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Maternal Protein Restriction Increases Respiratory and Sympathetic Activities and Sensitizes Peripheral Chemoreflex in Male Rat Offspring[1–3]

José L de Brito Alves,[4] Viviane O Nogueira,[4] Marinaldo P Cavalcanti Neto,[5,6] Andréia M Leopoldino,[5,6] Carlos Curti,[5,6] Débora SA Colombari,[7] Eduardo Colombari,[7] Almir G Wanderley,[8] Carol G Leandro,[4] Daniel B Zoccal,[8,9] and João H Costa-Silva[4,9]*

[4]Department of Physical Education and Sport Sciences, Federal University of Pernambuco, Vitoria de Santo Antão, Pernambuco, Brazil; Departments of [5]Physics and Chemistry and [6]Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; [7]Department of Physiology and Pathology, School of Dentistry of Araraquara, São Paulo State University, Araraquara, São Paulo, Brazil; and [8]Department of Physiology and Pharmacology, Federal University of Pernambuco, Pernambuco, Brazil

Abstract

Background: Maternal protein restriction in rats increases the risk of adult offspring arterial hypertension through unknown mechanisms.

Objectives: The aims of the study were to evaluate the effects of a low-protein (LP) diet during pregnancy and lactation on baseline sympathetic and respiratory activities and peripheral chemoreflex sensitivity in the rat offspring.

Methods: Wistar rat dams were fed a control [normal-protein (NP); 17% protein] or an LP (8% protein) diet during pregnancy and lactation, and their male offspring were studied at 30 d of age. Direct measurements of baseline arterial blood pressure (ABP), heart rate (HR), and respiratory frequency (Rf) as well as peripheral chemoreflex activation (potassium cyanide: 0.04%) were recorded in pups while they were awake. In addition, recordings of the phrenic nerve (PN) and thoracic sympathetic nerve (tSN) activities were obtained from the in situ preparations. Hypoxia-inducible factor 1α (HIF-1α) expression was also evaluated in carotid bifurcation through a Western blotting assay.

Results: At 30 d of age, unanesthetized LP rats exhibited enhanced resting Rf ($P = 0.001$) and similar ABP and HR compared with the NP rats. Despite their similar baseline ABP values, LP rats exhibited augmented low-frequency variability (~91%; $P = 0.01$). In addition, the unanesthetized LP rats showed enhanced pressor ($P = 0.01$) and tachypnoeic ($P = 0.03$) responses to peripheral chemoreflex activation. The LP rats displayed elevated baseline tSN activity (~86%; $P = 0.02$) and PN burst frequency (45%; $P = 0.01$) and amplitude (53%; $P = 0.001$) as well as augmented sympathetic ($P = 0.01$) and phrenic ($P = 0.04$) excitatory responses to peripheral chemoreflex activation compared with the NP group. Furthermore, LP rats showed an increase of ~100% in HIF-1α protein density in carotid bifurcation compared with NP rats.

Conclusion: Sympathetic-respiratory overactivity and amplified peripheral chemoreceptor responses, potentially through HIF-1α–dependent mechanisms, precede the onset of hypertension in juvenile rats exposed to protein undernutrition during gestation and lactation. *J Nutr* 2015;145:907–14.

Keywords: protein undernutrition, hypertension, sympathetic overactivity, peripheral chemoreflex, hypoxia-inducible factor 1 α

Introduction

Arterial hypertension is a major risk factor for cardiovascular dysfunction, which affects almost 1 billion people and is recognized as a major cause of morbidity and mortality worldwide (1). However, the underlying cause of hypertension has been difficult to identify due to its multifactorial nature.

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* To whom correspondence should be addressed. E-mail: joao.hcsilva@ufpe.br.

[2] Author disclosures: JL de Brito Alves, VO Nogueira, MP Cavalcanti Neto, AM Leopoldino, C Curti, DSA Colombari, E Colombari, AG Wanderley, CG Leandro, DB Zoccal, and JH Costa-Silva, no conflicts of interest.

