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Enhanced Nephrogenesis In Offspring of Water Restricted Rat Dams

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Abstract

Objective—Maternal water restriction (WR) may induce offspring plasma hypertonicity and enhanced vasopressin secretory responses. We determined effects of pregnancy WR on offspring body composition, renal morphology and blood pressure.

Study Design—Pregnant Sprague Dawley rats (n=21) were randomized to WR, pair-fed (PF) and control from embryo day (e) 10–21. Offspring body and organ weights and glomerular number and size were measured at birth and at 21 days of age. At 6 and 9 weeks, offspring blood pressure was determined.

Results—At 21 days of age, WR offspring glomerular number was increased (17%; $p<0.05$), while PF glomerular number was lower (4%) compared to controls. Systolic blood pressures were elevated in both WR and PF at both 6 and 9 weeks.

Conclusions—Pregnancy WR stimulates offspring nephrogenesis, suggesting an adaptive response to future dehydration. Programmed hypertension in WR and PF groups likely occurs via different mechanisms.

Keywords

Rat; dehydration; programming; glomeruli; hypertension

Introduction

Programming is a process whereby an insult or environmental exposure during a critical period of in utero or neonatal development results in a permanent change in offspring phenotype. Programming may exhibit as resetting of hormonal axes and feedback regulation, altered gene expression, perhaps mediated by epigenetic mechanisms, and/or altered organ structure.^{1–3} These manifestations are dependent upon the time in gestation and organ development, as well as the species in which the insult occurs. Studies of Barker et al^{1;4} and others have demonstrated a marked impact of human low birth weight on the incidence of offspring metabolic syndrome, including manifestations of obesity, diabetes, and hypertension. It has been postulated that gestational programming provides the organism survival benefits by

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permitting adaptations to a predicted extrauterine environment. Whereby genetic mutations require long periods of time, and are generally irreversible, programming may allow the organism to respond to comparatively short time period changes in the environment. Thus, the programmed phenotypic changes may be assessed in the prospective of survival benefits to the point of reproduction.^{5;6}

Throughout evolution, the most common stress on survival has been drought and the associated famine accompanying drought. In response to water restriction, dehydration is further associated with an anorexic response, and thus food intake may be reduced via both intrinsic and extrinsic mechanisms. Although drought conditions rarely occur in the developed world, they continue to occur in third world countries throughout the 20th and 21st centuries. In addition to drought, pregnant women are often exposed to conditions of dehydration, as evidenced by hyperemesis gravidarum, and/or maternal exercise.

Our laboratory has previously demonstrated programming effects of maternal water restriction in both the ovine and rodent models.⁷ In the offspring of water restricted rat dams, maternal water deprivation was associated with reduced maternal food intake, and resulted in low birth weight newborns. At 12 weeks of age, male offspring demonstrated significantly elevated plasma sodium and osmolality and blood hematocrit. In view of the resulting low birth weight following maternal dehydration and dehydration-anorexia, it remains unclear as to the primary effects of water restriction vs. the effects of secondary anorexia-mediated nutrient restriction. In the present study, we sought to differentiate the effects of water restriction and associated anorexia from the effects of matched nutrient restriction on offspring organ growth, plasma composition and systemic blood pressure.

Materials and Methods

Dams

A model of rat dam water-restriction during pregnancy and lactation was used. Studies were approved by the Animal Research Committee of LABioMed at Harbor-UCLA Medical and were in accordance with the American Association for Accreditation of Laboratory Care and National Institutes of Health guidelines. First-time-pregnant Sprague Dawley rats (Charles River Laboratories Inc, Hollister, CA) were housed in a facility with constant temperature and humidity and on a controlled 12-hour light/12-hour dark cycle. At embryo day (e) 10, dams were provided either ad libitum water and food intake (control; CTL, n=7), ad libitum food intake but water-restricted (WR, n=7) to produce an increment of ~6 mOsm/kg in plasma osmolality levels, or provided ad libitum water intake but food-restricted (pair fed; PF, n=7) to match the WR dams food intake. The regimens were followed from e10 to term (e21). Maternal food and water intake as well as body weights were monitored daily. Maternal plasma osmolality and sodium levels were determined at e21. Rat dams gave birth normally, and at day 1 after birth, pups were culled to eight (four males and four females) per litter to normalize rearing. One pup of each dam, in addition to the eight, was sacrificed by decapitation, and organs were collected. Once delivered, all dams were placed on ad libitum food and water intake.

