Renal Magnesium Handling Is Not Subject to Developmental Programming

Saleh H. Alwasel^{a, b} Vandana Sahajpal^c Nick Ashton^c

^aFetal Programming of Diseases Research Chair, ^bDepartment of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia; ^cFaculty of Life Sciences, University of Manchester, Manchester, UK

Key Words

Magnesium • Fetal programming • Kidney • Calcium • Hypertension

Abstract

Developmental programming of hypertension, induced by maternal protein restriction, is associated with enhanced urinary excretion of sodium and calcium in the rat. Although calcium and magnesium are reabsorbed via different pathways, renal calcium excretion often parallels magnesium output. Accordingly, the aim of the current study was to assess magnesium handling in rats exposed to a low-protein (LP) diet in utero. Wistar rats were fed a control (18%) or LP (9%) diet throughout pregnancy; offspring were weaned onto standard rat chow and studied at 4 weeks of age. Renal clearance measurements were made in both volume expanded and euvoalaemic anaesthetised rats; 24-hour magnesium turnover was also assessed in conscious animals. Plasma and total body magnesium content were measured. Total ($U_{Mg}V$) and fractional excretion (FE_{Mg}) of magnesium did not differ between control and LP rats under any of the experimental conditions. Neither plasma nor total body magnesium content differed between control and LP rats. Thus the hypercalciuria of LP rats is not mirrored by an increase in renal magnesium excretion. These data suggest that renal magnesium handling is not affected by developmental programming.

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Introduction

Developmental programming describes the phenomenon in which exposure to an adverse environment in utero has a life-long impact on the physiology of the offspring. Numerous studies in humans have shown that a poor maternal diet during pregnancy leading to a reduction in birth weight is associated with an increased risk of the child developing cardiovascular disease and diabetes mellitus in later life [1]. Similar effects can be induced in animal models by altering maternal food intake, facilitating the study of the underlying physiological mechanisms. One of the most common experimental approaches is to reduce dietary protein intake in the pregnant rat, which leads to a dose-related increase in offspring blood pressure [2].

A number of organs are affected by altered growth patterns in utero with the kidney being particularly susceptible to manipulation of the intrauterine environment. We [3] and others [4, 5] have shown that feeding pregnant rats a low-protein (LP) diet results in a 30–40% reduction in the number of nephrons developed by the offspring. This reduction in renal capacity is associated with changes in the renin-angiotensin system [3, 6, 7], and the renal handling of sodium [8] and calcium [9]. The latter is associated with a reduction in bone mineral content [10], bone mass, lower total serum calcium and inappropriately high concentrations of calcitropic hormones

[9]. Such a pattern of impaired skeletal development in early life when peak bone mass is achieved represents a risk factor for osteoporosis in old age [11].

The regulation of calcium homeostasis is closely linked with that of magnesium. Hypocalcaemia is a common feature of hypomagnesaemia: even modest reductions in plasma magnesium can lead to marked reductions in serum calcium concentrations [12]. Magnesium deficiency is also linked to osteoporosis [13]; the mechanism is unknown but may involve a reduction in bone extracellular fluid pH leading to demineralisation [14]. Altered magnesium homeostasis may therefore contribute to the perturbation in calcium regulation seen in our earlier study in LP exposed rats [9].

The effect of maternal protein restriction on magnesium homeostasis is unknown. Accordingly, the aim of the current study was to determine renal magnesium handling in rats exposed to a LP diet in utero. As extracellular fluid volume expansion is known to affect magnesium reabsorption by the kidney [15], we have utilised two different approaches to assess renal function in vivo. In the first set of experiments, we used a standard constant infusion approach which leads to the expansion of extracellular fluid; in the second we used a servo-controlled fluid replacement system which allows the precise control of extracellular fluid volume. In order to overcome the potential confounding effect of anaesthesia, we also measured 24-hour magnesium turnover in conscious rats. Finally, we measured total body magnesium content to establish whether any changes in the small exchangeable pool of magnesium in the plasma are reflected in the larger bone stores.

Methods

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and received local ethical approval.

Animals

Wistar rats (Harlan UK Ltd., Belton, UK) were paired in individual breeding cages and held in a room at 22–24°C with a 12 h light:12 h dark cycle. As soon as mating was confirmed by the presence of a sperm plug, the female's diet was switched from standard chow (Rat & Mouse Standard Diet, Bantin & Kingman Ltd., Hull, UK) to one which contained either 9% protein (LP) or 18% protein (control). The semi-synthetic diets were formulated as described previously [3]. On the day of birth, the dam's diet reverted to standard chow and the litter size was reduced to 8 animals. Male offspring were studied at 4 weeks of age.