[3] Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online contents at http://jn.nutrition.org.

[9] DBZ and JHC-S contributed equally to this work.

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Hypertension may arise from a combination of genetic factors and lifestyle-related behaviors (1). In addition, adverse events experienced in utero or during perinatal life (gestation, lactation, and early infancy) can affect the development of physiologic systems, leading to increased risk of hypertension and metabolic diseases later in life (2, 3).

For example, maternal undernutrition has been associated with low nephron number, kidney disease, insulin resistance, and obesity because of rapid weight gain in childhood or adolescence (4, 5). The biological phenomenon underlying these associations is known as phenotypic plasticity, which refers to the ability of a single genotype to produce variable behavioral, morphologic, and/or physiologic phenotypes (6) in individuals in response to different environmental circumstances encountered during development.

The offspring of rat dams subjected to a maternal low-protein (LP)[10] diet is a model that is often used to study the mechanisms of maternal undernutrition–related hypertension (6–8), which has been suggested to be associated with changes in the functioning of the sympathetic nervous system (9, 10). However, there is no direct evidence demonstrating that sympathetic vasoconstrictor tonus is elevated in rats subjected to a perinatal LP diet.

We recently showed that juvenile rats subjected to protein undernutrition during gestation and lactation exhibit increased baseline respiratory frequency associated with amplified ventilatory responses to hypoxia and hypercapnia (7). These findings indicated that the functioning of the respiratory network, in addition to that of the sympathetic nervous system, is affected by an LP diet during gestation and lactation, likely through a common excitation mechanism. In this regard, afferent inputs from peripheral chemoreceptors to the central nervous system, which are mainly generated in response to hypoxic stimuli, evoke reflex responses of sympatho-excitation and tachypnoea (11). Therefore, changes in the functioning of the peripheral chemoreflex may be involved in the exaggerated sympathetic and respiratory responses observed in the offspring of protein-restricted rats.

It has been shown that the sensitization of peripheral chemoreceptors is a risk factor for sympathetic overactivity and the development of arterial hypertension (12, 13). An important molecular mechanism involved in the enhanced sensory activity of peripheral chemoreceptors is the activation of hypoxia-inducible factor (HIF) (14, 15). Indeed, there is evidence that high expression of HIF-1α during early life is associated with an increased risk of developing hypertension (16, 17).

In this context, in the present study, we hypothesized that juvenile rats from dams subjected to protein undernutrition during pregnancy and lactation would exhibit enhanced baseline sympathetic and inspiratory motor activities associated with amplified respiratory and sympathetic responses to peripheral chemoreflex activation and enhanced HIF-1α concentrations in the carotid body peripheral chemoreceptors. This hypothesis was investigated in the offspring of protein-restricted dams before the onset of hypertension (7) to verify whether these changes are the cause or consequence of an increase in arterial pressure.

[10] Abbreviations used: HF, high frequency; HIF, hypoxia-inducible factor; HR, heart rate; KCN, potassium cyanide; LF, low frequency; LP, low protein; MAP, mean arterial pressure; NP, normal protein; PN, phrenic nerve; Rf, respiratory frequency; SAP, systolic arterial pressure; tSN, thoracic sympathetic nerve.

Methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Center (protocol 044454/2010–94) at the Federal University of Pernambuco and by the Animal Experimentation Ethics Committee of the School of Dentistry of Araraquara at São Paulo State University (protocol 21/2012), Brazil. All efforts were made to minimize animal discomfort and the number of rats used; in addition, we followed the Guidelines for the Care and Use of Laboratory Animals.

