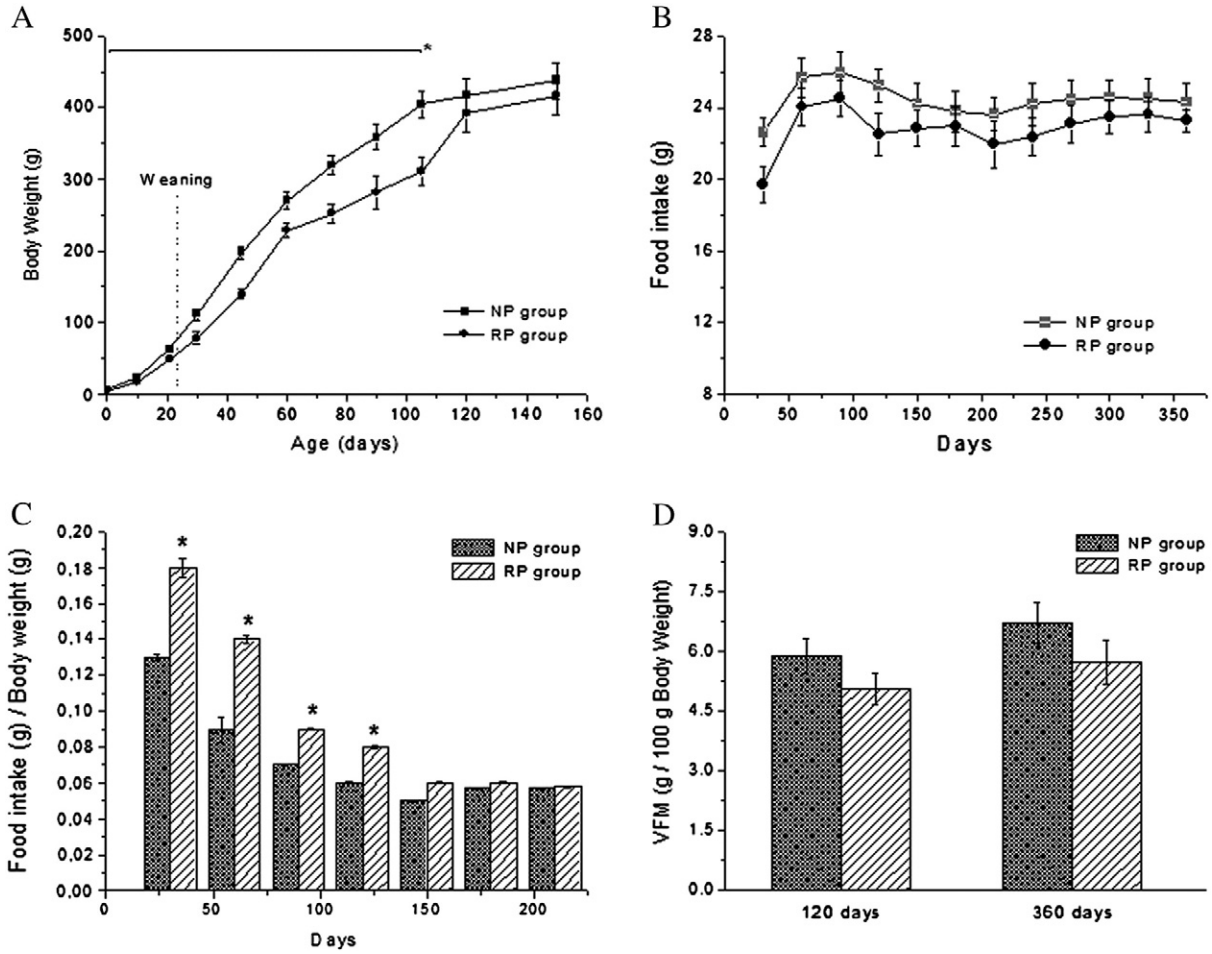

Fig. 1. Data from body weight and food intake measurements. A) Mean body weight of pups whose mothers were fed a control (NP) or low protein diet (RP) during gestation from day 1 until post-natal day (PDN) 150. B) Food intake of NP and RP pups after weaning until PDN 360. C) Post-weaning body weight normalized food intake in NP and RP offspring. D) Body composition: visceral fat mass (VFM) (g/100 g body weight). Values are means for at least twelve animals per group with standard deviation represented by vertical bars. * indicates that RP group is significantly different from NP group with $p<0.05$.

Material and methods

Animals

Adult female (90 days of age, n = 24) and male (90 days of age, n = 10) Wistar rats were obtained from the Central Stock breeder at the State University of Campinas — UNICAMP (Campinas, SP, Brazil). The animals were kept in a Central Biotherium of UNESP — Sao Paulo State University throughout the experimental period. The experimental protocol followed the Ethical Principles in Animal Research of the Brazil College of Animal Experimentation and was approved by the Bioscience Institute/UNESP Ethics Committee for Animal Experimentation (protocol: 08/09). A normal protein diet and a low protein diet were prepared as recommended by the American Institute of Nutrition (AIN 93-G) (Reeves et al., 1993). The diets are isoenergetic and their detailed composition is presented in Table 1.

Virgin female Wistar rats were mated overnight with established male breeders, and the day on which spermatozoa were present in a vaginal smear was designated as gestational day 1 (GD1). Pregnant dams were then randomized into two dietary groups: the control group (NP, n = 12) received a normal protein diet (containing 17% of protein), and the restricted group (RP, n = 12) received a low protein diet (a low protein formulation containing 6% of protein), ad libitum. The birth weight and ano-genital distance (AGD) of male pups were measured on post-natal day one (PND1). At 72 h post-birth, the litter size was standardized to eight pups (the gender ratio was kept as close to 1:1 as possible) per dam for both the control and restricted protein diet groups. This procedure maximizes lactation performance (Fischbeck and Rasmussen, 1987). After birth, all groups (mothers and offspring) had free access to normal protein diet for the remainder of the study. At PDN 22 the littermates were weaned and housed in isosexual groups. Post-weaning, body weight and food intake of the pups were measured weekly throughout the duration of the study. Food consumption was determined by measuring the difference in the amount of pellet food provided at the beginning of the interval from that remaining at the end of the interval.

On PND 30, 120 and 360, male rats (n = 12/group) were weighed and euthanized by decapitation. Blood samples were collected from ruptured cervical vessels. The urogenital complex (UGC — bladder, urethra, seminal vesicles and prostatic lobes) were dissected out and weighted. The ventral (VP) and dorsolateral (DLP) prostatic lobes were removed, weighted, and processed for histology. Those which were not easily dissected out of adherent tissue were not weighed. Visceral fat mass (VFM) – mesenteric, epididymal and retroperitoneal – was excised at DPN 120 and 360 and weighed for the evaluation of central adiposity (data were expressed as g fat/100 g body weight).

Hormone assay

Blood samples from each animal were collected at the time of euthanasia. Serum was obtained after centrifugation (2400×*g*, 20 min) and stored at −20 °C. The concentrations of the testosterone (T) and dihydrotestosterone (DHT) levels were determined by chemiluminescence (VITROS ECi-Johnson and Johnson Ultra-Sensitive Chemiluminescence analysis) using specific reagents (Johnson and Johnson Orthoclinical). The sensitivities of this assay were 0.02 and 0.04 ng/mL for T and DHT, respectively.

Histological procedures, stereological and morphometrical analysis

The prostatic lobes were fixed by immersion in 4% buffered formalin for 24 h, dehydrated in a series of graded ethanol and embedded in glycol methacrylate resin (Leica Historesin embedding kit), or they were clarified in xylene and embedded in Paraplast (Sigma Co, Saint Louis, MO). The resin sections of 3 μm were obtained and stained with hematoxylin–eosin (H&E) for morphological and morphometric analyses. Paraplast sections of 5 μm were stained

Table 3
Effects of maternal protein malnutrition on biometric values of the animals and rat prostatic lobes.