Offspring

Newborns were suckled ad libitum and at day 21 of age weaned to ad libitum food and water and housed individually. Body weights were recorded at 1 and 21 days of age, and at weekly intervals through 20 weeks. Daily water and food intake was monitored from 3 to 12 weeks of age. At 21 days of age (prior to weaning), one male and one female were sacrificed and blood was collected by cardiac puncture. All blood samples were measured for hematocrit and plasma electrolytes, osmolality, and aldosterone. At sacrifice (1 and 21 days of age), organs were

collected and wet weights of brain, heart, stomach, liver, pancreas, kidneys, and adrenals measured. Blood pressures were measured at 6 and 9 weeks of age. Once harvested, the kidneys were fixed in 10% neutral buffered formalin, and dissected in a plane perpendicular to the interpolar axis, yielding slices of 1mm which were embedded in paraffin. Computer-assisted image analysis was used to determine the cross-sectional area and number of glomeruli. Briefly, the kidney was fixed in 4% phosphate-buffered formalin, dissected in a plane perpendicular to the interpolar axis, and subsequently processed for paraffin embedding. 5 μ m sections were stained with hematoxylin-eosin stain and computer-assisted photomicrographs were captured at x20 magnification. Image PRO software (version 5.1) was used to determine the cross-sectional area and number of glomeruli per μ m² area. For each specimen, 3 sections were taken and from each section, 4 images were examined. The average value was thus determined for all specimens.

Analytical Methods

Plasma electrolyte levels were determined with a Nova 5 electrolyte analyzer (Nova Biomedical, Waltham, MA). Plasma osmolality was measured by freezing point depression on an Advanced Digimatic Osmometer (Model MO; Advanced Instruments, Needham Heights, MA). Hematocrit was determined in duplicate by collecting blood in heparinized microhematocrit tubes and centrifuging for 5 minutes (Autocrit II; Monoject Scientific, St. Louis, MO). Plasma aldosterone levels were measured by radioimmunoassay (ICN Pharmaceuticals, Costa Mesa, CA). Blood pressure was measured using a tail-cuff sphygmomanometer in offspring acclimated to daily handling, and the mean of four consecutive values determined.

Statistics

All values are expressed as mean $\pm$ SEM. Differences between groups were analyzed using analysis of variance (ANOVA) with Duncan's test post hoc analysis. All 1 day of age data analysis for males and females was combined. All other ages (21 days through 20 weeks) were analyzed separately for males and females.

Results

In WR dams, maternal water was restricted by ~50% to produce the desired 6 mOsm/kg increase in maternal plasma osmolality. WR dam food intake averaged ~25% less than controls, a value which was matched in the PF dams. At e21, in response to maternal water restriction, maternal WR plasma osmolality (WR 293 $\pm$ 3, PF 278 $\pm$ 3, CTL 286 $\pm$ 2 mOsm/kg, $p<0.05$) and sodium (WR 137.4 $\pm$ 0.8, PF 132.8 $\pm$ 0.8, CTL 134.4 $\pm$ 0.7 mEq/L, $p<0.01$) was significantly increased in comparison to PF and CTL, in concordance with the protocol objectives. PF animals demonstrated a small but nonsignificant decrease in maternal plasma sodium, though a significant decrease in plasma osmolality as compared to CTL dams.

There was no difference in litter size or pup survival among the three groups. Among the offspring, both the WR and PF were significantly smaller than CTL pups (WR 5.7 $\pm$ 0.1, PF 6.3 $\pm$ 0.1, CTL 7.1 $\pm$ 0.2 g, $p<0.01$). At three weeks of age (prior to weaning), catch up growth occurred such that there was no difference in the offspring weight among the three groups (Figure 1).

Organ weights obtained at 1 and 21 days of age demonstrated marked differences among the three groups (Table 1). At 1 day of age, despite the reduced body weights of WR and PF pups, there was evidence of brain sparing, with significantly increased percent brain/body weight. By 21 days of age catch up growth of PF and WR to CTL pups occurred and thus a difference in the absolute or percent brain/body weight among groups was no longer seen. In contrast,

though percent heart/body and adrenal/body weights were similar among the three groups at 1 day of age, WR offspring demonstrated cardiomegaly and adrenomegaly at 21 days, as compared to both PF and CTL animals. Although there were differences among the three groups in percent liver/body, pancreas/body, and stomach/body weights at 1 day of age, these values normalized by 21 days. There were no differences in absolute or percent kidney/body weight among the three groups at either 1 or 21 days of age. However, there were significant differences in glomerular number among the three groups at 21 days of age. Whereas, pair fed offspring had a significant decrease in the number of glomeruli, WR offspring demonstrated a significant increase in glomerular number (WR 2.3 ± 0.1 , PF 1.8 ± 0.1 , CTL 1.9 ± 0.1 per mm^2 , $p < 0.01$).