Animals and experimental groups. Virgin female albino Wistar rats (*Rattus norvegicus*) were obtained from the Academic Center of Vitoria de Santo Antão, Federal University of Pernambuco, Brazil. The rats were maintained in a room with a temperature of 22 ± 1°C and a controlled light-dark cycle (dark: 1800–0600 h). Standard laboratory feed pellets (52% carbohydrate, 21% protein, and 4% lipids, Labina; Purina Agriband) and water were consumed ad libitum up to the 3-mo point, when the rats were mated (2 females for 1 male). The day on which spermatozoa were identified in a vaginal smear was considered the date of conception, and pregnant rats were transferred to individual cages. Two experimental groups were designated according to diet manipulation: dams fed a 17% casein diet [normal-protein (NP) group; $n = 6$] and dams fed a 8% casein diet (LP group; $n = 6$) and water ad libitum.

The diets were mixed at the Laboratory of Experimental Nutrition–Academic Center of Vitoria de Santo Antão, Federal University of Pernambuco, according to the American Institute of Nutrition–AIN-93 diet (18, 19). The casein was previously analyzed and found to be 85% pure (85 g of protein for each 100 g of casein). The diets were isoenergetic and were fed during pregnancy and lactation. Both diets presented the same amount of vitamin and mineral mix. Only the amount of protein and carbohydrate was changed (18). Offspring were standardized as litters of 8 pups 48 h after birth. Male offspring were used in each litter and females were used only to standardize the size of each litter to 8 pups (7). At weaning, 3 or 4 male offspring from each litter were randomly housed in collective cages and received a standard diet ad libitum. At least 2 or 3 male offspring from each litter were used to compose the NP or LP groups and to perform the experimental protocol. All of the experiments were performed in 30-d-old juvenile rats.

Cardiovascular and respiratory evaluations in vivo. One day before the experiments, the NP ($n = 8$) and LP ($n = 11$) rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), and the femoral artery and vein were cannulated [Polyethylene Tubing (PE)-50 connected to PE-10; Clay Adams]. The catheters were filled with heparinized saline (NaCl 0.9%), tunneled subcutaneously, and exteriorized through the back of the neck. After surgery, the rats were administered an injection of ketoprofen (5 mg/kg, intraperitoneally), and a period of 24 h was allowed to pass until the rats had fully recovered from the surgical and anesthetic procedures. The next day, the mean arterial pressure (MAP) and heart rate (HR) of unanesthetized freely moving rats were recorded by connecting the arterial catheter to a pressure transducer. The signals were amplified (ML866/P, Power Lab; ADInstruments), sampled at 2 kHz, and digitalized by using appropriate software (LabChart7 Pro; ADInstruments). Recordings of the baseline pulsatile arterial pressure, MAP, and HR were made for 30–50 min. After 50 min of acclimatization and cardiovascular recordings, measurements of the respiratory frequency (Rf) were also performed by using the whole-body plethysmography method (20). Before recording baseline data, the rats were placed into a Plexiglas chamber (5 L) that was flushed with humidified room air and maintained at a temperature of 25°C. After this acclimatization period, the Rf was recorded as the airflow was suspended for short periods (3 min), and the pressure oscillations caused by breathing were captured by a pressure differential transducer connected to a signal amplifier (ML141 Spirometer, PowerLab; ADInstruments). The signals were then captured by an acquisition system and data analysis was performed (PowerLab; ADInstruments). All of the data were analyzed offline with the use of appropriate software (LabChart 7 Pro; ADInstruments).

After the baseline recordings of Rf and arterial pressure, the peripheral chemoreflex was activated through the intravenous injection of potassium cyanide (KCN; 40 μg/100 μL per rat; Merck) in accordance with previous reports (11, 21). At the end of the experiments,

the rats were killed with a 1-mL overdose of a mixture of ketamine (80 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally).

