Short- and long-term effects of a maternal low-protein diet on ventilation, O₂/CO₂ chemoreception and arterial blood pressure in male rat offspring

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Abstract

Maternal undernutrition increases the risk of adult arterial hypertension. The present study investigated the short- and long-term effects of a maternal low-protein diet on respiratory rhythm, O₂/CO₂ chemosensitivity and arterial blood pressure (ABP) of the offspring. Male Wistar rats were divided into two groups according to their mothers' diets during gestation and lactation: control (NP, 17% of casein) and low-protein (LP, 8% of casein) groups. Direct measurements of ABP, respiratory frequency (RF), tidal volume (V_T) and ventilation (VE), as well as hypercapnia (7% CO_2) and hypoxia (7% O_2) evoked respiratory responses were recorded from the awake male offspring at the 30th and 90th days of life. Blood samples were collected for the analyses of protein, creatinine and urea concentrations. The LP offspring had impaired body weight and length throughout the experiment. At 30 d of age, the LP rats showed a reduction in the concentrations of total serum protein (approximately 24%). ABP in the LP rats was similar to that in the NP rats at 30 d of age, but it was 20% higher at 90 d of age. With respect to ventilatory parameters, the LP rats showed enhanced RF (approximately 34%) and VE (approximately 34%) at 30 d of age, which was associated with increased ventilatory responses to hypercapnia (approximately 21% in VE) and hypoxia (approximately 82% in VE). At 90 d of age, the VE values and CO_2/O_2 chemosensitivity of the LP rats were restored to the control range, but the RF values remained elevated. The present data show that a perinatal LP diet alters respiratory rhythm and O₂/CO₂ chemosensitivity at early ages, which may be a predisposing factor for increased ABP at adulthood.

Key words: Protein undernutrition: Developmental plasticity: Ventilation: Hypertension

Epidemiological and experimental studies have shown that malnutrition during critical periods of embryonic development or in infant diet after birth is an important predisposing factor for the development of obesity, diabetes, CVD and hypertension in adulthood[1–3]. This phenomenon is referred to as 'developmental programming' or 'developmental origins of health and disease'[4]. In humans, the risk of developing late-onset diseases is increased in infants born prematurely (at <37 weeks of gestation), born from undernourished[2] or overnourished mothers[5] or born with low body weight (weighing <2500 g at birth)[2]. In the 1990s, Barker *et al.*[6] demonstrated that systolic blood pressure in older children is inversely related to their birth weight. In the 2000s, some epidemiological studies have confirmed that infants with low birth weight or intra-uterine growth restriction presented higher systolic and diastolic blood pressure than a control group as adults[7–9]. In a 2012 meta-analysis, low birth weight was associated with an increased OR (1·2) for the development of hypertension[10]. However, the mechanism by which low birth weight is linked to later hypertension is not yet well understood.

In experimental models, maternal low-protein diet during gestation and lactation is one of the most studied animal models of developmental origin of metabolic diseases[3,11,12]. For example, adult rats submitted to a perinatal protein undernutrition (5% protein) showed higher blood pressure, later glomerulogenesis and lower nephron numbers than their normoproteic controls[13]. The later consequence of kidney

injuries was associated with albuminuria and proteinuria, and these effects seem to be amplified with age[14]. Maternal exposure to a low-protein diet (8% of casein) during pregnancy and lactation induces growth restriction, age-dependent loss of glucose tolerance, insulin resistance and changes in lung structure and ventilatory function in adult offspring[3,15,16]. Thus, it can be suggested that there is some association between maternal undernutrition-induced short-term effects in respiratory function and the age of appearance of hypertension in adulthood. Recently, studies[17−19] have proposed that changes in the generation or modulation of respiratory function can contribute to the development of arterial hypertension. Our previous studies[17,20,21] have suggested that respiratory neurons located in the brainstem could modulate the cardiovascular system and that increased respiratory activity would lead to arterial hypertension via central pathways. Currently, it is believed that ventilatory responses elicited by the activation of these pathways are due to the intrinsic sensitivity to O_2/CO_2 and inputs from specific regions of the central nervous system and carotid bodies, which lead to increased respiratory drive[22−24]. Aside from the respiratory-related chemoreceptor responses, acute stimulation of central or peripheral respiratory chemoreceptors by hypercapnia or hypoxia produces increases in sympathetic activity and arterial pressure (AP)[25,26].

In the present study, we investigate the short- and long-term effects of a maternal low-protein diet on basal pulmonary ventilation (VE) and ventilatory control elicited by respiratory chemoreflex activation. Because disorders in the mechanisms of control of breathing, such as in the respiratory chemoreflex, are potential risk factors for the development of hypertension[27,28], our hypothesis was that a higher respiratory frequency (RF) and chemosensitivity to O_2/CO_2 at 30 d of age could be a potential hidden basis for an increased arterial blood pressure at 90 d of age.

Methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Centre (process no. 23 076·044454/2010-94), Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals[29].

Animals

A total of seventeen virgin female albino Wistar rats (Rattus norvegicus) were obtained from the Academic Centre of Vitoria de Santo Antao (CAV), Federal University of Pernambuco, Brazil. The animals were maintained at a room temperature of 22 ± 1°C with a controlled light–dark cycle (dark 18.00–06.00 hours). A standard laboratory chow diet (52% carbohydrate, 21% protein and 4% lipids – Labina; Purina Agriband) and water were given ad libitum. At 90 d of age, rats were mated (two females for one male). The day on which spermatozoa were present in a vaginal smear was designated as the day of conception (day 1 of pregnancy). Pregnant rats were then transferred to individual cages.

The following two groups were formed based on dietary manipulations: mothers fed a 17% casein diet (normal-protein group, NP, n 8) and mothers fed an 8% casein isoenergetic diet (low-protein group, LP, n 9) ad libitum. Offspring were standardised as litters of eight pups 48 h after birth. Male offspring were used in each litter and females were used only to standardise the size of each litter to eight pups. During the suckling period, the mothers continued to be provided with the experimental diet, either 8% of casein or 17% of casein. Diets were made at the Laboratory of Experimental Nutrition-CAV, Federal University of Pernambuco according to the American Institute of Nutrition −(AIN-93)[30]. Casein had previously been analysed and showed 85% purity (85 g protein for each 100 g casein). The diets were isoenergetic, and only the amount of protein was changed (Table 1). At weaning, three or four male offspring from each litter were randomly housed in collective cages and received a standard diet (Labina; Purina Agriband) ad libitum. At least two male offspring from each litter were used to evaluate either the short-term effects (at 30 d of age: NP, n 7 and LP, n 8) or the long-term effects (at 90 d of age: NP, n 7 and LP, n 8).