Renal Function – Conscious Animals

Four-week-old male rats (control $n = 14$, LP $n = 11$) were housed individually in metabolic cages provided with stainless steel mesh bottoms and a urine collection pan underneath attached to a glass collection flask. Rats were kept in a thermoregulated room at 22–24°C and approximately 18% relative humidity, with a controlled 12 h light:dark period. Water and food (0.096 mmol Na⁺, 0.179 mmol K⁺, 0.185 mmol Ca²⁺, 0.074 mmol Mg²⁺ per gram) were provided ad libitum. One day was allowed for adaptation to the cage conditions followed by 5 consecutive days of urine collection. Cages and urine collection flasks were cleaned thoroughly with deionised water after the collection of urine. Once urine was collected, samples were centrifuged, weighed and stored at 4°C. At the end of the experiment, animals were sacrificed humanely by stunning and decapitation to allow collection of trunk blood which was spun at 2,500 g for 10 min. Plasma was removed and stored at –20°C prior to analysis for Mg²⁺ concentration (see below).

Renal Function – Anaesthetised Animals

Inactin (thiobutabarbital sodium, 100 mg/kg i.p.) anaesthetised rats were prepared for renal clearance measurements. Briefly, a jugular catheter was implanted for the continuous infusion of 0.154 M NaCl. A carotid catheter was implanted to allow continuous recording of arterial blood pressure, using a data acquisition system (PowerLab 800/s, ADInstruments, Hastings, UK), and withdrawal of blood samples. A catheter was also implanted in the urinary bladder to facilitate collection of urine. A tracheotomy was performed to assist breathing and a rectal probe was inserted to monitor body temperature, which was maintained at 37°C by means of a heated surgical table.

Animals were then divided at random into two groups. The first group of rats (control $n = 12$, LP $n = 12$) received a continuous i.v. infusion of 0.154 M NaCl at 50 $\mu\text{l min}^{-1}$ 100 g body weight⁻¹ (volume expanded rats). The second group of animals (control $n = 15$, LP $n = 15$) received an i.v. infusion of 0.154 M NaCl at a rate matched to spontaneous urine output (euvolaemic rats). This was achieved by means of a servo-controlled fluid replacement system which monitors urine flow rate over a defined period (15 min) and then adjusts a computer-controlled infusion pump to match the rate of infusion to urine output over the subsequent 15-min period [16]. This prevents expansion of extracellular fluid volume and allows renal clearance measurements to be made in a euvolaemic state.

Following surgery, both groups of animals received a bolus of ³H-inulin (4 μCi , Amersham Biosciences UK Ltd., Little Chalfont, UK) via the jugular vein cannula. ³H-inulin was infused, as a marker of glomerular filtration rate, at 0.3 $\mu\text{Ci/h}$ in all animals. For the first group of volume expanded rats, the ³H-inulin was simply added to the 0.154 M NaCl infusion; for the second group of fluid-balanced rats, ³H-inulin was infused via a second, slow (1 ml/h) infusion pump at a rate less than the spontaneous urine flow rate. All animals were allowed a 2-hour equilibration period after which urine samples were collected every 15 min for a further hour. A blood sample (300 μl) was taken at 30 min, mid-way through the urine collection period; a similar volume of 0.154 M NaCl was replaced via the jugular catheter. Animals were sacrificed humanely at the end of the experiment. Urine and plasma samples were analysed for Mg²⁺ by atomic absorption spectroscopy (Solaar S Series, Thermo Elemental (Unicam Ltd.), Cam-

Fig. 1. Total body magnesium (a), urinary magnesium excretion rate (U_{MgV}) (b) and magnesium balance (c) in conscious 4-week-old control (C; $n = 14$, open bars) and LP ($n = 11$, solid bars) male rats held in metabolism cages for 5 days. Data are shown as the mean $\pm$ SEM; statistical analysis was by the two-tailed t test.

bridge, UK); 3H -inulin was determined using a 1900CA Tri-Carb Liquid Scintillation Analyser (Canberra Industries, Meriden, Conn., USA) β -counter.

Total Body Magnesium Content

Control ($n = 6$) and LP ($n = 6$) rats were sacrificed by an overdose of Inactin (thiobutabarbital sodium), weighed and dried to a constant weight at $80^\circ C$ in an oven (Griffin Oven, Griffin & George Ltd., Wembley, UK) over 10 days. Dried carcasses were then transferred to aluminium oxide crucibles and ashed in a muffle oven at $675^\circ C$ for 24 h. The resulting ash was dissolved in concentrated nitric acid before further dilution in deionised water and the measurement of Mg^{2+} as described above.