An indirect evaluation of the autonomic modulation of vascular resistance and cardiac function was performed through an analysis of the variability in the arterial pressure and HR in the frequency domain (22). Oscillations of arterial pressure and HR at the low-frequency (LF) range are representative of the modulatory effects of sympathetic activity controlling vascular tonus and heart activity, whereas oscillations at the high-frequency (HF) range are associated with a respiratory or parasympathetic modulation of blood vessels or the heart, respectively (22–24). To reach this goal, a beat-by-beat time series of the systolic arterial pressure (SAP) and HR were extracted from the baseline cardiovascular recordings (10-min epochs) of the pulsatile arterial pressure of the NP and LP rats (Chart Pro; ADInstruments), and the overall variability of these series was assessed through fast Fourier transformation infrared spectroscopy (Cardioseries software, version 2.4) (25). The power of the oscillatory components obtained from the rats belonging to the NP and LP groups was quantified in 2 frequency bands: LF (0.20–0.75 Hz) and HF (0.75–3.0 Hz) (22, 26).

In situ working heart-brainstem preparation. Juvenile rats at 30 d of age [NP (n = 6) and LP (n = 8)] were deeply anesthetized with halothane (Astra Zeneca), such that the withdrawal responses to noxious pinching of the tail and paw were absent. The rats were then transected caudally to the diaphragm and submerged in cooled Ringer solution (in mM: 125 NaCl, 24 $NaHCO_3$, 3 KCl, 2.5 $CaCl_2$, 1.25 $MgSO_4$, 1.25 KH_2PO_4, and 10 dextrose). They were made insentient by decerebration at the precollicular level, skinned, and had the descending aorta isolated. Preparations were then transferred to a recording chamber, where the descending aorta was cannulated and perfused retrogradely with modified Ringer solution containing lactate (2 mM), an oncotic agent (1.25% polyethylene glycol; Sigma), and a neuromuscular blocker (vecuronium bromide, 3–4 μg/mL; Cristalia) by using a roller pump (Watson-Marlow 502s) via a double-lumen cannula. Perfusion pressure was maintained in the range of 50–70 mm Hg by adjusting the rate flow between 21 and 25 mL/min and by adding vasopressin to the perfusate (6–12 nM; Sigma), as previously described (27). Electrical activity in all nerves was recorded by using glass suction bipolar electrodes held by a micromanipulator (Narishige). Left phrenic nerve (PN) discharges were recorded from the central end and its rhythmic ramping activity gave a continuous physiologic index of preparation viability. Thoracic sympathetic nerve (tSN) activity was recorded from the thoracic sympathetic chain at the level of T10–T12. All of the signals were amplified, band-pass filtered (0.05–5 kHz), and acquired with an A/D converter (CED 1401; Cambridge Electronic Design) on a computer using Spike2 software (version 7; Cambridge Electronic Design). Peripheral chemoreceptors were stimulated by injections of KCN (0.05%, 50 μL) into the descending aorta of the working heart-brainstem preparation via the perfusion cannula, as previously described (28).

All of the analyses of rectified and integrated (50-ms) signals were performed off-line by using the Spike 2 software with custom-written scripts. Before analyses, PN and tSN recordings were subtracted from the electrical noise obtained after the death of the working heart-brainstem preparation (induced by turning the pump off). For baseline measurements, PN activity was assessed by its frequency (cycles per minute), amplitude (μV), burst duration (inspiratory time, s), and burst interval (expiratory time, s). tSN activity was assessed by its mean activity (μV) and by the amplitude of inspiratory-related bursts (μV), which was calculated by the value difference between the maximal and lowest activity observed during inspiratory and postinspiratory phases. With respect to the changes induced by peripheral chemoreflex activation, the phrenic frequency reflex response was assessed by the difference between the baseline frequency and the peak of response observed after the stimulus (ΔPN; expressed in cycles per minute). The sympathetic response was assessed by the measurement of the AUC and expressed as percentage change (ΔtSN; in %) in relation to the activity before the stimulus.