The body weight of pups was recorded at birth and on 30 and 90 d of age with an appropriate balance (model AS-1000; Marte), having an error range of 0·01 g. The body length of pups was recorded by the measurement of external surfaces (nose-to-anus length) with calipers (0·01 mm accuracy). The body weight and body length of pups were used to determine the anthropometrical parameter of the Lee index (cube root of body weight (g)/nose-to-anus length (cm)) and BMI (body weight (g)/square of nose-to-anus length (cm)).

Measurements of serum albumin, total protein, urea and creatinine

At 27 or 87 d of age, offspring from both groups were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg), and blood samples (approximately 1 ml) were collected by plexus retro-orbital disruption. Serum samples were collected for the quantification of albumin, total protein, urea and creatininewith commercial kits (Labtest Diagnostica). Globulin values were

Table 1. Nutritional composition of the diets (g/100 g diet)

Nutrients	Normal protein (17% of protein)	Low protein (8% of protein)
Casein (85%)*	20	9·41
Dextrin maize starch	13	13·2
Cellulose	5	5
Sucrose	10	10
Maize starch	39·74	50·34
Soyabean oil	7	7
Choline	0·25	0·25
Met	0·3	0·3
Vitamin mix	1	1
Mineral mix	3·5	3·5
Energy density (kJ/g)	16·26	16·26

* Casein showed 85% purity (85 g protein for each 100 g casein).

Table 2. Somatic parameters (body weight, body length, BMI and Lee index) and serum biochemical parameters (albumin, globulin, total protein, urea and creatinine) of rats from dams submitted to a normoproteic (NP, 17 % of protein, *n* 14) or low-protein diet (LP, 8 % of protein, *n* 16) during pregnancy and lactation

(Mean values with their standard errors)

	Birth				30 d				90 d			
	NP		LP		NP		LP		NP		LP	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Somatic parameters												
Body weight (g)	6·1	0·1	5·2*	0·1	83·1	3·2	65·7*	2·7	306·2	5·9	273·8*	7·9
Body length (cm)	5·0	0·1	4·6*	0·1	13·2	0·2	11·9*	0·1	22·7	0·2	22·1*	0·2
BMI (body weight (g)/body length (cm)^2)	0·27	0·01	0·24*	0·01	0·47	0·02	0·46	0·02	0·59	0·01	0·56	0·01
Lee index (cube root of body weight (g)/body length (cm))	3·90	0·05	3·69*	0·07	3·30	0·05	3·39	0·05	2·97	0·03	2·94	0·04
Biochemical parameters												
Albumin (g/l)	–	–	–	–	0·33	0·01	0·29*	0·01	0·39	0·01	0·374	0·02
Total protein (g/l)	–	–	–	–	0·64	0·02	0·49*	0·02	0·68	0·023	0·68	0·024
Globulin (g/l)	–	–	–	–	0·31	0·02	0·197*	0·02	0·31	0·03	0·30	0·03
Urea (mmol/l)	–	–	–	–	2·71	0·06	3·20*	0·12	2·27	0·08	2·77*	0·12
Creatinine (mmol/l)	–	–	–	–	0·026	0·001	0·036*	0·002	0·019	0·001	0·026*	0·003

* Mean values were significantly different from those of the NP group at each age ($P < 0.05$; unpaired Student's *t* test).

obtained by subtracting albumin values from total protein values. The animals were fasted overnight.

Measurements of ventilation during basal, hypercapnia and hypoxia challenges

Measurements of VE were obtained at either 30 or 90 d of age using the whole-body plethysmography method as described by Malan[31]. Before recording the data, the animals were placed into the Plexiglas chamber (5 litres) for a period of acclimatisation (approximately 60 min), and the chamber was flushed with humidified air and maintained at 25°C. Afterwards, to record the measurements of VE, air flow was suspended for short periods (3 min), and pressure oscillations caused by the breathing of the animals were captured by an apparatus connected to the chamber, with a pressure differential transducer and a signal amplifier (ML141 spirometer, PowerLab; ADInstruments). Then, the signal was captured into an acquisition system for data analysis (PowerLab; ADInstruments).

After recording the baseline VE measurements, the respiratory responses to CO_2 were induced by flushing a hypercapnic gas mixture (7 % CO_2, 21 % O_2 and N_2 balance; Linde Gas) into the plethysmographic chamber. This gas mixture was
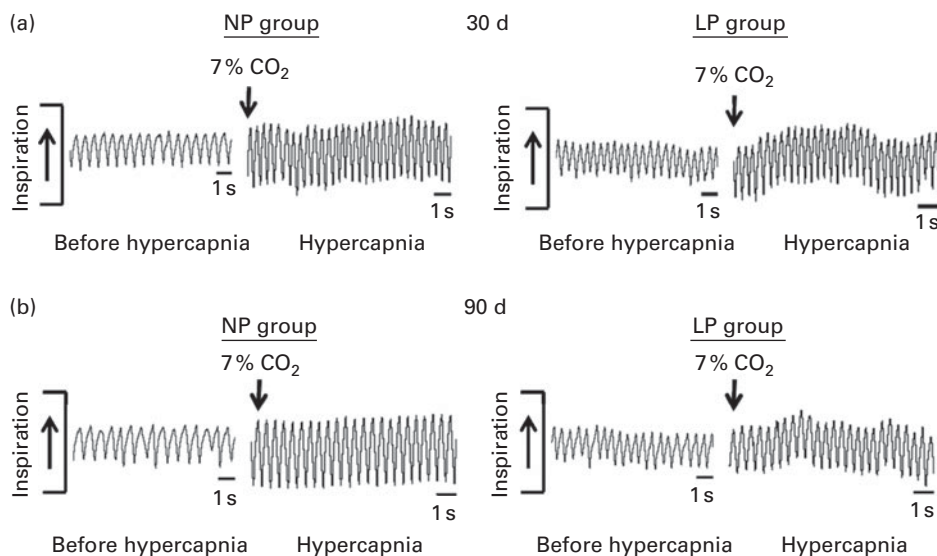
Fig. 1. Representative tracings of rats at rest and during hypercapnia at 30 and 90 d of age. Representative tracing of ventilation before (before hypercapnia) and during the last minute of hypercapnia exposure (hypercapnia) in (a) 30- and (b) 90-d-old representative rats from dams submitted to either a normoproteic diet (NP group, 17 % of protein) or a low-protein diet (LP group, 8 % of protein) during pregnancy and lactation. All pups were fed a standard chow diet at weaning.

flushed into the chamber at a flow of 3 litres/min over the course of 5 min.