At 21 days of age, WR pups demonstrated elevated plasma sodium while PF pups exhibited relative hyponatremia as compared to CTL (WR 141.2 ± 0.4 , PF 134.6 ± 0.6 , CTL 138.2 ± 0.3 mEq/L, $p < 0.01$). In conjunction with plasma hyponatremia, female WR offspring demonstrated significantly elevated plasma aldosterone as compared to both PF and CTL (WR 414 ± 38 , PF 251 ± 13 , CTL 260 ± 14 pg/ml, $p < 0.01$), while the male offspring showed a similar trend (WR 272 ± 31 , PF 171 ± 31 , CTL 225 ± 34 pg/ml, $p > 0.05$). Plasma chloride and osmolality were significantly decreased in PF as compared to WR and CTL respectively (WR 108 ± 0.5 , PF 105 ± 0.5 , CTL 107 ± 0.5 mEq/L, $p < 0.01$); (WR 297.7 ± 0.7 , PF 293.4 ± 0.7 , CTL 298.8 ± 0.7 mOsm/Kg, $p < 0.01$). No significant differences were found in hematocrit values.

WR and PF pups demonstrated significantly elevated systolic pressures at both 6 weeks (WR 131 ± 3 , PF 131 ± 3 , CTL 121 ± 3 mmHg, $p < 0.05$) and 9 weeks (WR 137 ± 3 , PF 137 ± 3 , CTL 127 ± 3 mmHg, $p < 0.05$) of age as compared to CTL, though there was no difference between the WR and PF groups.

Comment

The results of the present study demonstrate unique programming effects of gestational water restriction (and accompanying dehydration-anorexia) in comparison to matched nutrient restriction alone. These results confirm previous studies in the rat in which maternal hyponatremia programs offspring plasma hyponatremia.⁷ The findings of programmed hypertension, adrenomegaly and cardiomegaly indicate potentially adverse consequences as well as potentially beneficial survival effects in response to gestational dehydration.

In response to maternal water restriction, rat dams demonstrated the expected increases in plasma sodium and osmolality. The WR and thus the PF group consumed approximately 25% less food than CTL, with this reduction remaining relatively constant over the course of the water restriction. Whereas PF dams demonstrated no change in plasma sodium, there was a small but significant decrease in plasma osmolality compared to CTL. In humans, meal-related food intake induces a significant increase in plasma osmolality, thought to be a result of fluid entry into the gastrointestinal tract.⁸ As blood samples were drawn in the morning, several hours following the 12 h dark period during which rodents ingest the majority of food, it is unlikely that diet induced hyperosmolality in the CTL animals accounts for the relatively reduced osmolality in PF dams. In rodents, food deprivation induces a vasopressin-independent increase in renal water reabsorption⁹ which may reduce plasma osmolality. Additionally, decreased nutrient and electrolyte intake may contribute to the reduced plasma osmolality.

As predicted, offspring of both WR and PF animals were significantly growth restricted at birth. The more marked reduction in the weight of WR pups suggests an additive effect of water restriction beyond that of matched nutrient restriction. As wet and dry body weights were not determined, it is possible that WR offspring were relatively dehydrated at birth contributing to the reduced body weight. However, the water loss associated with a 1–2% increase in plasma sodium is unlikely to explain the 20% reduction in body weight, as compared to CTL.

Alternatively, reduced maternal water intake and potentially reduced plasma volume may further impair maternal to fetal nutrient transfer beyond that of maternal nutrient restriction. By 21 days of offspring age and through 20 weeks, there were no differences in the body weights of the offspring, indicating rapid catch-up growth among the WR and PF groups, though no development of obesity.

In previous studies, we demonstrated that 50% maternal nutrient restriction during the same period of gestation results in a marked degree of intrauterine growth restriction, more significant than that induced by the 25% pair fed nutrient restriction in the present group (17% vs 12% reduction, respectively).¹⁰ Growth restricted offspring of dams 50% nutrient restricted during pregnancy exhibited rapid catch-up growth resulting in marked obesity (increased body weight, % body fat), a finding not observed in the PF or WR pups in the present study. The absence of the development of obesity in the PF group suggests that there is a threshold low birth weight for the development of offspring obesity that was likely not reached in the PF group.