Evaluation of HIF-1α protein density. Under normoxic conditions, separate groups of NP (n = 6) and LP (n = 7) rats that were not subjected to any surgical procedure were killed by an overdose of ketamine (80 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally) for collection of the carotid bifurcation at 30 d of age. The tissues were flash-frozen in liquid nitrogen and stored at −80°C until use. The carotid bifurcation samples were pooled respectively to obtain a sufficient amount of protein. The samples were then sonicated, and protein extracts were obtained in radioimmunoprecipitation assay buffer (50 mM Tris pH 7.6, 150 mM NaCl, 1% SDS, 0.5% sodium deoxycholate, 0.5% NP-40) with protease and phosphatase inhibitor cocktails (Sigma-Aldrich). Protein concentration was determined by using the Bradford method (Bio-Rad Laboratories). Ninety micrograms of protein was submitted to 8% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (GE HealthCare). The membranes were blocked for 1 h by using Tris-buffered saline containing 10% Tween 20 (TBS-T) and 5% (wt: vol) nonfat dry milk (blocking buffer). Antibodies against HIF-1α (mouse monoclonal H1α67, GR45835-1; AbCam) and GAPDH (2118S; Cell Signaling) were used. The antibody against HIF-1α was used diluted 1:500 in TBS-T with 5% of BSA (Sigma-Aldrich). Anti-mouse secondary antibody was diluted 1:5000 in blocking buffer. Blots were developed by using the chemiluminescent ECL Western Blotting System

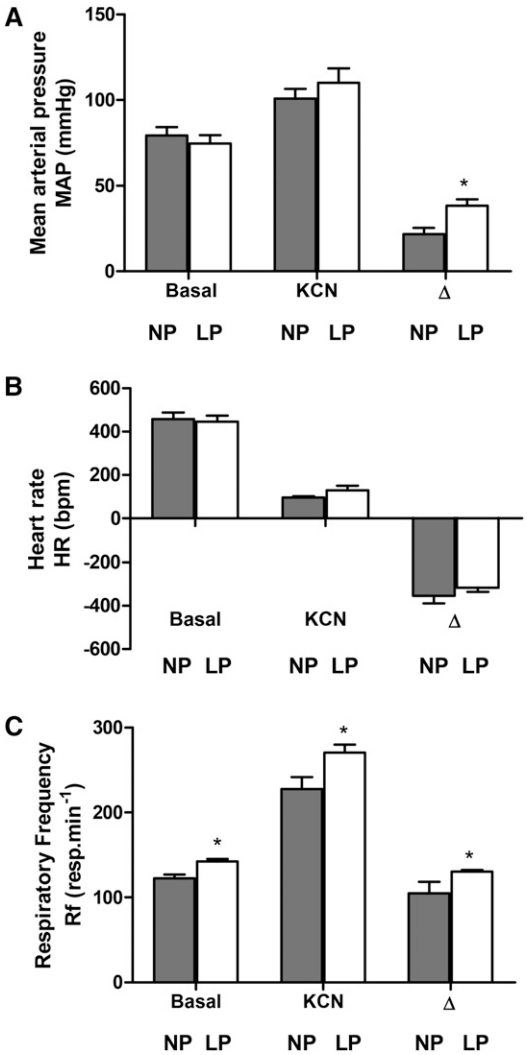

FIGURE 1 MAP (A), HR (B), and Rf (C) of 30-d-old male rat pups of dams fed an NP or an LP diet during pregnancy and lactation. Values are means ± SEMs, n = 8–11. *Different from NP, $P < 0.05$ (unpaired Student's t test). bpm, beats per minute; HR, heart rate, KCN, potassium cyanide; LP, offspring of experimental rat dams fed a low-protein diet (8% protein); MAP, mean arterial pressure; NP, offspring of control rat dams fed a normoproteic diet (17% protein); resp, respirations; Rf, respiratory frequency.

(GE HealthCare). The bands were quantified by densitometry with the use of Image J software (NIH; http://rsbweb.nih.gov/ij/), and the relative densities of HIF-1α were normalized by their respective controls.