Parameters	Experimental groups					
	NP30	RP30	NP120	RP120	NP360	RP360
Biometric data (n = 12)						
Body weight (g) — day of euthanasia	140.68 ± 10.63	98.42 ± 6.21[a]	405.81 ± 37.66	391.31 ± 14.41	526.17 ± 54.07	501.53 ± 29.61
Urogenital complex absolute weight (g)	0.31 ± 0.01	0.23 ± 0.02[a]	3.24 ± 0.12	2.78 ± 0.10[a]	3.64 ± 0.13	3.55 ± 0.12
Urogenital complex relative weight (g)	0.24 ± 0.01	0.20 ± 0.01[a]	0.82 ± 0.03	0.71 ± 0.04[a]	0.72 ± 0.09	0.68 ± 0.03
Hormone plasma levels (n = 10)						
Testosterone (ng/dl)	38.23 ± 6.11	24.64 ± 5.72[a]	202.16 ± 58.53	185.91 ± 66.81	165.31 ± 28.13	133.62 ± 32.21
Dihydrotestosterone (ng/dl)	5.80 ± 2.30	3.50 ± 0.80[a]	26.21 ± 3.19	18.74 ± 4.18[a]	19.62 ± 2.13	12.51 ± 2.36[a]
Ventral prostate (n = 8)						
VP absolute weight (g)	–	–	0.46 ± 0.06	0.35 ± 0.06[a]	0.56 ± 0.09	0.48 ± 0.06
VP relative weight (g)	–	–	0.11 ± 0.01	0.09 ± 0.02[a]	0.11 ± 0.01	0.10 ± 0.01
Epithelial height (μm)	10.21 ± 0.98	8.82 ± 0.91[a]	23.03 ± 1.09	21.01 ± 0.92	20.55 ± 0.51	19.94 ± 0.92
Collagen fibers volume fraction (%)	5.82 ± 0.62	8.37 ± 0.54[a]	3.23 ± 0.72	5.95 ± 0.91[a]	3.48 ± 0.18	5.97 ± 0.32[a]
Epithelial cells proliferation index (%)	5.42 ± 0.11	8.33 ± 0.61[a]	0.53 ± 0.03	0.61 ± 0.06	0.62 ± 0.06	0.86 ± 0.05
Dorsolateral prostate (n = 8)						
DLP absolute weight (g)	–	–	0.32 ± 0.03	0.25 ± 0.05[a]	0.34 ± 0.09	0.35 ± 0.06
DLP relative weight (g)	–	–	0.08 ± 0.01	0.06 ± 0.01[a]	0.07 ± 0.01	0.07 ± 0.01
DP epithelial height (μm)	9.91 ± 0.85	7.83 ± 0.63[a]	20.28 ± 0.53	19.75 ± 0.42	18.26 ± 0.78	17.95 ± 0.93
DP collagen fibers volume fraction (%)	3.01 ± 0.31	3.76 ± 0.89	3.89 ± 0.94	4.12 ± 0.98	3.30 ± 0.32	3.89 ± 0.41
DP epithelial cells proliferation index (%)	7.91 ± 0.32	11.63 ± 1.23[a]	0.63 ± 0.02	0.68 ± 0.03	0.59 ± 0.01	0.66 ± 0.04
LP epithelial height (μm)	11.28 ± 0.57	9.89 ± 0.61[a]	20.11 ± 0.72	19.70 ± 0.57	20.02 ± 0.73	19.85 ± 0.64
LP collagen fibers volume fraction (%)	3.81 ± 0.31	5.26 ± 0.89[a]	5.42 ± 0.65	8.43 ± 0.87[a]	5.21 ± 1.04	7.75 ± 1.07[a]
LP epithelial cells proliferation index (%)	9.04 ± 0.84	17.58 ± 1.42[a]	0.53 ± 0.03	0.59 ± 0.06	0.65 ± 0.03	0.78 ± 0.04

NP30, NP120 and NP360 (normal-protein diet group died at day 30, 120 and 360, respectively); RP30, RP120 and RP360 (restrict-protein diet group died at day 30, 120 and 360, respectively); Urogenital complex (bladder; urethra, seminal vesicle; ventral, dorsolateral and anterior prostatic lobes); VP (ventral prostate); DLP (dorsolateral prostate); DP (dorsal prostate); LP (lateral prostate). Values are expressed as mean ± SD.

[a] Indicates that RP group is significantly different from NP group with $p<0.05$.

with picrosirius for collagen fibers (type I and type III collagen fibers) and stereological analysis (Junqueira et al., 1979).

The sections were analyzed in a Leica DMLB 80 microscope connected to a Leica DC300FX camera. The digitalized images, obtained by using the image analyzer Leica Q-win software Version 3 for Windows, were used for stereological–morphometric analysis.

The mean of epithelial heights of the prostatic lobes was determined from a total of 1200 random measurements, at 10 different points, in 20 different fields (40×) and from six different animals (sections stained by H&E). The mean of the collagen fibers' volume fraction in the prostatic lobes was determined by a red color automatic detection, in 10 different microscopic fields (20×) and from 10 different individual prostatic lobe sections. The collagen volume fraction was determined as a percentage of red-stained areas per total prostatic area.

To determine the relative proportions of the prostate components (epithelium, stroma and lumen), stereological analysis was carried out using Weibel et al. (1966) multipurpose graticulate with 130 points and 60 test lines. Random measurements were performed in 10 different fields (20×) and from six different individual prostatic lobe sections. The relative values were determined by counting the coincident points of the test grid and dividing them by the total number of points. To avoid erroneous interpretations of the morphometric data, all measurements were made in the intermediate regions of the prostate lobes, which represent the major portion of the prostatic

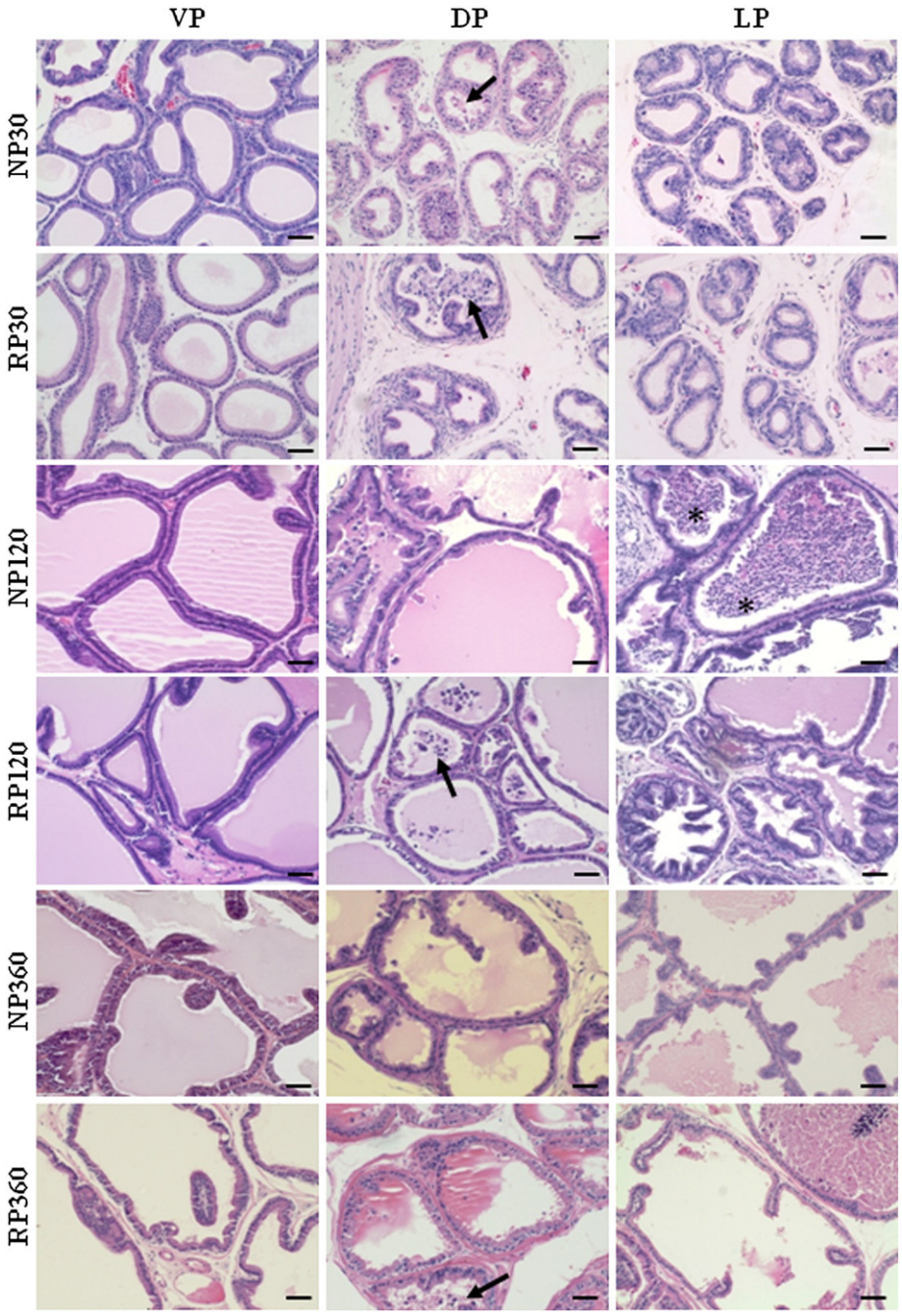

Fig. 2. Representative sections of the ventral (VP), dorsal (DP) and lateral (LP) prostatic lobes from the normal (NP) and restricted (RP) protein diet groups, stained with hematoxylin–eosin. NP group: Tall columnar cells with basal nuclei and evident nucleoli. The clear supranuclear areas probably represent the Golgi complex. Prostatic stroma with blood vessels, smooth muscle cells and thin layer of collagen fibers surrounding the glandular acini. RP group: Note lighter, reduced glandular acini, some areas with epithelial atrophy and hypertrophic stroma with increased collagen fibers. Inflammatory infiltration (*) in LP was observed in both of the groups. Arrow shows cells detached from the DP epithelium. Scale bars = 50 μm.

ductal system in rats (Lee et al., 1990; Nemeth and Lee, 1996). Image acquisition, all quantitative measurements, and the identification of animal and experimental groups were performed in a blind manner by two independent investigators.