The respiratory responses to O_2 were performed by flushing a hypoxic gas mixture (7 % O_2 and N_2 balance; Linde Gas) into the plethysmographic chamber. This gas mixture was flushed into the chamber at a flow of 3 litres/min over the course of 3 min, as previously standardised in our laboratory.

Analysis of respiratory parameters

RF, tidal volume (V_T) and VE were determined in the room air condition (control baseline) and during the hypercapnic or hypoxic challenge. All data were analysed off-line with Lab-Chart software (LabChart 7 Pro; ADInstruments). A period of 10 s was selected for the determination of mean RF. V_T was calculated from the pressure oscillation caused by the breathing of rats and using the formula described previously by Malan[31]. VE was obtained by multiplying RF by V_T, and it is presented at ambient barometric pressure and at body temperature and pressure saturated.

Measurements of arterial blood pressure

At 48 h after the respiratory parameters were recorded, the NP and LP offspring (at 32 or 92 d of age) were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg) for inserting a femoral arterial catheter (PE-50 connected to PE-10). The catheter was filled with heparinised saline (NaCl 0·9 %) and exteriorised through the animal's back. After surgery, the animals were injected with ketoprofen (5 mg/kg, intra-peritoneally) and allowed to recover for 18 h before the beginning of the recording experiments. After this period, the arterial blood pressure and heart rate (HR) were recorded in unanaesthetised animals by connecting the femoral catheter to the pressure transducer. The signal was amplified (ML866/P, Power Lab; ADInstruments), sampled at 2 kHz, digitised and recorded in the microcomputer with appropriate software (LabChart 7 Pro; ADInstruments).

Each animal was placed in the recording chamber for a period of acclimatisation (approximately 60 min), pulsatile AP was recorded for 30 min at rest and the values of mean AP and HR were calculated from this recording period. For the determination of mean values of AP and HR, a period of 5–10 min was selected for each animal. All data were analysed off-line in appropriate software (LabChart 7 Pro; ADInstruments). At the end of the experiments, the animals were killed by decapitation.

Statistical analysis

Each experimental group included at least two animals from each litter. Bartlett's test was performed to evaluate data homogeneity in respiratory, cardiovascular, biochemical and murinometric parameters, and statistical results supported the use of a parametric test. Thus, significant differences between and within the groups were assessed by unpaired and paired Student's t tests, respectively. The significance level was fixed at $P < 0.05$. Data are expressed as means with their standard errors. Statistical analysis was performed using GraphPad Prism 5.0® software (GraphPad Software, Inc.).

Results

Pups from mothers submitted to a LP diet had lower birth weight, body length and BMI than the normoproteic group (Table 2). The reduced body weight in the LP group was maintained at 30 and 90 d of age, but with normalised values for BMI and the Lee index. In addition, there were no differences in the food intake between the groups at 30 d (NP 9·6 (SEM 0·9) v. LP 9·7 (SEM 0·9) g/100 g per 24 h) and 90 d (NP 9·3 (SEM 0·6) v. LP 8·2 (SEM 0·7) g/100 g per 24 h). Serum concentrations of albumin, total protein and globulin
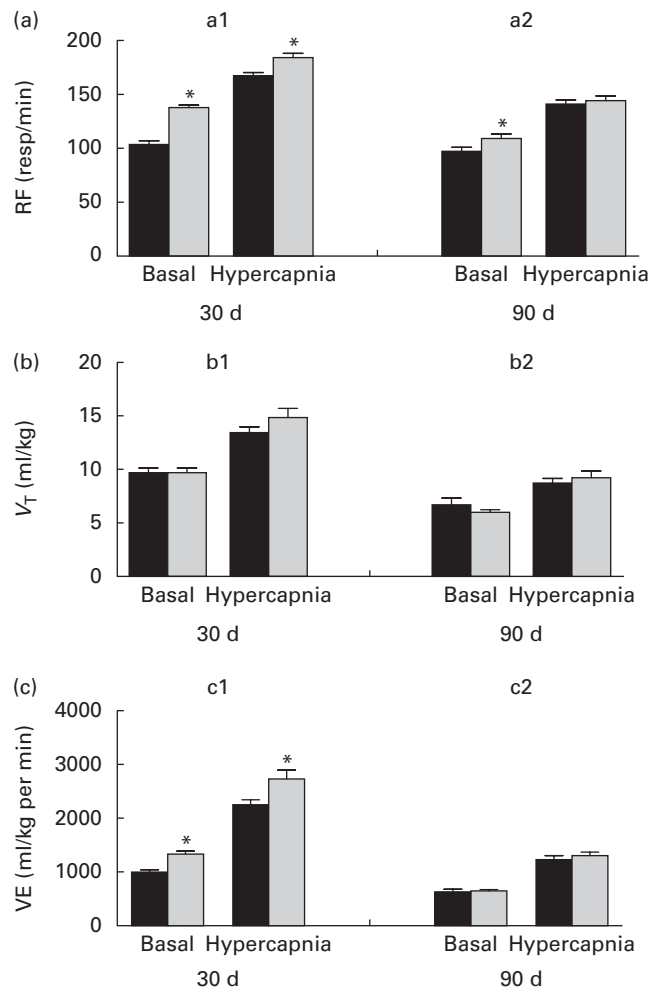
Fig. 2. Perinatal protein undernutrition increased ventilation (VE) at rest and during hypercapnia at 30 d and increased respiratory frequency (RF) at 90 d. Evaluation of (a) RF, (b) tidal volume (V_T) and (c) VE before (basal) and after hypercapnia (7 % CO_2) in 30- (a1, b1 and c1; normoproteic diet (NP), n 7 and HP, n 8) and 90-d-old rats (a2, b2 and c2; NP, n 7 and high-protein diet (HP), n 8) from dams submitted to a NP diet (17 % of protein, ■) or a LP diet (8 % of protein, □) during pregnancy and lactation. All pups were fed a standard chow diet at weaning. Values are means, with their standard errors represented by vertical bars. * Mean values were significantly different from those of the NP group in each condition (basal or hypercapnia) and at each age (30 or 90 d) ($P < 0.05$; unpaired Student's t test).
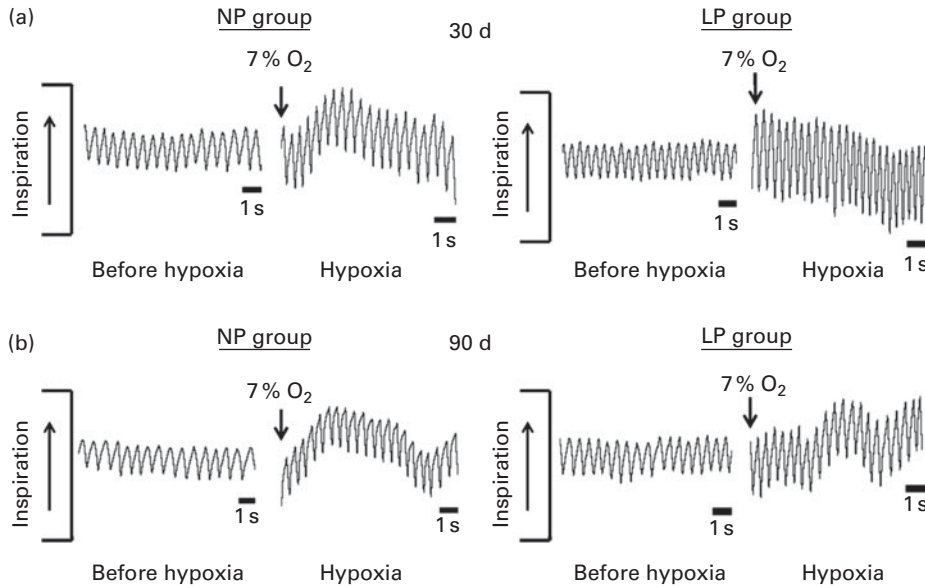
Fig. 3. Representative tracings of rats at rest and during hypoxia at 30 and 90 d of age. Representative tracing of ventilation before (before hypoxia) and during the last minute of hypoxia exposure (hypoxia) in (a) 30- and (b) 90-d-old representative rats from dams submitted to a normoproteic diet (NP group, 17 % of protein) or a low-protein diet (LP group, 8 % of protein) during pregnancy and lactation. All pups were fed a standard chow diet at weaning.