Statistical analysis. Each experimental group included at least 2 rats from each litter. Bartlett's test was performed to evaluate data homogeneity of the respiratory and sympathetic variables, and statistical results supported the use of a parametric test. Thus, the significance of the difference between groups was assessed by unpaired Student's *t* test. The significance level was fixed to $P < 0.05$. The data are expressed as means with associated SEs. Statistical analysis was performed by using GraphPad Prism 5.0 software.

Results

Ponderal gain. Pups from mothers subjected to an LP diet had lower birth weight than did the NP group (NP vs. LP: 6.3 ± 0.1 vs. 5.3 ± 0.2 g; $P = 0.04$). Furthermore, the reduced body weight of the LP group was maintained until 30 d of age (NP vs. LP: 86.3 ± 2.9 vs. 62.5 ± 2.6 g; $P = 0.01$).

Conscious rats. Representative baseline and chemoreflex-evoked changes in MAP, HR, and Rf of unanesthetized rats at 30 d of age are shown in **Supplemental Figures 1** and **2.** The baseline MAP ($P = 0.83$; **Figure 1**A) and HR ($P = 0.95$; Figure 1B) were similar between LP and NP rats. However, baseline Rf was higher in the LP group than in the NP group ($P = 0.001$; Figure 1C). Despite the similar baseline values, the autonomic modulation of arterial pressure and HR was altered in LP rats. As can be observed in the representative spectra of SAP (**Figure 2**A), LP rats exhibited an augmented magnitude of oscillation at the LF range ($P = 0.01$; Figure 2B) but not at the HF range ($P = 0.75$; Figure 2C) compared with NP rats. In relation to pulse interval, the LF:HF ratio (an index of sympathetic:parasympathetic balance to the heart) was enhanced in the LP group ($P = 0.001$; Figure 2D).

Peripheral chemoreflex activation with KCN (intravenous) produced pressor, bradycardic, and tachypnoeic responses in both NP and LP groups. The increase in MAP ($P = 0.01$; Figure 1A) and Rf ($P = 0.03$; Figure 1C) were significantly greater in the LP group than in the NP group. In contrast, the magnitude of decrease in HR of both groups were similar ($P = 0.32$; Figure 1B).

tSN and PN activities in situ. Baseline recordings of the PN and tSN activities of representative 30-d-old rats are shown in **Figure 3**A. The LP rats presented higher levels of tSN amplitude than did NP rats ($P = 0.02$; Figure 3B). Average tSN levels were not significantly different between groups ($P = 0.35$; Figure 3C). Furthermore, LP rats exhibited a larger PN burst frequency ($P = 0.01$; Figure 3E) and amplitude ($P = 0.001$; Figure 3D) in comparison to NP rats. The LP rats also showed shorter inspiratory (NP vs. LP: 1.4 ± 0.1 vs. 1.1 ± 0.1 s; $P = 0.04$) and expiratory (NP vs. LP: 4.3 ± 0.6 vs. LP: 2.6 ± 0.2 s; $P = 0.01$) times compared with the NP group. Together, these findings showed augmented sympathetic and inspiratory motor activities for LP rats at baseline conditions.

The activation of peripheral chemoreceptors elicited responses of sympatho-excitation and increased PN burst frequency in the in situ preparations of 30-d-old rats from the NP and LP groups, as shown in **Figure 4**A. We verified that LP rats exhibited greater increases in PN burst frequency ($P = 0.04$; Figure 4B) and higher sympatho-excitatory responses ($P = 0.01$; Figure 4C) than did NP rats.

HIF-1α expression in carotid bifurcation. Bands corresponding to HIF-1α were observed at 120 kDa in samples of carotid bifurcations from NP and LP rats. HIF-1α protein density, which was normalized by GAPDH density, was 119% higher (NP vs. LP: 0.30 vs. 0.67 arbitrary units) in carotid bifurcation samples from LP rats compared with the samples from NP rats, as shown in **Figure 5**.