The histopathological classification of prostate lesions presented in the experimental animals was accomplished according to the Bar Harbor Classification System for the mouse prostate, developed by National Cancer Institute's Mouse Models of the Human Cancers Consortium, Prostate Steering Committee (Shappell et al., 2004). Five histological sections of the prostatic distal-intermediate regions (eight animals each experimental group) were examined (n = 40 microscopic sections) to quantify the incidence of histopathological lesions including: inflammatory infiltrate (into stromal or luminal spaces), epithelial dysplasia, prostatic intra-epithelial neoplasia (PIN) and focal hyperplasia associated with inflammatory cells (inflammatory reactive atypia). Results were expressed in percentage of total animals.

In situ cell apoptosis detection by TUNEL assay

Apoptosis detection was based in a reaction of in situ Terminal Deoxynucleotidyl Transferase Mediated Biotinylated UTP Nick End-Labeling (TUNEL). Detection was performed using the FragEL™ DNA (Calbiochem, CA, USA) according to the manufacturer's directions. The specimens were counterstained with Hematoxylin and mounted with Permount. The epithelial cells were counted in 10 random microscopic fields (40×) from 6 different ventral, dorsal and lateral prostatic lobe sections from both control and restricted groups. Approximately 10,000 cells were counted per experimental group. TUNEL index was expressed as a percentage of total cells counted (number of TUNEL-positive cells × 100/total cell number).

Immunohistochemistry

Sections of Paraplast® embedded prostates of 4 μm were collected on silanized glass. Antigen retrieval was achieved using a decloaker for 20 min. After washing, the slides were blocked with 3% hydrogen peroxide in methanol for 10 min followed by 3% bovine serum albumin (BSA) in PBS for 1 h at room temperature. Slides were then incubated with monoclonal anti-Rat-Ki-67 antibody (clone MIB-5, Dako, Carpinteria, CA) and polyclonal rabbit anti-androgen receptor antibody (Santa Cruz Biotechnology, CA, USA) at a 1:100 dilution in 1% BSA in PBS and incubated overnight at 4 °C. After washing with PBS, the slides were incubated for 1 h at room temperature with goat anti-mouse IgG-HRP or goat anti-rabbit IgG-HRP antibody (Santa Cruz Biotechnology, CA) diluted 1:200 in 1% BSA in PBS. Chromogen color development was carried out with 3,3′-diaminobenzidine tetrahydrochloride, slides were counterstained with Harris's hematoxylin. Negative control was performed by omitting the primary antibody incubation step.

Epithelial cell proliferation (Ki-67) index

To quantitatively evaluate Ki-67-immunostained nuclei (proliferation index), the number of positive epithelial cells was counted in 10 microscopic fields (40×) of the ventral, dorsal and lateral prostatic lobe sections from 6 different animals from each experimental group. The results are expressed as a percentage of total cells counted (number of labeled nuclei × 100/total number cell). Approximately 10,000 cells were counted per experimental group.

Western blotting analysis of androgen receptor, PCNA and PAR-4

Frozen samples of urogenital complex (without bladder, seminal vesicles and coagulating gland) at PND30, ventral and dorsal rat prostatic lobes at PDN120 and PDN360 from NP and RP experimental groups were mechanically homogenized in 50 mM Tris–HCl buffer pH 7.5 plus 0.25% Triton-X 100 by Polytron for 30 s at 4 °C, centrifuged, and protein was extracted on supernatant and quantified as per Bradford methods (Bradford, 1976). A protein sample (70 μg) was loaded into 10% SDS-PAGE under reducing conditions. After electrophoresis, the proteins were transblotted onto a nitrocellulose membrane (Sigma-CO™, Saint Louis, MO, USA). The blot was blocked with 5% bovine serum albumin (BSA) in TBST (10 mM Tris–HCl, pH 7.5; 150 mM NaCl; 0.1% Tween-20) for 1 h. The blot was then incubated overnight at 4 °C with 3% BSA in TBST containing a 1:1000 dilution of monoclonal mouse anti-PCNA (Proliferating cell nuclear antigen, Santa Cruz Biotechnology™, CA, USA), or polyclonal goat anti-PAR-4 (Prostate apoptosis response 4, Santa Cruz Biotechnology™, CA, USA), or polyclonal rabbit anti-androgen receptor (Santa Cruz Biotechnology™, CA, USA), or polyclonal goat anti-beta actin (Santa Cruz Biotechnology™, CA, USA) primary antibodies. The blot membranes were then washed five times for 20 min in TBST and incubated for 1 h at room temperature with respective secondary peroxidase-conjugated antibodies. After washing in TBS-T, antibody location was detected using chemiluminescence substrate kit (Pierce ECL Western Blotting Substrate — GE Healthcare) according to the manufacturer's instructions. The substrates were removed from membranes and ECL signals were captured by CCD camera (ImageQuant LAS 4000 mini®, GE Healthcare™). The integrated optical density (IOD) of the target proteins bands was measured using the Image J software downloaded from the NIH website (http://rsb.info.

Table 4
Relative frequency of prostate compartments.

Parameters (%)	Experimental groups					
	NP30	RP30	NP120	RP120	NP360	RP360
Ventral prostate (n = 8)						
Epithelium	29.53 ± 3.15	31.32 ± 5.34	33.53 ± 4.32	31.32 ± 3.25	34.14 ± 5.21	36.65 ± 4.54
Stroma	11.51 ± 1.52	25.53 ± 1.55[a]	23.95 ± 3.65	26.74 ± 2.83	18.18 ± 3.41	29.72 ± 4.31[a]
Lumen	59.01 ± 4.86	43.22 ± 6.26[a]	44.32 ± 3.28	42.01 ± 3.43	47.87 ± 5.12	34.58 ± 5.39[a]
Dorsal prostate (n = 8)						
Epithelium	34.82 ± 3.31	34.72 ± 3.41	30.56 ± 4.54	29.82 ± 2.85	29.35 ± 2.62	31.67 ± 3.87
Stroma	26.75 ± 1.72	32.13 ± 1.81	28.73 ± 3.65	29.12 ± 1.84	22.93 ± 2.22	28.76 ± 3.78
Lumen	42.54 ± 3.23	33.15 ± 4.87	47.21 ± 3.62	47.35 ± 2.43	47.85 ± 4.74	40.57 ± 4.23
Lateral prostate (n = 8)						
Epithelium	34.43 ± 2.52	35.52 ± 1.86	26.87 ± 5.77	26.34 ± 3.15	25.23 ± 2.62	26.69 ± 4.76
Stroma	25.23 ± 1.92	36.85 ± 1.52[a]	20.72 ± 4.43	29.54 ± 1.42	23.75 ± 2.42[a]	37.67 ± 5.33[a]
Lumen	40.45 ± 2.55	29.76 ± 2.13[a]	53.22 ± 5.22	44.31 ± 2.32	51.13 ± 4.61	37.52 ± 4.51[a]

NP30, NP120 and NP360 (normal-protein diet group died at day 30, 120 and 360, respectively); RP30, RP120 and RP360 (restrict-protein diet group died at day 30, 120 and 360, respectively). Values are expressed as mean ± SD.

[a] Indicates that RP group is significantly different from NP group with $p<0.05$.

nih.gov.ij/) to compare the protein levels. The androgen-receptor, PCNA and PAR-4 expression was normalized to the β-actin values and the results were expressed as means ± SD.

Statistical analysis

All statistical analyses were carried out using INSTAT software (version 3.0; GraphPad, Inc., San Diego, CA). According to the type of the data, either a non-parametric Mann–Whitney test or a T test (with Welch's correction factor) was used to examine the significance of any difference between groups. The results were presented as the mean ± S.E.M. Differences were considered statistically significant when p-value was <0.05.