were reduced in the LP pups at 30 d of age but not at 90 d of age. Serum concentrations of urea and creatinine were increased at both 30 and 90 d of age.

Breathing recordings of one representative rat from the NP and LP groups during the basal and hypercapnic conditions at 30 d and 90 d of age are shown in Fig. 1 ((a) and (b)). At 30 d of age, in the basal condition, the LP group had increased RF (Fig. 2(a)) and VE (Fig. 2(c)) when compared with the NP group, but there were no changes in V_T at rest (Fig. 2(b)). At 90 d of age, in the basal condition, the LP group still showed elevated RF (Fig. 2(a)), but the V_T and VE values had returned to normal levels (Fig. 2(b) and (c)). As expected, the activation of respiratory chemoreceptors by hypercapnia (CO_2 7 %) produced an increase in RF, V_T and VE values in both the NP and LP groups (Fig. 2). At 30 d of age, the LP group exhibited higher values of RF (Fig. 2(a1)) and VE (Fig. 2(c1)) during hypercapnia than did the NP group. No difference was found in V_T values during hypercapnia (Fig. 2(b1)). At 90 d of age, the LP and NP groups had similar respiratory responses to hypercapnia (Fig. 2(a2), (b2) and (c2)).

Breathing recordings of representative rats from the NP and LP groups during the basal and hypoxic conditions, at 30 and 90 d of age, are shown in Fig. 3((a) and (b)). At 30 d of age under the basal condition, the LP group had elevated RF (Fig. 4(a1)) and VE (Fig. 4(c1)) when compared with the NP group, but they showed no changes in V_T values at rest (Fig. 4(b1)). At 90 d of age under the basal condition, the LP group still showed elevated RF (Fig. 4(a2)), but their V_T and VE values had returned to normal levels (Fig. 4(b2) and (c2)). As expected, the activation of respiratory chemoreceptors by hypoxia (O_2 7 %) produced an increase in RF, V_T and VE values in both the NP and LP groups (Fig. 4). However, these responses were more pronounced in the LP pups

at 30 d of age (Fig. 4(a1), (b1) and (c1)). At 90 d of age, both the groups responded at the same magnitude when stimulated by hypoxia (Fig. 4(a2), (b2) and (c2)).

The pulsatile AP, mean AP and HR recordings of representative rats from the NP and LP groups at 30 and 90 d of age are shown in Fig. 5((a) and (b)). At 30 d of age, there were no differences between the groups in terms of baseline values of systolic AP, diastolic AP, mean AP and HR (Fig. 6(A)–(D), respectively). At 90 d of age, the levels of AP in the LP animals were significantly increased, but there were no changes in the levels of HR (Fig. 6).