Discussion

In the present study, we investigated the effects of perinatal protein restriction on baseline and chemoreflex-evoked control of arterial pressure, Rf, sympathetic and phrenic activities, and HIF-1α expression in the carotid bodies. The main findings of this study showed that protein restriction during perinatal development produced in 30-d-old rats *1*) increased baseline

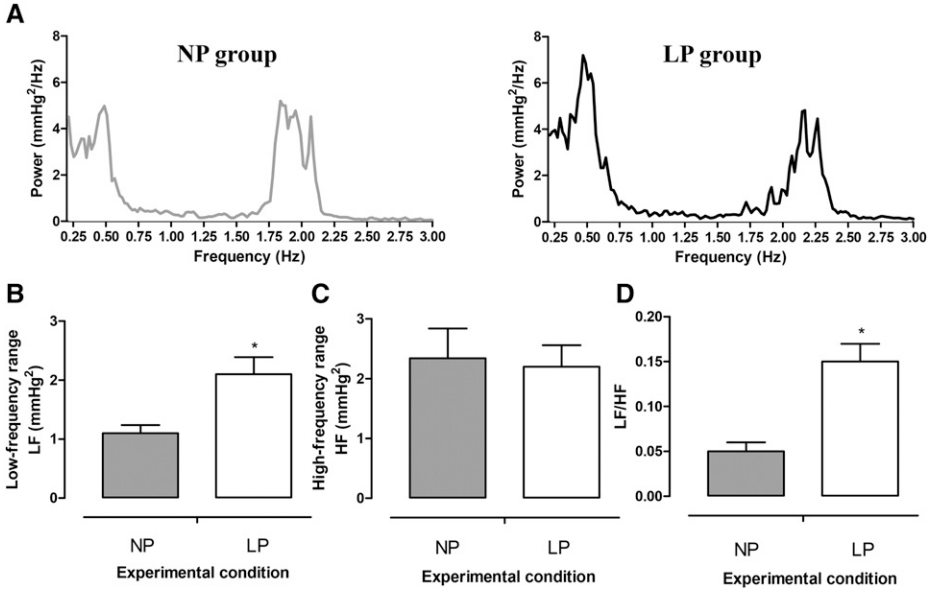

FIGURE 2 Representative spectra of SAP (A), average magnitudes of LF (B) and HF (C) components of SAP, and the LF/HF index of PIs (D) of 30-d-old male rat pups of dams fed an NP or an LP diet during pregnancy and lactation. Values are means ± SEMs, n = 8–11. *Different from NP, $P < 0.05$ (unpaired Student's *t* test). HF, high-frequency band; LF, low-frequency band; LF/HF, index of sympathetic/parasympathetic balance to the heart; LP, offspring of experimental rat dams fed a low-protein diet (8% protein); NP, offspring of control rat dams fed a normoproteic diet (17% protein); PI, pulse interval; SAP, systolic arterial pressure.