Results

Reproductive performance of the dams, birth weight and DAG of the male offspring

As shown in Table 2, the RP and NP rat dams had equivalent body weights at the beginning of pregnancy; however, dams fed a low protein diet gained significantly less weight during pregnancy, despite no change in food intake. The lower weight gain did not affect the litter size, male/female distribution and gestational length (data not shown). At birth, both absolute and relative distances of AGD in the RP rats were lower compared with those in the NP pups. At PND1 the RP male pups were significantly lighter (approximately 33% lower) than the NP pups (Table 2).

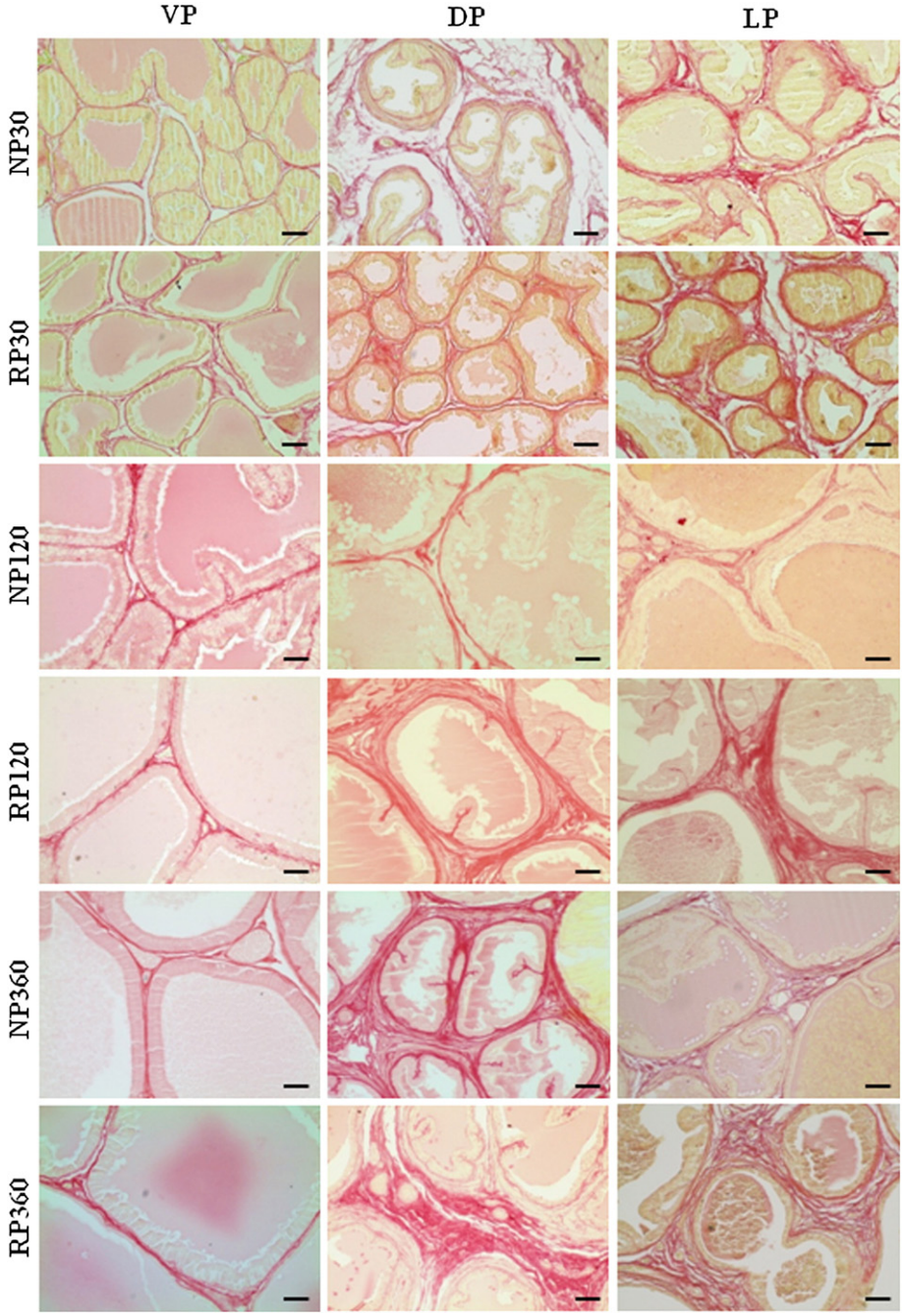

Fig. 3. Representative sections of the ventral (VP); lateral (LP) and dorsal (DP) prostatic lobes from the normal (NP) and restricted (RP) protein diet groups, stained by picrosirius. Collagen fibers are stained in red. The RP prostatic lobes show increased area of collagen fibers compared with that of the NP group, mainly in the lateral lobe. Scale bars = 30 μm.

Neonatal and post-natal growth profile and food intake of the male offspring

Body weight and food intake of pups whose mothers were submitted to protein restriction during gestation are shown in Fig. 1. Despite initiating maternal nutritional rehabilitation with normal protein diet afterbirth, intrauterine protein malnutrition produced long-term reductions in the body weight of male offspring, in which showed a slower growth rate (Fig. 1A). This difference between NP and RP offspring persisted until adulthood (Fig. 1A). A significant catch up growth in body weight was observed between PND100 and PND120, in which the RP animals gained approximately 90 g, while the NP animals gained approximately 30 g (Fig. 1A). As shown in Fig. 1B there was no difference in food consumption between NP and RP male offspring. However, when food intake was normalized to body weight, the low protein offspring consumed significantly more food per gram body weight compared to the control offspring. This indicates that the low protein offspring are in a state of hyperphagia. The hyperphagia was evident until PDN 150 (Fig. 1C). Instead of Fig. 1D presenting no statistically difference in content of visceral fat mass (VFM) between NP and RP groups, there was a trend for the RP group to have a lower VFM (−20%) at DPN120 and 360.

Serum hormone levels

The serum levels of T were significantly reduced in the RP group at PND30, while the serum levels of DHT were decreased in all of the RP groups compared with that of the age-matched control group (Table 3).

Prostatic lobe weight

The absolute and relative weights of the UGC were significantly lower in the RP animals at PND30 and PND120 compared with those of the age-matched control group (Table 3). Similarly, a significant reduction in the absolute and relative weights of VP and DLP was observed in the RP group, at PND120. However, at PND360, there were no differences in these parameters between the experimental groups (Table 3).

Table 5
Effects of maternal protein malnutrition on histopathological lesions incidence in rat prostatic lobes.

Parameters	Experimental groups			
	NP120	RP120	NP360	RP360
Ventral prostate (n = 8)				
Prostatitis (%)	0	2/6 (33%)[a]	1/6 (17%)	3/6 (50%)[a]
Epithelial dysplasia (%)	0.11 ± 0.01	0.28 ± 0.01[a]	10.41 ± 1.03	32.35 ± 2.98[a]
Dorsal prostate (n = 8)				
Prostatitis (%)	0	0	0	1/6 (17%)
Epithelial dysplasia (%)	0	0	0.73 ± 0.08	1.06 ± 0.32[a]
Lateral prostate (n = 8)				
Prostatitis (%)	2/6 (33%)	4/6 (66%)[a]	3/6 (50%)	6/6 (100%)[a]
Epithelial dysplasia (%)	0.21 ± 0.01	0.25 ± 0.01	9.12 ± 1.05	21.41 ± 2.08[a]

NP30, NP120 and NP360 (normal-protein diet group died at day 30, 120 and 360, respectively); RP30, RP120 and RP360 (restrict-protein diet group died at day 30, 120 and 360, respectively). Results are expressed as mean ± SD.
[a] Indicates that RP group is significantly different from NP group with $p<0.05$.

Morphological and morphometrical analyses

The prostatic lobes from the RP group presented similar morphological aspects as those observed in the control group. However, all the prostatic lobes from the RP group offspring showed an apparent slight reduction in the luminal diameter of prostatic glands and an increase in the stromal compartment (Fig. 2). Morphometric analysis confirmed these data and showed a significant reduction in the epithelial cell height of VP, DP and LP in the RP group at PND30, whereas no differences were found in the other ages (Table 3). Similarly, at PND30 and PND360, there was a reduction in the luminal diameter and an increase in the stromal compartment in VP and LP in the RP group compared with those in the NP group (Table 4).