Discussion

We investigated the short- and long-term effects of feeding a LP diet during gestation and lactation on basal pulmonary VE and ventilatory control elicited by respiratory chemoreflex activation. The main finding of the present study is that protein restriction during perinatal development differentially alters the VE and ventilatory responses to respiratory chemoreflex activation in an age-dependent manner. Actually, although there were differences between the control and perinatal LP animals in response to hypercapnia and hypoxia at 30 d of age, no changes were found in ventilatory responses at 90 d of age. However, since disorders in the mechanisms of control of breathing are potential risk factors for the development of hypertension[27,28], the high RF and chemosensitivity to O_2/CO_2 at 30 d of age were associated with increased arterial blood pressure at 90 d of age. These observations indicate that the short-term maternal LP diet induced changes in the ventilatory frequency. It can be suggested that there is a relationship between this early change and the onset of high blood pressure in adulthood.
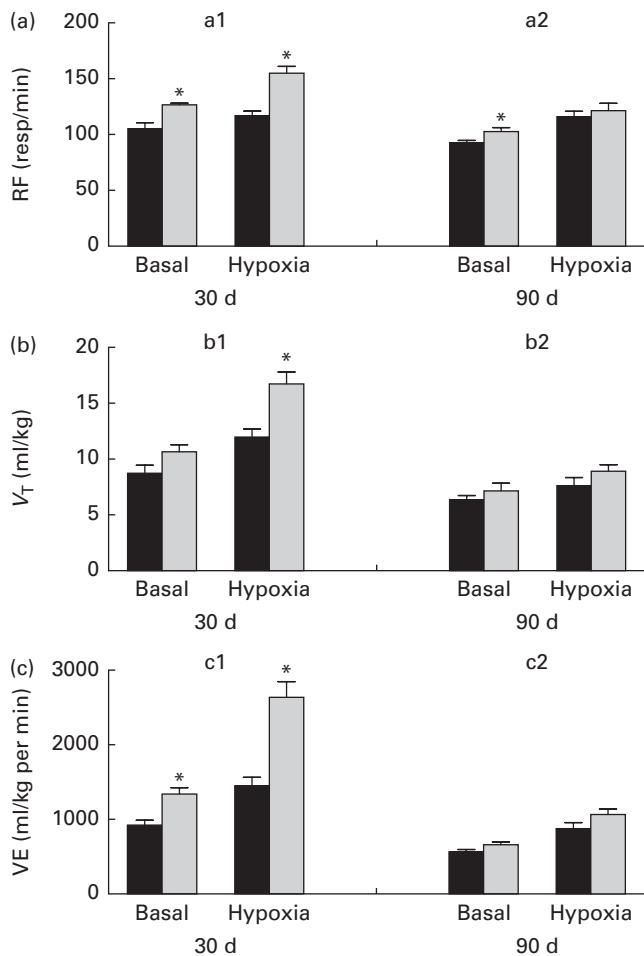
Fig. 4. Perinatal protein undernutrition increased ventilation (VE) at rest and during hypoxia at 30 d and respiratory frequency (RF) at 90 d. Evaluation of (a) RF, (b) tidal volume (V_T) and (c) VE, before (basal) and after hypoxia (7 % O_2) in 30- (a1, b1 and c1; normoproteic diet (NP), n 7 and high-protein diet (HP), n 8) and 90-d-old rats (a2, b2 and c2; NP, n 7 and HP, n 8) from dams submitted to a NP (17 % of protein, ■) or a low-protein diet (LP, 8 % of protein, □) during pregnancy and lactation. All pups were fed a standard chow diet at weaning. Values are means, with their standard errors represented by vertical bars. *Mean values were significantly different from those of the NP group in each condition (basal or hypoxia) and at each age (30 or 90 d) ($P<0.05$; unpaired Student's t test).

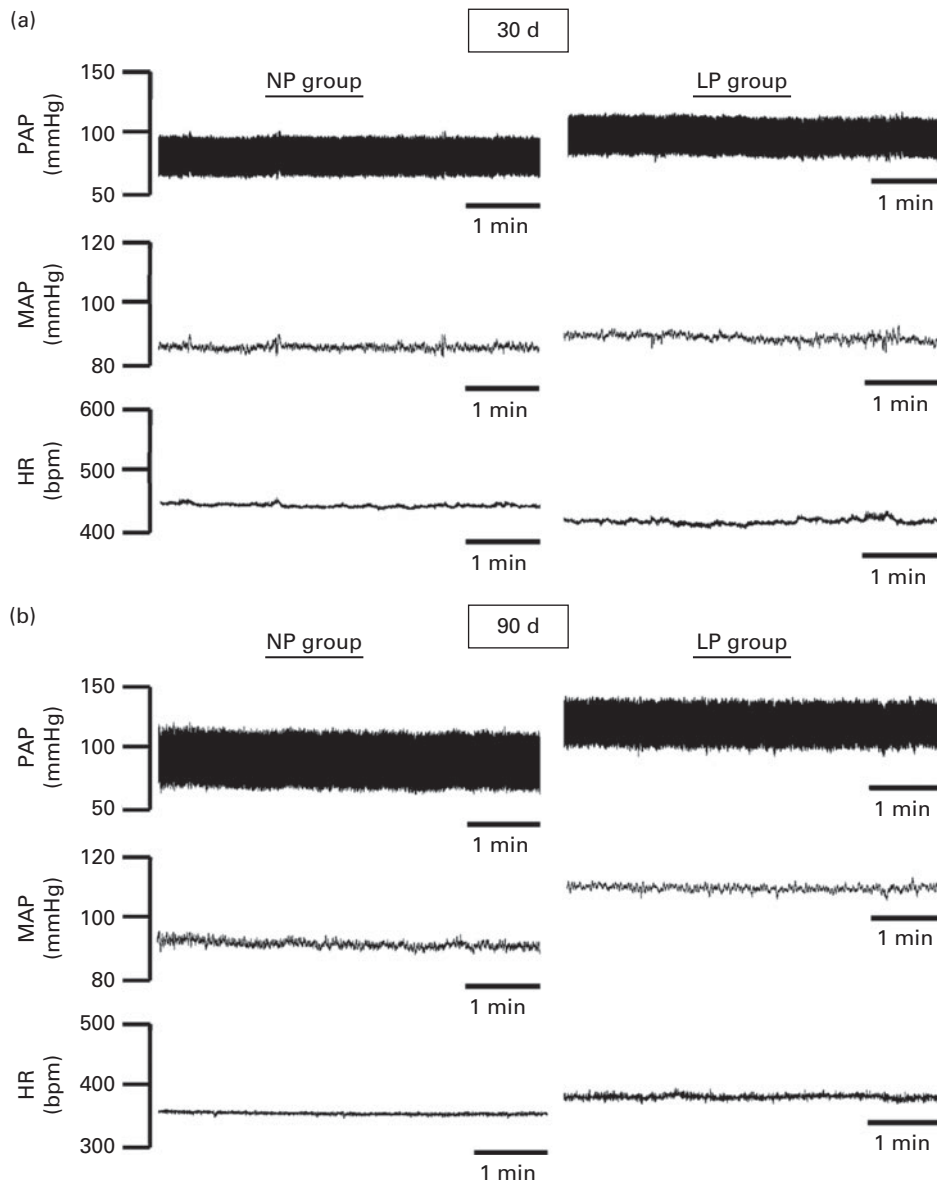that renal excretion is permanently disturbed, as observed in previous studies[38,39].