The organ specific changes and particularly that of the kidney, indicates marked differences among the three groups. The increased cardiac weight of WR pups at 21 days of age implies cardiomegaly, and potentially adverse effects. One may typify the WR offspring as exhibiting hypernatremic hypertension in conjunction with elevated aldosterone levels. In contrast, the PF group demonstrated normonatremic hypertension associated with significantly reduced glomerular number (4%). The reduced glomerular number in PF group is consistent with studies of nutrient deprived sheep and rats suggesting that the hypertension expressed in these animals may be, in part, of a renal etiology. It is unclear whether the reduced glomerular number is a result of a lack of glomerular formation, or an induction of glomerular sclerosis in response to the nutrient restriction. The relative increased in glomerular size suggests glomerular hyperperfusion, although GFR was not measured in the present study. In contrast, the WR offspring demonstrated a significant *increase* in glomerular number (17%). Thus, the effects of plasma hypernatremia and secondary endocrine responses counteract the effects of nutrient restriction on glomerular growth or sclerosis. We postulate that increased glomerular number permits the organism optimal urinary concentrating ability in response to dehydration, either as a result of reduced individual glomerular perfusion or as a result of the increased ability to maintain an increased medullary osmotic gradient.

Notably, human epidemiologic studies indicate a U-shaped curve for the programmed effect of low birth weight on offspring cardiovascular disease.^{11;12} However, an important covariate is the degree of weight gain during the first year of life. In our previous studies, we demonstrated that an increased rate of catch-up growth during the first 21 days of life predicted the offspring obesity phenotype. Whereas 50% food restricted pups demonstrated a rapid catch-up growth, exceeding the weight of the control group at 21 days of age, the PF offspring in the present study equaled the weight of controls at 21 days of age. Although the WR pups were growth restricted to a similar degree as that previously noted by 50% food restriction, they too did not develop offspring obesity by 20 weeks of age, perhaps consistent with the unique effects of dehydration and dehydration-anorexia. Of note, in the earlier study, 50% nutrient restricted offspring were cross fostered to dams fed ad libitum during pregnancy and lactation, while the current study maintained both WR and PF pups with their own dams (fed ad libitum during lactation). It is likely that water and/or nutrient restriction during pregnancy reduced subsequent lactation milk supply and thus limited the rate of catch-up growth in the present study. Nevertheless, these results demonstrate that low birth weight alone is not sufficient for the programming of offspring obesity; rather, programming effects are dependent upon additional mechanisms.

At 21 days of age, WR offspring demonstrated plasma hypernatremia. These results are consistent with our previous studies indicating the programmed hypernatremia in offspring of water restricted rat dams.⁷ The small though significant decrease in PF plasma sodium appears to be a similar programming response to the relative plasma osmolality noted in PF dams, although this degree of hyponatremia is likely not clinically significant. WR pups demonstrated elevated plasma aldosterone which may be the putative factor underlying the plasma sodium increase, and is consistent with the increased adrenal weight noted at 21 days of age, although we did not examine the relative cortex from medullary weight. Determination of the responsible mechanism(s) for hyper- and hyponatremia in WR and PF offspring, respectively, would require assessment of plasma osmolality setpoints for vasopressin secretion and potentially studies of urinary concentrating abilities.

Importantly, the present study demonstrates the programming of offspring systolic hypertension in both WR and PF pups at six weeks and nine weeks of age. As discussed above, 50% nutrient restriction results in evidence of offspring hypertension by approximately 3 months of age in conjunction with obesity.¹⁰ As the pair fed (25% nutrient restriction) offspring in the present study demonstrated hypertension by 6 weeks of age, though not obesity, these results suggest independent mechanisms contributing to these phenotypic expressions. Recent preliminary data from our laboratory indicate reduced mesenteric branching and altered extracellular matrix (i.e., increased collagen, reduced elastin) in low birth weight offspring, likely contributing to the development of hypertension.¹³ Despite the similar degrees of hypertension, the evidence of cardiomegaly in the WR though not PF offspring suggest varying etiologies or responses to hypertension among these two groups. Plasma hypernatremia has been associated with increased central sympathetic drive originating at the nucleus of solitary tract.¹⁴ Although heart rate was not measured in the current study, a sympathetically increased heart rate and accompanying cardiac output, in the presence of systemic hypertension, may contribute to cardiomegaly in the hypernatremic offspring.¹⁵ The manifestation of obesity, in contrast, may result from impaired development of hypothalamic anorexigenic pathways as a result of low plasma leptin levels during critical growth periods.^{16–18} Although the degree of body weight reduction was similar in WR and 50% NR rats, we did not quantify body composition (i.e., % body fat) or plasma leptin levels. Thus, organ specific and phenotype specific responses to nutrient restriction may have varying thresholds dependent upon the organ.