Picrosirius staining showed collagen fibers adjacent to the epithelium, in the interacinar stroma, and around smooth muscle cells in VP, DP and LP in all of the groups (Fig. 3). In VP and LP from the RP

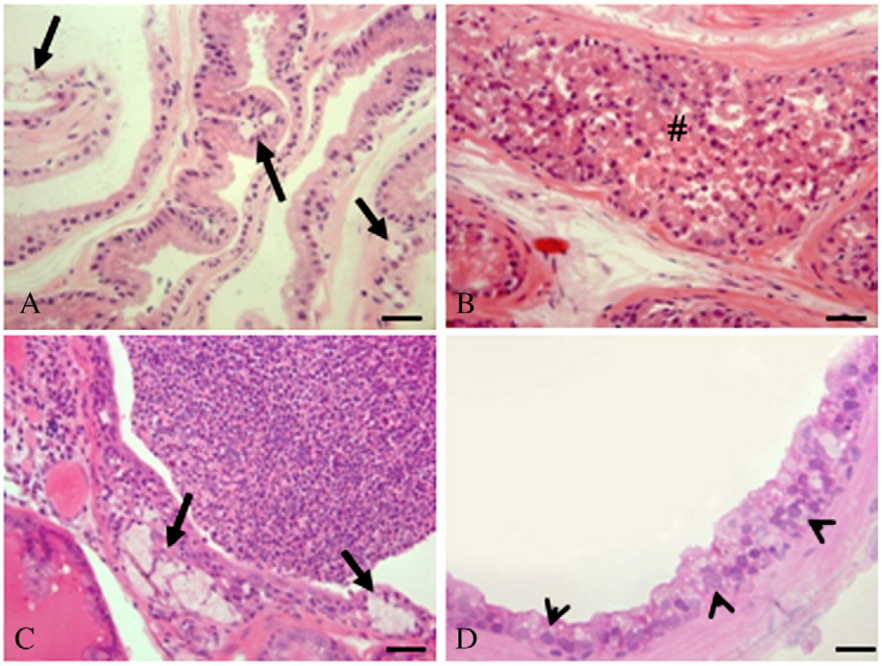

Fig. 4. Representative sections of VP (A), DP (B) and LP (C–D) for morphological alterations from the RP360 group, stained with H&E. A. Note reduced epithelial height and epithelium metaplastic cells (arrows); B. DP with epithelial cells (#) filling out the lumen. C. Stromal hypertrophy with a great amount of collagen fiber (*) disorganization and atrophy of the secretory epithelium. D. Cluster of metaplastic cells (arrow) beside inflammatory cells (prostatitis) at the lumen. Scale bars = 30 μm.

group, an increase in the distribution of collagen fibers was observed (Fig. 3). At PND30 and PND360, morphometric analysis confirmed an increase in collagen volume in VP and LP from the RP group compared with that of the age-matched group (Table 3).

Histopathological examination showed that prostatic lesions were more frequent in the RP group. Dysplasic epithelium (clear epithelial cells with vacuolated cytoplasm and atypical basal nuclei) in VP and LP was observed. Inflammatory infiltrates were observed into three different areas: perivascular (inflammatory cells tightly around the blood capillaries), stromal (inflammatory cells in the stroma between and around the gland or periglandular) and glandular (inside the lumen of the gland). The incidence of prostatitis in LP was more frequent and severe in the RP group at PND360. Such incidence was associated with epithelial alterations, reactive stromal hypertrophy with collagen fibers deposition and changes of glandular architecture (Fig. 4; Table 5). No histopathological alterations were found in the prostatic lobes at PND30 from both experimental groups (data not shown).

Epithelial cell proliferation and apoptosis

Fig. 5 shows the results of immunostaining for Ki-67 in rat prostates. The number of positive nuclei in the VP, DP and LP epithelium from the RP group was evidently increased at PND30 compared with that of the age-matched control group. No positive staining was observed in the negative control (data not shown). The quantitative analysis confirmed a higher Ki-67 index at PND30 in the VP, DP and LP epithelium from the RP group compared with age-matched control group (Table 3).

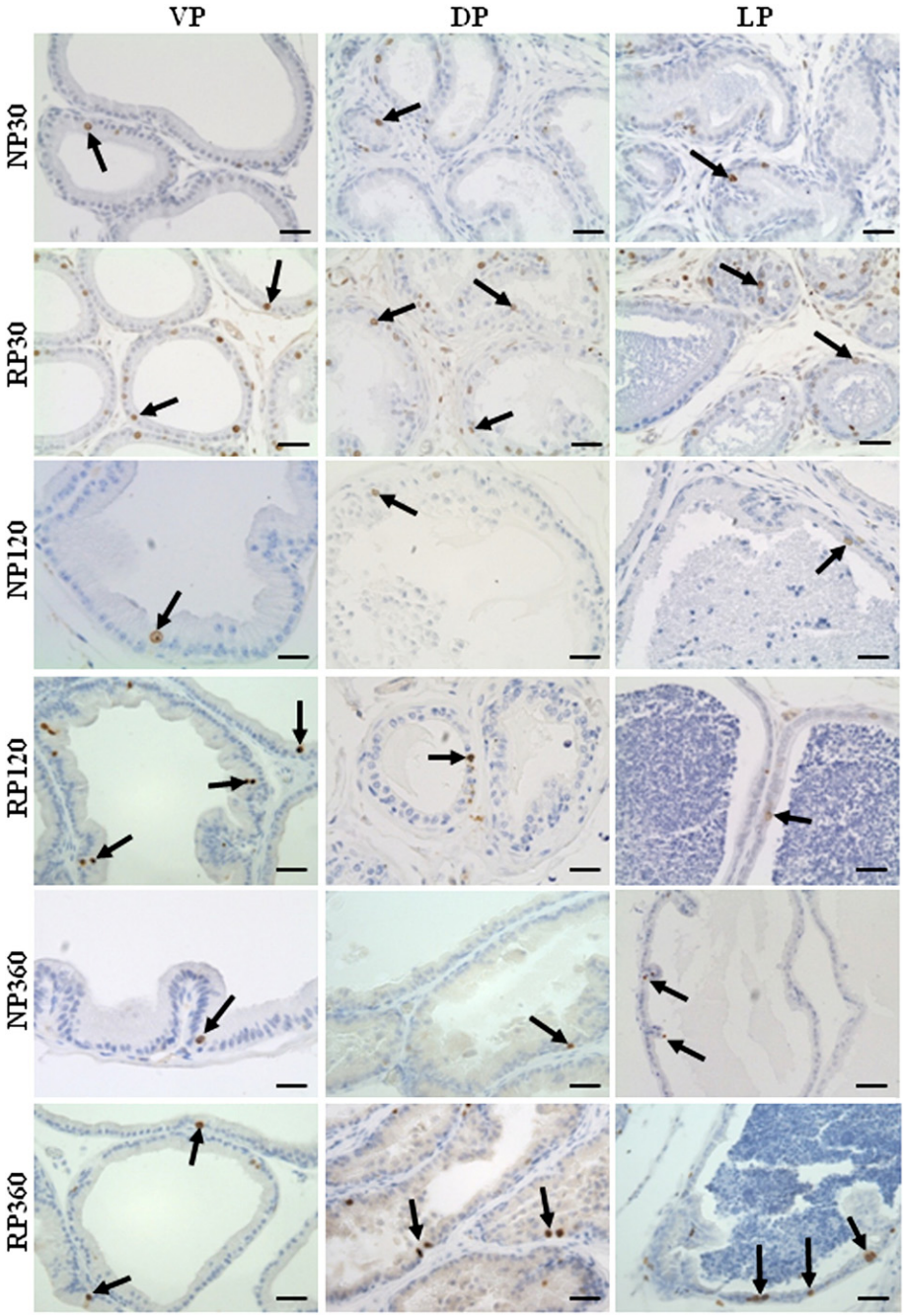

Fig. 5. Representative sections of the ventral (VP), dorsal (DP) and lateral (LP) prostatic lobes from the normal (NP) and restricted (RP) protein diet groups, immunostained for Ki-67. The arrows point to nuclei positive for Ki-67. Note an increased number of Ki-67 positive cells in the RP30 group compared with that in the NP30 group. Scale bars = 30 μm.