Recent studies[17−19] have indicated that changes in the generation or modulation of respiratory function can contribute to the development of arterial hypertension. It has been shown that spontaneously hypertensive rats exhibited amplified respiratory–sympathetic coupling in early life and changes in baseline respiratory rhythm before developing hypertension[18]. Our previous studies[17,20,21] have suggested that respiratory neurons located in the brainstem could modulate the cardiovascular system and that increased respiratory activity would lead to arterial hypertension via central pathways. Herein, we showed that maternal protein restriction during pregnancy and lactation leads to relevant short-term effects on the respiratory function of the offspring, such as increased baseline RF (up to 28 %) and VE (up to 40 %). These short-term effects elicited by maternal protein restriction are important findings and strongly suggest changes in respiratory–sympathetic coupling in early life[17,21], which would contribute to raising arterial blood pressure in the adult life of these animals.

Central or peripheral respiratory chemoreceptors play important roles in acid–base homeostasis and respiratory control. These cells are sensitive to changes in O_2/CO_2 levels in the body and enhance breathing in response to decreases in O_2 levels or increases in CO_2 levels[23,40,41]. In addition, acute stimulation of these respiratory chemoreceptors elicited by hypoxia or hypercapnia increases sympathetic activity and AP[22−25,40]. It is well established that the critical period for the development of the respiratory system includes the entirety of the fetal period as well as postnatal life until day 15 in rats[24,42,43]. During this period, there is intense development and maturation of chemoreceptors and the respiratory network, and any environmental perturbations in that critical period can alter the development of the respiratory system[43]. Herein, maternal protein restriction during pregnancy and lactation led to increased sensitisation of respiratory chemoreceptors in early age, with high ventilatory responses to hypoxia and hypercapnia. However, after exposure to a NP diet (after weaning), O_2/CO_2 chemosensitivity returned to normal levels in adulthood. These findings demonstrated that maternal protein undernutrition induces increased VE and O_2/CO_2 chemosensitivity in early life. In this context, a study by Abdala et al.[28] has proposed that hypertension is critically dependent on chemoreceptor input in hypertensive conditions and is modulated by the respiratory rhythm generator in juvenile rats[18,44,45]. Thus, considering that offspring from protein-restricted dams had increased chemoreceptor sensitivity, the present data strongly suggest that increased arterial blood pressure in adulthood could be dependent on the short-term effects elicited by maternal protein restriction. This thereby provides insights into the mechanisms that underlie the development of arterial hypertension in perinatal protein-restricted individuals.

The effects on respiratory rhythm seem to be unchangeable and could be related to the changes in the gene expression of chemosensory transducers, modifying the cellular phenotype in chemosensitive cells[23,45]. These effects could induce

Previous studies[32−34] have demonstrated that perinatal LP diet exposure leads to lower weight in the offspring. Furthermore, maternal low protein intake during pregnancy leads to smaller birth values of growth indices such as body length, BMI and Lee index. A low supply of protein in the maternal diet during pregnancy could induce a deficit in the maternal–fetal amino acid transport through the placenta, leading to the restriction of fetal growth and producing a low birth weight[35,36]. After birth, maternal protein restriction during lactation affects protein levels in the maternal milk[37], which could reduce the protein intake of pups and produces a reduction of serum protein levels in the offspring at 30 d of age. It is interesting to note that serum urea and creatinine levels remained high in 90-d-old LP offspring, suggesting

Fig. 5. Representative tracings of baseline arterial pressure of rats at 30 and 90 d of age. Representative tracing of baseline pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in (a) 30- and (b) 90-d-old rats from dams submitted to a normoproteic (NP, 17 % of protein) or a low-protein diet (LP, 8 % of protein) during pregnancy and lactation. All pups were fed a standard chow diet at weaning.

permanent modifications in neuronal activity and the respiratory pattern generator as well as in O_2/CO_2 chemosensitivity in the brainstem. These abnormalities in gene expression of the organs and central nervous system of protein-restricted offspring are worthy of future investigation.

Studies performed in young offspring from protein-restricted dams have shown changes in gene expression involved in aspartate and glutamate metabolism[46], which play an important role in the excitatory neurotransmission of the central nervous system[47,48]. Recently, it has been demonstrated that rats submitted to malnutrition have altered central glutamatergic pathways and that these disruptions could be involved in the development of arterial hypertension in that experimental model[49]. Furthermore, studies in hypertensive animals have shown the involvement of glutamatergic

mechanisms in the brainstem[17,50,51] and a decreased expression of genes encoding antioxidant enzymes and an increased expression of pro-oxidant enzymes, leading to elevated oxidative stress and increased AP[52]. This suggests that genes related to redox homeostasis could also be important keys to understanding the mechanisms underlying the development of hypertension. In addition, studies have suggested that maternal protein undernutrition could induce increases in the expression of hypoxia-inducible factor 1 α in the offspring and could be a risk factor for the development of arterial hypertension[53–56].

In conclusion, the present data showed that short-term effects induced by a protein-restricted diet during the perinatal period included increased respiratory rhythm and O_2/CO_2 chemosensitivity, which could contribute to the development
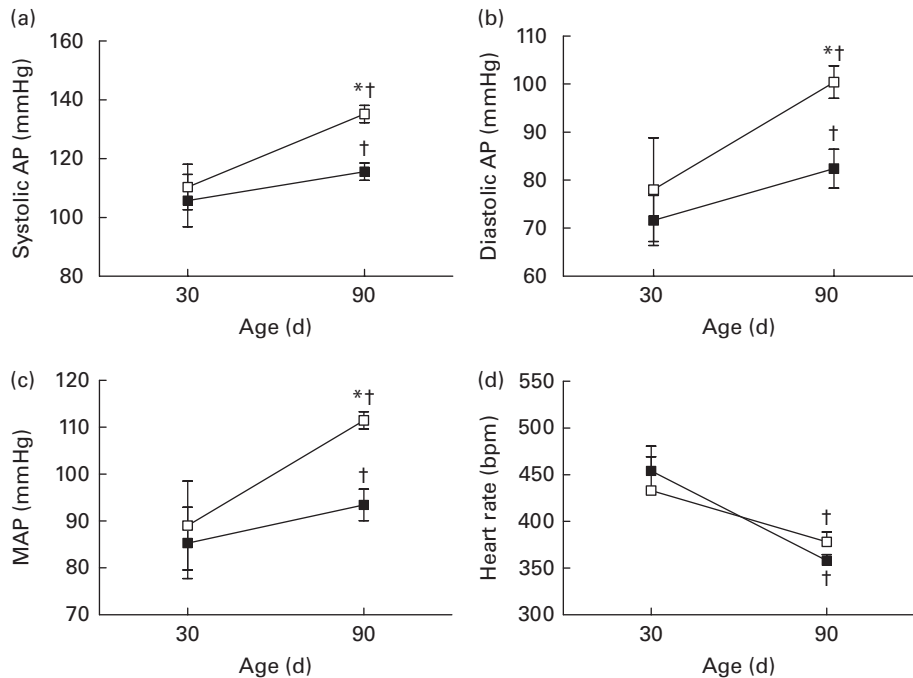
Fig. 6. Perinatal protein undernutrition increased arterial pressure at 90 d. Evaluation of (a) systolic arterial pressure (systolic AP), (b) diastolic arterial pressure (diastolic AP), (c) mean arterial pressure (MAP) and (d) heart rate in 30- and 90-d-old rats from dams submitted to a normoproteic (NP, 17 % of protein, n 13, ─■─) or a low-protein diet (LP, 8 % of protein, n 9, ─□─) during pregnancy and lactation. All pups were fed a standard chow diet at weaning. * Mean values were significantly different from those of the NP group at specific age ($P < 0.05$; unpaired Student's t test). † Mean values were significantly different from those at 30 d of age ($P < 0.05$; paired Student's t test).