Statistical Analysis

Data are shown as the mean $\pm$ SEM. Statistical comparisons were by two-tailed t test or one-way ANOVA and Tukey's test, as appropriate (SPSS version 16.0, SPSS UK Ltd., Surrey, UK). Significance was assumed when $p < 0.05$.

Results

Body weight did not differ between control (111.2 ± 3.0 g, $n = 41$) and LP (116.4 ± 4.1 g, $n = 38$, $p = 0.31$) rats aged 4 weeks. However, mean arterial blood pressure, measured in the anaesthetised animals, was significantly higher in LP rats (control $n = 27$, 86 ± 4 vs. LP $n = 27$, 104 ± 3 mmHg, $p < 0.001$).

Plasma magnesium concentrations did not differ between control and LP rats (table 1). Extracellular fluid volume status had no effect on the plasma magnesium concentration of control animals ($F_{2,40} = 1.2$, $p = 0.3$); however, volume status did affect magnesium concentration in LP rats ($F_{2,37} = 4.4$, $p = 0.02$). The plasma magnesium concentration of volume-expanded LP rats was significantly lower than that of conscious LP rats ($p < 0.05$).

Table 1. Plasma magnesium concentrations (mmol/l) in 4-week-old rats exposed to a maternal low protein diet in utero

	Plasma magnesium	
	control	low protein
Conscious rats	0.79 ± 0.04 (14)	0.83 ± 0.04 (11)
Anaesthetised volume-expanded rats	0.69 ± 0.03 (12)	$0.66 \pm 0.04^*$ (12)
Anaesthetised euvoalaemic rats	0.75 ± 0.07 (15)	0.79 ± 0.05 (15)

Data are shown as the mean $\pm$ SEM; the number of animals in each group is shown in parentheses. Statistical analysis was by one-way ANOVA and Tukey's post-hoc test.

* $p < 0.05$ LP conscious vs. LP volume expanded.

Total body magnesium content did not differ between control and LP rats (fig. 1a, $p = 0.6$). Urinary magnesium excretion (fig. 1b, $p = 0.85$) and magnesium balance (dietary intake – urinary excretion, fig. 1c, $p = 0.28$) did not differ between conscious control and LP rats held in metabolism cages for 5 days.

During the acute renal clearance experiments, differences were seen in the magnesium excretion rates according to the extracellular volume status of the animals; however, LP rats did not differ from control animals under either condition (fig. 2a, c). Urinary magnesium excretion was 4-fold higher in volume expanded rats compared with euvoalaemic animals ($F_{3,53} = 7.2$, $p < 0.001$). Urine flow rates were also greater in volume expanded rats (control $n = 12$, 63.6 ± 8.9 ; LP $n = 12$, 69.5 ± 9.9 $\mu l \min^{-1} 100$ g body weight $^{-1}$) compared with euvoalaemic animals (control $n = 15$, 17.1 ± 2.6 ; LP $n = 15$, 15.9 ± 1.9 $\mu l \min^{-1} 100$ g body weight $^{-1}$, $F_{3,53} = 21.9$, $p < 0.0001$).

Fig. 2 Urinary magnesium excretion rate (U_{MgV}) and fractional excretion of magnesium (FE_{Mg}) in anaesthetised volume-expanded (**a, b**) control ($n = 12$, open bars) and LP ($n = 12$, solid bars) rats, and in euvoaemic (**c, d**) control (C; $n = 15$, open bars) and LP ($n = 15$, solid bars) rats. Data are shown as the mean $\pm$ SEM; statistical analysis was by one-way ANOVA and Tukey's test.

Fractional excretion of magnesium (FE_{Mg}), which represents the proportion of filtered magnesium that is excreted, did not differ between control and LP rats irrespective of extracellular fluid volume status (fig. 2b, d). However, FE_{Mg} was greater in control and LP rats under volume expanded conditions compared with euvoaemic animals ($F_{3,53} = 3.7$, $p = 0.017$).

Discussion

This study shows that magnesium homeostasis is not affected by exposure to a LP diet in utero. Neither plasma nor total body magnesium content differed between control and LP rats. Similarly, the renal handling of magnesium did not differ between control and LP rats both under euvoaemic and volume expanded conditions. These data suggest that magnesium homeostasis is not influenced by developmental programming through maternal dietary manipulation. This is in marked contrast to calcium homeostasis which shows a number of important changes in the LP rat. These include a reduction in total plasma calcium concentration, increased urinary calcium excretion and a reduction in bone mass [9].