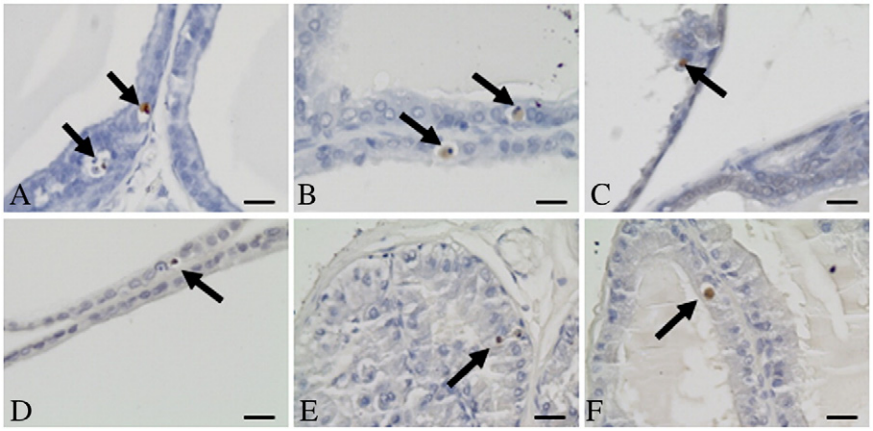

Fig. 6. Representative image of TUNEL reaction in sections of the rat ventral (A and D), dorsal (B and E) and lateral (C and F) prostatic lobes from the normal protein diet (A–C) and low protein diet (D–F) groups at post-natal day 120. The arrows indicate typical positively stained apoptotic epithelial cells that were used to determine the TUNEL index. Scale bars = 30 μm.

Fig. 6 shows the typical morphology of apoptotic cells stained for TUNEL found in the prostatic lobes epithelium from both experimental groups at PND120. Quantitative analysis showed that the apoptotic index (TUNEL index) of epithelial cells was about 0.4% and without statistically significant differences between the groups in all ages studied (data not shown).

Androgen receptor

Androgen receptor immunostaining was detected in the nuclei of columnar and basal epithelial cells of the three prostatic lobes from the NP and RP groups (Fig. 7). AR immunostaining was apparently less intense in the nuclei of prostatic epithelial cells from LP in the RP group at PND30 (Fig. 7).

Western blotting

The expression of AR, PCNA and PAR-4 in the VP and DP tissue from the different experimental groups was evaluated by western blot (Fig. 8). There was no difference in the expression of AR in the urogenital complex and prostatic lobes between groups (Fig. 8B). The analysis of PAR-4 also showed no differences in the expression of this protein related to apoptosis in the prostatic lobes between the groups (Fig. 8C). On the other hand, the analysis of PCNA expression also confirmed the increased index of cellular proliferation in the UGC from RP offspring at PDN30 (Fig. 8D).

Discussion

Nutrient requirement for the organism depends on developmental state, reproductive activity, and age. Protein requirement for gestation and lactation as a percentage of the diet is similar for growth of weanling rats — about 12% — when highly digestible protein of balanced amino acid pattern is used (Benevenga et al., 1995). In this sense, fetal programming caused by a maternal low-protein diet affects the development of several organs, including the reproductive system (Leonhardt et al., 2003; Zambrano et al., 2005; Guzman et al., 2006; Faria et al., 2008; Ramos et al., 2010; Qasem et al., 2012). However, the effect of this programming in adulthood and during aging is unclear, in part because of the possibility of reversion or the compensation of these adverse effects observed earlier in life (Langley-Evans and McMullen, 2010). Therefore, to our knowledge, this is the first study addressing the effect of fetal programming on rat ventral, dorsal and lateral prostatic lobes throughout lifespan.

In our experiment, which only half of the needed protein was provided (6%), MPM did not affect gestation length or litter size. These observations are similar to other reports in the literature evaluating reproductive performance of the pregnant rats fed with an 8% protein diet (Langley and Jackson, 1994; Langley-Evans, 2000). Despite similar litter sizes in both experimental groups, evidence of fetal programming was observed in the RP group by the decreased body weight at birth and reduced AGD in male offspring. These results are consistent with previous reports (Fagundes et al., 2007; Gosby et al., 2009).

MPM was associated with a prolonged reduction in body weight, which in this study, reached the age-matched control group values by the age of 4 months. Other investigators have also demonstrated that the long-term decrease in this parameter represents the imprinting effect of MPM (Desai et al., 2005; Zambrano et al., 2005; Cherala et al., 2006; Fagundes et al., 2007; Qasem et al., 2012). However, the mechanism(s) underlying this imprinting effect is not clearly understood. Furthermore, post-weaning male low protein offspring demonstrated hyperphagia as evidenced by higher food consumption per gram of body weight and this hyperphagia persisted into adulthood. Other investigators have also reported hyperphagia in offspring after maternal exposure to low protein diet during pregnancy and lactation (Fagundes et al., 2007; Coupe et al., 2009; Qasem et al., 2012). Instead of no statistical difference in visceral fat mass between groups, the present study showed that there was a trend for this parameter to be lower in RP male offspring. Previous studies have shown that protein restriction during lactation programmed lower visceral fat mass and total body fat, which are responsible for the lower body weight in the adult offspring (Fagundes et al., 2007; Qasem et al., 2012).

AGD has been used as a good external biomarker for correct prenatal androgen exposure because it is regulated by testosterone from fetal testes (Graham and Gandelman, 1986; Swan et al., 2005). Thus, the decreased AGD in male RP offspring observed in our study suggests an impaired intrauterine androgenic signaling, as proposed by Page et al. (2001). This deficiency in the androgen stimuli persisted in the RP offspring at PND30, which presented a reduction in the serum levels of T and DHT. These results are also in agreement with other studies (Zambrano et al., 2005; Guzman et al., 2006), in which offspring from malnourished mothers presented reduced levels of LH and T, even after being fed a normal protein diet. However, epithelial cell proliferation in the prostatic lobes from the RP group was increased at PND30, suggesting that other factors or hormones in addition to androgens may be acting on cell proliferation in the prostate. Certain growth factors, such as insulin-like growth-factor-1 (IGF-1), keratinocyte growth factor (KGF) and epidermal growth factor (EGF), can stimulate AR and activate AR target genes in the absence of androgen (Culig et al., 1994). These growth factors are ligands for receptor tyrosine kinases, and they initiate complex intracellular signaling cascades (Feldman and Feldman, 2001). This is one of the mechanisms that contribute to prostatic carcinoma progression in an androgen-independent manner (Culig et al., 1994). A similar mechanism has been observed in breast and ovarian cancer,

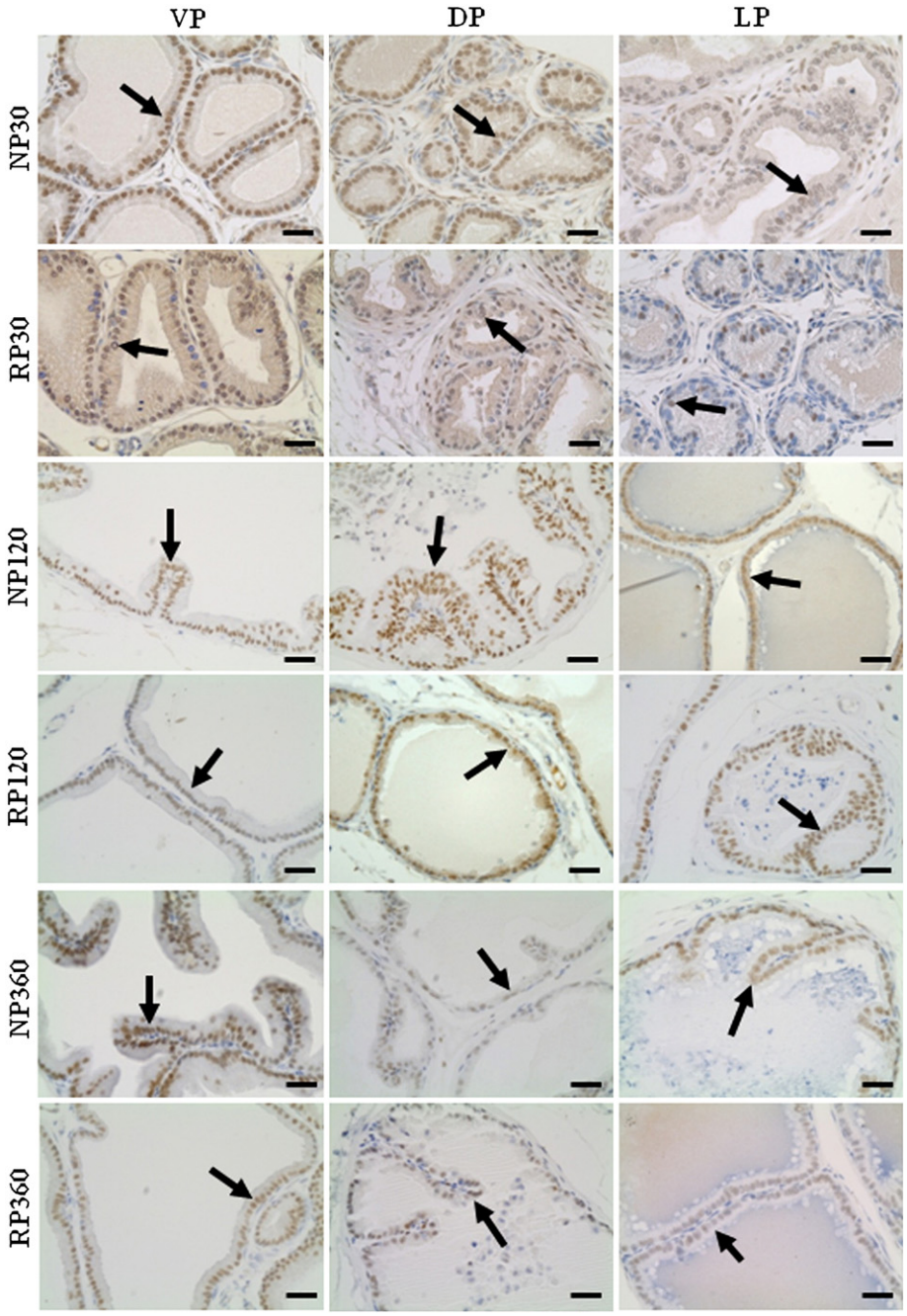

Fig. 7. Representative sections of the ventral (VP), dorsal (DP) and lateral (LP) prostatic lobes from the normal (NP) and restrict (RP) protein diet groups immunostained for androgen receptor. Scale bars = 30 μm.