of long-term effects, such as increased AP in adulthood. These findings provide insights into the effects of maternal protein restriction and suggest that increased O_2/CO_2 chemosensitivity is one possible risk factor for the development of arterial hypertension in perinatal protein-restricted animals.

Acknowledgements

References

1. Gluckman PD, Lillycrop KA, Vickers MH, *et al.* (2007) Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc Natl Acad Sci U S A* **104**, 12796–12800.
2. Barker DJ, Osmond C, Kajantie E, *et al.* (2009) Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol* **36**, 445–458.
3. Ozanne SE & Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. *Nature* **427**, 411–412.
4. Gluckman PD & Hanson MA (2004) The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab* **15**, 183–187.
5. Cnattingius S, Villamor E, Lagerros YT, *et al.* (2012) High birth weight and obesity – a vicious circle across generations. *Int J Obes (Lond)* **36**, 1320–1324.
6. Barker DJ, Hales CN, Fall CH, *et al.* (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* **36**, 62–67.
7. Fattal-Valevski A, Bernheim J, Leitner Y, *et al.* (2001) Blood pressure values in children with intrauterine growth retardation. *Isr Med Assoc J* **3**, 805–808.
8. Latini G, De Mitri B, Del Vecchio A, *et al.* (2004) Foetal growth of kidneys, liver and spleen in intrauterine growth restriction: "programming" causing "metabolic syndrome" in adult age. *Acta Paediatr* **93**, 1635–1639.
9. Shankaran S, Das A, Bauer CR, *et al.* (2006) Fetal origin of childhood disease: intrauterine growth restriction in term infants and risk for hypertension at 6 years of age. *Arch Pediatr Adolesc Med* **160**, 977–981.
10. Mu M, Wang SF, Sheng J, *et al.* (2012) Birth weight and subsequent blood pressure: a meta-analysis. *Arch Cardiovasc Dis* **105**, 99–113.
11. Burdge GC, Lillycrop KA, Jackson AA, *et al.* (2008) The nature of the growth pattern and of the metabolic response to fasting in the rat are dependent upon the dietary protein

and folic acid intakes of their pregnant dams and post-weaning fat consumption. *Br J Nutr* **99**, 540–549.

12. Fidalgo M, Falcao-Tebas F, Bento-Santos A, *et al.* (2012) Programmed changes in the adult rat offspring caused by maternal protein restriction during gestation and lactation are attenuated by maternal moderate-low physical training. *Br J Nutr* **109**, 449–456.

13. Villar-Martini VC, Carvalho JJ, Neves MF, *et al.* (2009) Hypertension and kidney alterations in rat offspring from low protein pregnancies. *J Hypertens Suppl* **27**, S47–S51.

14. Keijzer-Veen MG, Schrevel M, Finken MJ, *et al.* (2005) Micro-albuminuria and lower glomerular filtration rate at young adult age in subjects born very premature and after intra-uterine growth retardation. *J Am Soc Nephrol* **16**, 2762–2768.

15. Karadag A, Sakurai R, Wang Y, *et al.* (2009) Effect of maternal food restriction on fetal rat lung lipid differentiation program. *Pediatr Pulmonol* **44**, 635–644.

16. Leandro CG, da Silva Ribeiro W, Dos Santos JA, *et al.* (2012) Moderate physical training attenuates muscle-specific effects on fibre type composition in adult rats submitted to a perinatal maternal low-protein diet. *Eur J Nutr* **51**, 807–815.

17. Costa-Silva JH, Zoccal DB & Machado BH (2012) Chronic intermittent hypoxia alters glutamatergic control of sympathetic and respiratory activities in the commissural NTS of rats. *Am J Physiol Regul Integr Comp Physiol* **302**, R785–R793.

18. Simms AE, Paton JF, Pickering AE, *et al.* (2009) Amplified respiratory-sympathetic coupling in the spontaneously hypertensive rat: does it contribute to hypertension? *J Physiol* **587**, 597–610.

19. Simms AE, Paton JF, Allen AM, *et al.* (2010) Is augmented central respiratory-sympathetic coupling involved in the generation of hypertension? *Respir Physiol Neurobiol* **174**, 89–97.

20. Costa-Silva JH, Silva PA, Pedi N, *et al.* (2009) Chronic under-nutrition alters renal active Na^+ transport in young rats: potential hidden basis for pathophysiological alterations in adulthood? *Eur J Nutr* **48**, 437–445.

21. Costa-Silva JH, Zoccal DB & Machado BH (2010) Glutamatergic antagonism in the NTS decreases post-inspiratory drive and changes phrenic and sympathetic coupling during chemoreflex activation. *J Neurophysiol* **103**, 2095–2106.

22. Forster HV & Smith CA (2010) Contributions of central and peripheral chemoreceptors to the ventilatory response to CO_2/H^+. *J Appl Physiol* **108**, 989–994.

23. Huckstepp RT & Dale N (2011) Redefining the components of central CO_2 chemosensitivity – towards a better understanding of mechanism. *J Physiol* **589**, 5561–5579.

24. Wong-Riley MT, Liu Q & Gao XP (2013) Peripheral–central chemoreceptor interaction and the significance of a critical period in the development of respiratory control. *Respir Physiol Neurobiol* **185**, 156–159.