The majority of magnesium within the body is found in bone (60%), with less than 2% in the extracellular fluid and only 0.3% found in plasma [17]. Despite the small quantity of magnesium in the plasma, magnesium homeostasis is regulated primarily by the kidneys. Approx-

imately 70% of magnesium in the plasma is ultrafiltrable; 70–80% being free Mg^{2+} and the remainder complexed with filtrable anions such as oxalate, phosphate and citrate [18]. Only a small proportion of the filtered magnesium is reabsorbed by the proximal tubule (15%), via a paracellular pathway, in contrast with calcium and other ions which are reabsorbed in greater quantities (60%) in this part of the nephron.

Up to 70% of the filtered magnesium is reabsorbed in the loop of Henle. There is some evidence of reabsorption by the descending limb, but the majority of magnesium is reabsorbed in the thick ascending limb (TAL). In the rat, this is limited entirely to the cortical TAL (cTAL) with no reabsorption of magnesium, or calcium, in the medullary TAL (mTAL) [19]. Magnesium reabsorption across the cTAL is passive, via a paracellular route mediated by claudin-16 and claudin-19 [20, 21]. It is driven by a positive lumen voltage which is maintained by active sodium reabsorption. Apical sodium uptake is mediated by the $Na^+K^+2Cl^-$ co-transporter (NKCC2 or BSC1) and basolateral efflux by the $Na^+K^+ATPase$. Claudin-16 has been shown to mediate leakage of sodium back into the lumen, generating a lumen positive potential difference which drives magnesium (and calcium) reabsorption [22]. In this context it is interesting to note that expression of NKCC2 is increased in the LP rat [8, 23], which has led to the suggestion that sodium reabsorption is greater in LP animals [23]. However, we have shown that sodium excretion is increased, not decreased [8], which calls into

question the functional activity of the excess NKCC2 expressed by LP rats. The current data lend further support to the notion that greater NKCC2 expression does not correlate with increased transport activity as magnesium excretion did not differ between LP and control animals.

The distal convoluted tubule (DCT) accounts for only 5% of magnesium reabsorption [24]; however, this segment plays an important role in determining urinary magnesium concentration as it is the last site of magnesium reabsorption by the nephron. Magnesium reabsorption by the DCT is an active transcellular process, separate from that which mediates calcium reabsorption [25]. The transient receptor potential melastatin subtype 6 (TRPM6), the only channel identified to date that mediates transepithelial magnesium transport [26, 27], is localised to the apical membrane of the early DCT [28]. Transepithelial calcium reabsorption via TRPV5, the renal epithelial calcium channel, occurs downstream in the late DCT and connecting tubule [29]. Regulation of TRPM6 is not fully understood, however 17β -oestradiol [30] and epidermal growth factor [30] have been shown to act as magnesiotropic hormones which stimulate TRPM6. Raised intracellular magnesium can reduce TRPM6 activity [28] as can the receptor for activated C-kinase (RACK1) which is expressed predominantly in the kidney in the DCT [31]. RACK1 has been shown to play a role in magnesium-dependent suppression of TRPM6 activity, thereby preventing magnesium overload during reabsorption [32].

The DCT sodium chloride co-transporter NCC has been shown to influence TRPM6 expression. Gitelman's syndrome, an autosomal-recessive condition arising through mutations in NCC, is associated with sodium wasting and hypomagnesaemia [33]. NCC-knockout mice display hypomagnesaemia and renal magnesium wasting which are associated with down regulation of TRPM6 expression [34]. NCC expression has been reported to be increased in the LP rat [23], although we did not see any change at the mRNA level [8]. Whether this affects magnesium reabsorption by the DCT in the LP rat remains to be determined, but it seems unlikely given that there is no further change in tubular fluid magnesium content downstream of the DCT and that the final urinary excretion rate of magnesium does not differ between LP and control animals.

In conclusion, the current study shows that, unlike calcium [9] and sodium [8], renal handling of magnesium is not affected by exposure to a LP diet in utero. Despite increased expression of NKCC2 in the LP rat TAL [8, 23], activity of the co-transporter does not seem to be increased; as a result there does not appear to be an increase in the driving force for magnesium reabsorption in this part of the nephron. Fine tuning of the final urinary magnesium content by the distal tubule also appears to be unaffected, suggesting that developmental programming does not alter expression or activity of TRPM6 or its regulators in the final part of the nephron.

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