in which estrogen receptor (ER) activation by growth factors in the absence of estrogen can occur (Pietras et al., 1995). During prostatic development, similar pathways could be activated when androgens/estrogens are low and could stimulate the growth and differentiation of the gland. In addition, some authors have shown that somatotrophic factors, such as prolactin, retinoic acid, estrogen and growth hormones, as well as the interaction between stroma and epithelium may be involved in prostatic development (Webber, 1981; Prins et al., 2001). These possible androgen-independent mechanisms of prostate growth will be issue of future studies of our group.

The ventral and dorsolateral prostatic weights from the RP group offspring were significantly lower until PND120, suggesting a prolonged delay in the maturation of the prostatic lobes. The prostate weight reduction observed in our study is probably related to a decrease in serum androgen levels and reduced epithelial height and epithelial cell secretory activity. This observation is in agreement with Zambrano et al. (2005) study. Previous study on maternal low protein diet during lactation also observed reduction in the dorsal prostate weight at PND21 (Ramos et al., 2010).

Notably, apart from the similarity between the groups in prostate weight and plasma androgen levels at PND360, the prostate gland from RP animals exhibited important differences in epithelial dysplasia, collagen fibers density, prostatitis incidence and severity compared to NP group. These differences reveal that this type of maternal malnutrition promotes other effects on prostate gland physiology beyond just prostatic tissue growth delay. Several studies have demonstrated that alterations in androgens signaling during fetal/neonatal period may be involved in an increased susceptibility to prostate diseases with aging (Risbridger et al., 2005; Prins et al., 2006; Un-no et al., 2007). The increased incidence of epithelial dysplasia suggests impaired control

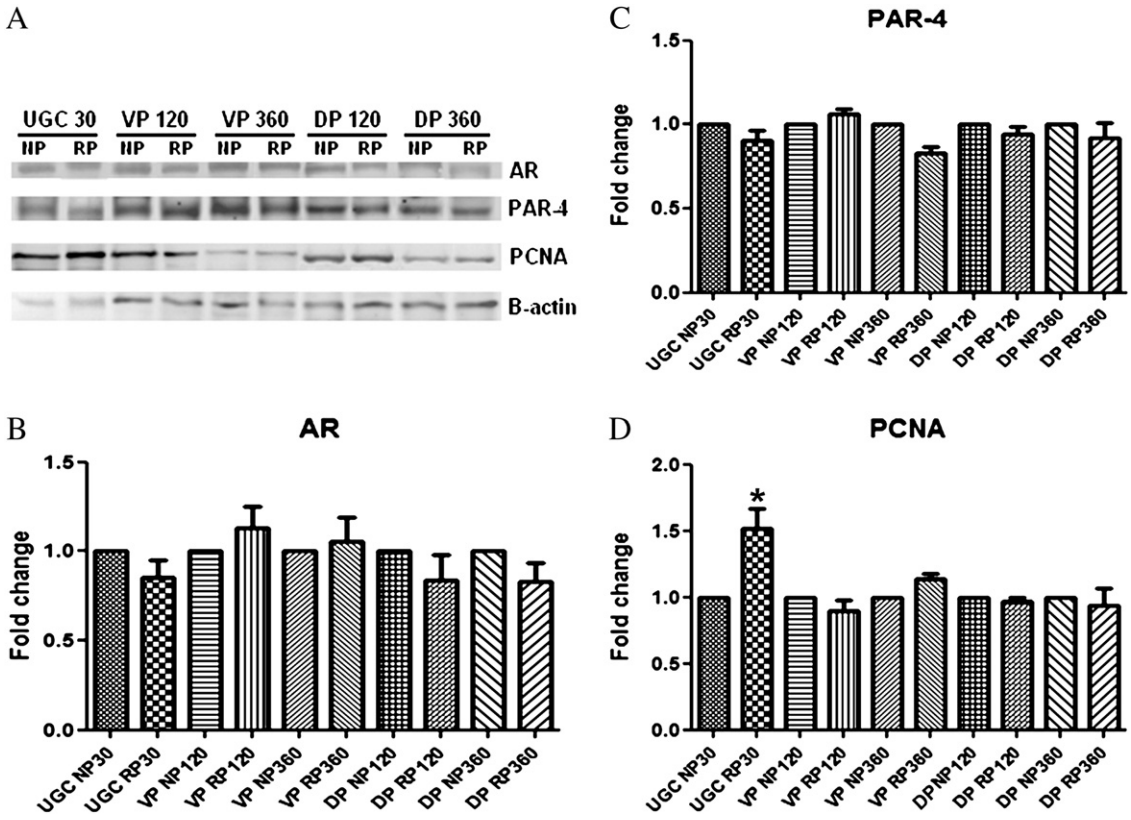

Fig. 8. A) Representative Western blotting for androgen receptor (AR), prostate apoptosis response-4 (PAR-4), proliferating cell nuclear PCNA and beta-actin expression in the rat urogenital complex (UGC), ventral (VP) and dorsal (DP) prostatic lobes from the control (NP) and low protein (RP) groups. B) Densitometric analysis of the AR bands and (C) PAR-4 bands showed no differences between the groups in both prostatic lobes. D) Densitometric analysis of the PCNA bands showing a significant higher expression only in the UGC from the RP at PDN30. Results are expressed as mean ± SD. * indicates that RP group is significantly different from NP group with $p<0.05$.

of epithelial cell differentiated status. Androgens play a crucial role in the maintenance of the differentiated state of prostate epithelial cells and low androgen levels observed in the RP animals may lead to phenotypic instability of prostatic epithelial cells (Marker et al., 2003; Shidaifat et al., 2004; Justulin et al., 2010). However, if the dysplasic epithelium will evolve into neoplasic lesions remains to be determined. On the one hand, increased incidence of prostatitis also suggests unbalanced regulation of inflammatory processes in these glands. Sustained inflammatory microenvironment has been associated with initiation and progression of epithelial dysplasia (Elkahwaji et al., 2009). In this scenario, besides epithelial dysplasia, repeated tissue damage and repair due inflammation can result in increased collagen deposition in the stroma, as observed in LP from RP animals (Sivakumar and Das, 2008).

Conclusion

In conclusion, our results suggest that adequate nutritional status during embryonic/fetal development is important for both prostate morphogenesis during early life as well as homeostasis of the gland throughout the lifespan. In addition, MPM can increase prostate susceptibility to prostatitis and epithelial dysplasia with aging.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by São Paulo Research Foundation, grants no. 09/50204-6 and 09/52747-7. This article represents part of the PhD thesis developed by JCR to the Institute of Biosciences (UNESP), Brazil.