25. Guyenet PG, Stornetta RL, Abbott SB, *et al.* (2010) Central CO_2 chemoreception and integrated neural mechanisms of cardiovascular and respiratory control. *J Appl Physiol* **108**, 995–1002.

26. Moreira TS, Takakura AC, Colombari E, *et al.* (2006) Central chemoreceptors and sympathetic vasomotor outflow. *J Physiol* **577**, 369–386.

27. Passino C, Giannoni A, Milli M, *et al.* (2010) [Recent knowledges on chemosensitivity to hypoxia and hypercapnia in cardiovascular disease]. *Recenti Prog Med* **101**, 308–313.

28. Abdala AP, McBryde FD, Marina N, *et al.* (2012) Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. *J Physiol* **590**, 4269–4277.

29. Bayne K (1996) Revised Guide for the Care and Use of Laboratory Animals available. American Physiological Society. *Physiologist* **39**, 199, 208–211.

30. Reeves PG, Nielsen FH, Fahey GC, *et al.* (1993) 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-76A rodent diet. *J Nutr* **123**, 1939–1951.

31. Malan A (1973) Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. *Respir Physiol* **17**, 32–44.

32. Lopes de Souza S, Orozco-Solis R, Grit I, *et al.* (2008) Perinatal protein restriction reduces the inhibitory action of serotonin on food intake. *Eur J Neurosci* **27**, 1400–1408.

33. Falcao-Tebas F, Bento-Santos A, Fidalgo MA, *et al.* (2012) Maternal low-protein diet-induced delayed reflex ontogeny is attenuated by moderate physical training during gestation in rats. *Br J Nutr* **107**, 372–377.

34. Orozco-Solis R, Lopes de Souza S, Barbosa Matos RJ, *et al.* (2009) Perinatal undernutrition-induced obesity is independent of the developmental programming of feeding. *Physiol Behav* **96**, 481–492.

35. Harding JE (2001) The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol* **30**, 15–23.

36. Metcoff J, Cole TJ & Luff R (1981) Fetal growth retardation induced by dietary imbalance of threonine and dispensable amino acids, with adequate energy and protein-equivalent intakes, in pregnant rats. *J Nutr* **111**, 1411–1424.

37. Grigor MR, Allan J, Carne A, *et al.* (1986) Milk composition of rats feeding restricted litters. *Biochem J* **233**, 917–919.

38. Nwagwu MO, Cook A & Langley-Evans SC (2000) Evidence of progressive deterioration of renal function in rats exposed to a maternal low-protein diet *in utero*. *Br J Nutr* **83**, 79–85.

39. Harrison M & Langley-Evans SC (2009) Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *Br J Nutr* **101**, 1020–1030.

40. Guyenet PG, Stornetta RL & Bayliss DA (2010) Central respiratory chemoreception. *J Comp Neurol* **518**, 3883–3906.

41. Wenker IC, Benoit JP, Chen X, *et al.* (2012) Nitric oxide activates hypoglossal motoneurons by cGMP-dependent inhibition of TASK channels and cGMP-independent activation of HCN channels. *J Neurophysiol* **107**, 1489–1499.

42. Putnam RW, Conrad SC, Gdovin MJ, *et al.* (2005) Neonatal maturation of the hypercapnic ventilatory response and central neural CO_2 chemosensitivity. *Respir Physiol Neurobiol* **149**, 165–179.

43. Davis SE, Solhied G, Castillo M, *et al.* (2006) Postnatal developmental changes in CO_2 sensitivity in rats. *J Appl Physiol* **101**, 1097–1103.

44. Zoccal DB, Simms AE, Bonagamba LG, *et al.* (2008) Increased sympathetic outflow in juvenile rats submitted to chronic intermittent hypoxia correlates with enhanced expiratory activity. *J Physiol* **586**, 3253–3265.

45. Tan ZY, Lu Y, Whiteis CA, *et al.* (2010) Chemoreceptor hypersensitivity, sympathetic excitation, and overexpression of ASIC and TASK channels before the onset of hypertension in SHR. *Circ Res* **106**, 536–545.

46. McNeil CJ, Hay SM, Rucklidge GJ, *et al.* (2009) Gene and protein expression profiles in the foetal liver of the pregnant rat fed a low protein diet. *Genes Nutr* **4**, 189–194.

47. Rubio ME & Juiz JM (1998) Chemical anatomy of excitatory endings in the dorsal cochlear nucleus of the rat: differential synaptic distribution of aspartate aminotrans-

ferase, glutamate, and vesicular zinc. *J Comp Neurol* **399**, 341–358.

48. Fuchs SA, Berger R, Klomp LW, et al. (2005) D-Amino acids in the central nervous system in health and disease. *Mol Genet Metab* **85**, 168–180.

49. Rodrigues FA, Chianca-Jr DA & Gonçalves Fernandes L (2012) Malnutrition affects the pressor response to microinjection of L-glutamate into the RVLM of awake rats. *Biol Res* **45**, 337–343.

50. Moraes DJ, Zoccal DB & Machado BH (2012) Sympathoexcitation during chemoreflex active expiration is mediated by L-glutamate in the RVLM/Bötzinger complex of rats. *J Neurophysiol* **108**, 610–623.

51. Moraes DJ, Zoccal DB & Machado BH (2012) Medullary respiratory network drives sympathetic overactivity and hypertension in rats submitted to chronic intermittent hypoxia. *Hypertension* **60**, 1374–1380.

52. Nanduri J, Makarenko V, Reddy VD, et al. (2012) Epigenetic regulation of hypoxic sensing disrupts cardiorespiratory homeostasis. *Proc Natl Acad Sci U S A* **109**, 2515–2520.

53. Ito T, Funamoto K, Sato N, et al. (2012) Maternal undernutrition induces the expression of hypoxia-related genes in the fetal brain. *Tohoku J Exp Med* **226**, 37–44.

54. Ito T, Tanabe K, Nakamura A, et al. (2011) Aberrant expression of hypoxia-inducible factor 1alpha in the fetal heart is associated with maternal undernutrition. *Tohoku J Exp Med* **224**, 163–171.

55. Ratcliffe PJ (2002) From erythropoietin to oxygen: hypoxia-inducible factor hydroxylases and the hypoxia signal pathway. *Blood Purif* **20**, 445–450.

56. Lee SH, Wolf PL, Escudero R, et al. (2000) Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* **342**, 626–633.