These results imply marked differences in the mechanisms of programmed hypertension among PF and WR offspring. Although not specifically attributed to gestational programming, human studies are consistent with differing etiologies of hypertension.^{19–21} Whereas, African Americans exhibit an increased incidence of sodium dependent hypertension and associated renal impairment,²² other populations appear to have hypertension secondary to alternative mechanisms. Thus, the potential therapeutic interventions and responses for humans in regards to antihypertensive medications differ according to their phenotype. Clinical studies evaluating those who suffer dehydration secondary to diarrhea during infancy demonstrate that these individuals are predisposed to adult hypertension.^{23;24} The evolutionary benefit of this phenomenon has been proposed to be a salt retention mechanism which would protect against sudden fluid loss. Stress and its associated steroid response can potentially affect plasma concentrating ability by altering concentration gradients in Henle's loop via permanent changes in kidney medullary structure and function.²⁵ Studies in the ovine model similarly demonstrate the effects of gestational hypernatremia on offspring plasma sodium. Three week old lambs exhibit a reset, higher plasma osmolality/sodium set point for vasopressin secretion, and presumably thirst.^{26;27} Programmed ovine fetuses also demonstrate an enhanced vasopressin secretory response (slope) with hypernatremia, despite the elevated plasma sodium threshold, again pointing to evidence of a potential survival advantage of water restriction in response to offspring exposure to drought.

In summary, the results of the present study confirm the impact of gestational dehydration on programmed offspring hypernatremic hypertension. These offspring responses, specifically increased glomerular number, may have developed to facilitate offspring survival (to reproductive age) under expected drought conditions. However, following ages of reproduction, these phenotypic changes (e.g., hypertension) may predispose to significant adult morbidities. An understanding of the gestational origins of hypertension, and specifically sodium-dependent hypertension, may provide for directed therapeutics, and perhaps early life preventative strategies.

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Figure 1.

Male (top panel) and female (bottom panel) rat weight of Control (●), water restricted (○), and pair fed (▼) offspring from birth to 20 wks.

Table 1

Offspring Organ Weights

Organ	Age	Absolute Organ Weights			Percent Organ/Body Weights (%)		
		CTL	WR	PF	CTL	WR	PF
Brain (g)	1d	0.26±0.00	0.24±0.00	0.25±0.00	3.70±0.04	4.18±0.06**	3.92±0.04**
	21d	1.41±0.02	1.40±0.01	1.45±0.02	2.86±0.06	2.93±0.08	2.87±0.05
Heart (g)	1d	0.04±0.00	0.03±0.00	0.04±0.00	0.64±0.02	0.60±0.02	0.61±0.01
	21d	0.30±0.01	0.36±0.01*	0.31±0.01	0.59±0.01	0.73±0.02**	0.61±0.02
Liver (g)	1d	0.30±0.01	0.24±0.01	0.29±0.01	4.22±0.06	4.11±0.09	4.64±0.13**
	21d	1.92±0.09	1.83±0.08	1.92±0.04	3.84±0.07	3.81±0.07	3.79±0.05
Pancreas (g)	1d	0.02±0.00	0.02±0.00	0.02±0.00	0.22±0.01	0.27±0.01*	0.27±0.02
	21d	0.16±0.02	0.16±0.01	0.16±0.01	0.32±0.03	0.34±0.02	0.32±0.01
Stomach (g)	1d	0.06±0.00	0.04±0.00	0.04±0.00	0.82±0.04	0.66±0.03*	0.71±0.04
	21d	0.39±0.02	0.40±0.01	0.40±0.01	0.78±0.02	0.83±0.02	0.80±0.02
Adrenals (mg)	1d	---	---	---	---	---	---
	21d	0.18±0.02	0.23±0.02*	0.20±0.01	0.04±0.00	0.05±0.00*	0.04±0.00

Absolute organ weights represent wet organ weights. Percent organ/body weights represent (organ weight/body weight)×100. Organ weights of Control (CTL), water restricted (WR), and pair fed (PF) offspring at 1 and 21 days of age.

* P<0.05 vs Control

** P<0.01 vs Control

--- too small for measurement