References

Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. Nutrition 1997;13:807–13.
Bateson P, Barker DJ, Clutton-Brock T, Deb D, D'Udine D, Foley RA. Developmental plasticity and human health. Nature 2004;430:419–21.
Benevenga NJ, Calvert C, Eckhert CD, Fahey GC, Greger JL, Keen CL, et al. Nutrient requirements of the laboratory rat. In: Benevenga NJ, Calvert C, Eckhert CD, Fahey GC, Greger JL, Keen CL, et al, editors. Nutrient requirements of laboratory animals. 4th ed. Washington: National Academy Press; 1995. p. 11–79.
Bertram CE, Hanson MA. Animal models and programming of the metabolic syndrome. Br Med Bull 2001;60:103–21.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
Cherala G, Shapiro BH, D'mello AP. Two low protein diets differentially affect food consumption and reproductive performance in pregnant and lactating rats and long-term growth in their offspring. J Nutr 2006;136:2827–33.
Coupe B, Frt I, Darmaun D, Parnet P. The timing of "catch-up growth" affects metabolism and appetite regulation in male rats born with intrauterine growth restriction. Am J Physiol Regul Integr Comp Physiol 2009;297:R813–24.
Cowin PA, Foster P, Pedersen J, Hedwards S, McPherson SJ, Risbridger GP. Early-onset endocrine disruptor-induced prostatitis in the rat. Environ Health Perspect 2008;116:923–9.
Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 1994;54:5474–8.
Desai M, Gayle D, Babu J, Ross MG. Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. Am J Physiol Regul Integr Comp Physiol 2005;288:R91–6.
Dimosthenopoulos C. Malnutrition. In: Katsilambros N, Dimosthenopoulos C, Kontogianni M, Manglara E, Poulia K, editors. Clinical nutrition in practice. Chichester: Wiley-Blackwell; 2010. p. 37–44.
Elkahwaji JE, Hauke RJ, Brawner CM. Chronic bacterial inflammation induces prostatic intraepithelial neoplasia in mouse prostate. Br J Cancer 2009;101:1740–8.
Fagundes AT, Moura EG, Passos MC, Oliveira E, Toste FP, Bonomo IT, et al. Maternal low-protein diet during lactation programmes body composition and glucose homeostasis in the adult rat offspring. Br J Nutr 2007;98:922–8.
Faria TS, Brasil FB, Sampaio FJ, Ramos CF. Maternal malnutrition during lactation alters the folliculogenesis and gonadotropins and estrogen isoforms ovarian receptors in the offspring at puberty. J Endocrinol 2008;198:625–34.

Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
Fernandez-Twinn DS, Ozanne SE, Ekizoglou S, Doherty C, James L, Gusterson B, et al. The maternal endocrine environment in the low-protein model of intra-uterine growth restriction. Br J Nutr 2003;90:815–22.
Fernandez-Twinn DS, Ekizoglou S, Gusterson BA, Luan J, Ozanne SE. Compensatory mammary growth following protein restriction during pregnancy and lactation increases early-onset mammary tumor incidence in rats. Carcinogenesis 2007;28:545–52.
Fischbeck KL, Rasmussen KM. Effect of repeated reproductive cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. J Nutr 1987;117:1967–75.
Gardner DS, Ozanne SE, Sinclair KD. Effect of the early-life nutritional environment on fecundity and fertility of mammals. Philos Trans R Soc Lond B Biol Sci 2009;364:3419–27.
Gosby AK, Stanton LML, Maloney CA, Thompson M, Briody J, Baxter RC, et al. Postnatal nutrition alters body composition in adult offspring exposed to maternal protein restriction. Br J Nutr 2009;101:1878–84.
Graham S, Gandelman R. The expression of ano-genital distance data in the mouse. Physiol Behav 1986;36:103–4.
Guzman C, Cabrera R, Cardenas M, Larrea F, Nathanielsz PW, Zambrano E. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. J Physiol 2006;572:97-108.
Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979;11:447–55.
Justulin Jr LA, Della-Coleta HH, Taboga SR, Felisbino SL. Matrix metalloproteinase (MMP)-2 and MMP-9 activity and localization during ventral prostate atrophy and regrowth. Int J Androl 2010;33:696–708.
Langley SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci 1994;86:217–22.
Langley-Evans SC. Critical differences between two low protein diet protocols in the programming of hypertension in the rat. Int J Food Sci Nutr 2000;51:11–7.
Langley-Evans SC, Alexander B, McArdle HJ, Sloboda DM. Developmental origins of health and disease. J Nutr Metab 2012;2012:838640. http://dx.doi.org/10.1155/2012/838640.
Langley-Evans SC, McMullen S. Developmental origins of adult disease. Med Princ Pract 2010;19:87–98.
Lee C, Sensibar JA, Dudek SM, Hiipakka RA, Liao ST. Prostatic ductal system in rats: regional variation in morphological and functional activities. Biol Reprod 1990;43:1079–86.
Leonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, Dupouy JP. Effects of perinatal maternal food restriction on pituitary–gonadal axis and plasma leptin level in rat pup at birth and waning and on timing of puberty. Biol Reprod 2003;68:390–400.
Lins MC, Moura EG, Lisboa PC, Bonomo IT, Passos MCF. Effects of maternal leptin treatment during lactation on the body weight and leptin resistance of adult offspring. Regul Pept 2005;127:197–202.
Marker PC, Donjacour AA, Dahiya R, Cunha GR. Hormonal, cellular, and molecular control of prostatic development. Dev Biol 2003;253:165–74.
McMillen IC, MacLaughlin SM, Muhlhausler BS, Gentili S, Duffield JL, Morrison JL. Developmental origins of adult health and disease: the role of periconceptional and foetal nutrition. Basic Clin Pharmacol Toxicol 2008;102:82–9.
Nemeth JA, Lee C. Prostatic ductal system in rats: regional variation in stromal organization. Prostate 1996;28:124–8.
Nijland MJ, Ford SP, Nathanielsz PW. Prenatal origins of adult disease. Curr Opin Obstet Gynecol 2008;20:132–8.
Page KC, Sottas CM, Hardy MP. Prenatal exposure to dexamethasone alters Leydig cell steroidogenic capacity in immature and adult rats. J Androl 2001;22:973–80.
Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram ND, Ramos L, et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995;10:2435–46.
Prins GS, Birch L, Habermann H, Chang WY, Tebeau C, Putz O, et al. Influence of neonatal estrogens on rat prostate development. Reprod Fertil Dev 2001;13:241–52.
Prins GS, Huang L, Birch L, Pu Y. The role of estrogens in normal and abnormal development of the prostate gland. Ann NY Acad Sci 2006;1089:1-13.
Qasem RJ, Yablonski E, Li J, Tang HM, Pontiggia L, D'mello AP. Elucidation of thrifty features in adult rats exposed to protein restriction during gestation and lactation. Physiol Behav 2012;105:1182–93.
Rae MT, Kyle CE, Miller DW, Hammond AJ, Brooks AN, Rhind SM. The effects of undernutrition, in utero, on reproductive function in adult male and female sheep. Anim Reprod Sci 2002;72:63–71.
Ramos CF, Babinski MA, Costa WS, Sampaio FJ. The prostate of weaned pups is altered by maternal malnutrition during lactation in rats. Asian J Androl 2010;12:180–5.
Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993;123:1939–51.
Risbridger GP, Almahbobi GA, Taylor RA. Early prostate development and its association with late-life prostate disease. Cell Tissue Res 2005;322:173–81.
Santos AM, Ferraz MR, Teixeira CV, Sampaio FJ, Ramos CF. Effects of undernutrition on serum and testicular testosterone levels and sexual function in adult rats. Horm Metab Res 2004;36:27–33.
Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, et al. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the bar harbor meeting of the mouse models of Human Cancer Consortium Prostate Pathology Committee. Cancer Res 2004;64:2270–305.
Shidaifat F, Daradka M, Al-Omari R. Effect of androgen ablation on prostatic cell differentiation in dogs. Endocr Res 2004;30:327–34.
Sivakumar P, Das AM. Fibrosis, chronic inflammation and new pathways for drug discovery. Inflamm Res 2008;57:410–8.
Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in ano-genital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 2005;113:1056–61.
Un-no T, Hayami S, Nobata S, Sudoko H, Honma S, Fujita K, et al. Neonatal exposure to estrogen in the Wistar rat decreases estrogen receptor-beta and induces epithelial proliferation of the prostate in the adult. Urol Int 2007;79:345–51.
Untergasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: age-related tissue-remodeling. Exp Gerontol 2005;40:121–8.
Vicente LL, Moura EG, Lisboa PC, Costa AMA, Amadeu T, Mandarim-de-Lacerda CA, et al. Malnutrition during lactation is associated with higher expression of leptin receptor in pituitary of the adult offspring. Nutrition 2004;20:924–8.
Webber MM. Polypeptide hormones and the prostate. Prog Clin Biol Res 1981;75B:63–88.
Weibel ER, Kistler GS, Scherle WF. Practical stereological methods for morphometric cytology. J Cell Biol 1966;30:23–38.
Wu G, Imhoff-Kunsch B, Girard AW. Biological mechanisms for nutritional regulation of maternal health and fetal development. Paediatr Perinat Epidemiol 2012;26:4-26.
Zambrano E, Rodrigez-González GL, Guzmán C, García-Becerra R, Boeck L, Díaz L, et al. A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. J Physiol 2005;563:275–84.
Zambrano E, Bautista CJ, Deás M, Martinez-Samayoa PM, González-Zamorano M, Ledesma H, et al. A maternal low protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. J Physiol 2006;571:221–30.