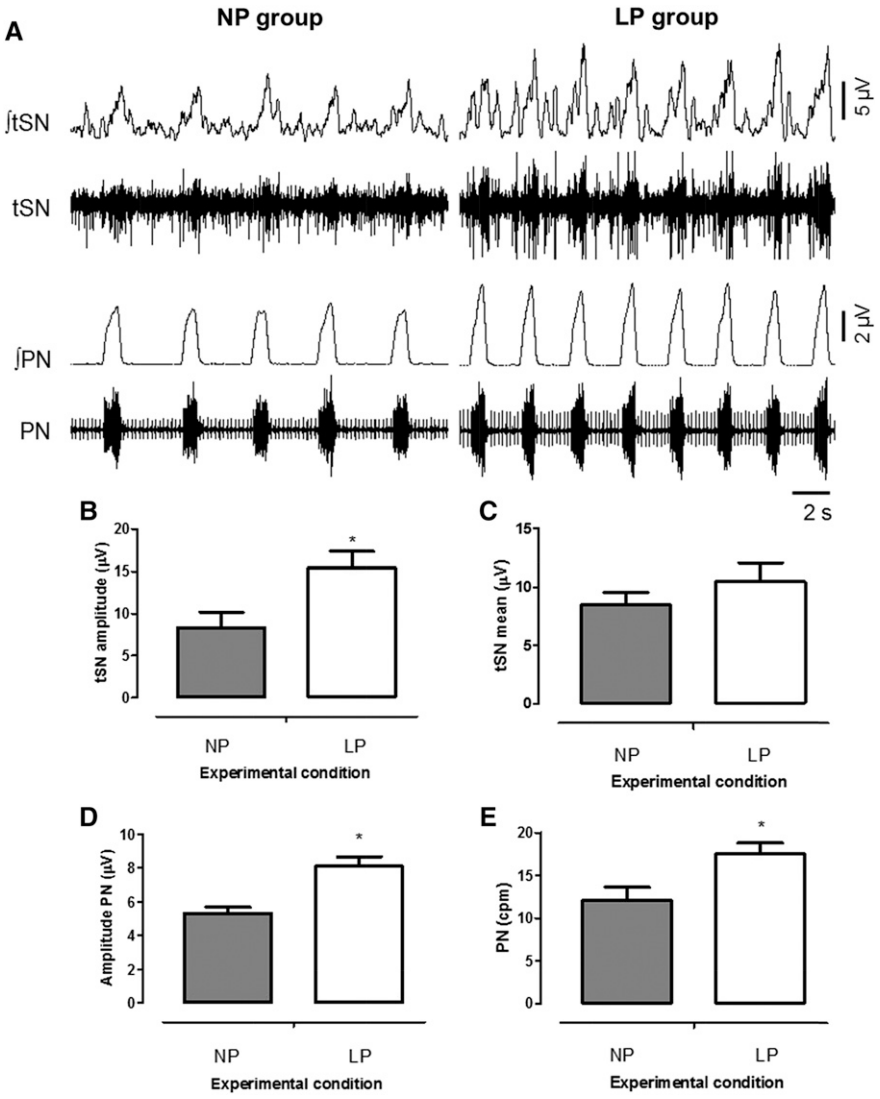

FIGURE 3 Representative tracings showing raw and integrated PN and tSN activities (A), average of baseline tSN amplitude (B), tSN mean (C), PN amplitude (D), and PN mean (E) for 30-d-old male rat pups of dams fed an NP or an LP diet during pregnancy and lactation. Values are means ± SEMs, $n = 6$–8. *Different from NP, $P < 0.05$ (unpaired Student's t test). cpm, cycles per minute; LP, offspring of experimental rat dams fed a low-protein diet (8% protein); NP, offspring of control rat dams fed a normoproteic diet (17% protein); PN, phrenic nerve; tSN, thoracic sympathetic nerve; ∫, integrated activity.

ventilation, as evidenced by greater respiratory frequency in vivo and higher phrenic burst frequency and amplitude in situ; *2*) showed normal arterial pressure but augmented sympathetic activity at rest, as indicated by enhanced LF modulation of arterial pressure in unanesthetized rats and elevated levels of thoracic sympathetic activity in in situ preparations; and *3*) amplified sympatho-excitatory and tachypnoeic responses to peripheral chemoreceptor stimulation combined with enhanced HIF-1α expression in the carotid bodies, suggesting a sensitization of the carotid peripheral chemoreceptors. Altogether, our data bring new insights into the etiologic mechanisms underlying the development of arterial hypertension in protein-restricted rats, highlighting a critical role of the sympathetic nervous system and peripheral chemoreceptors.

Although a relation between maternal protein restriction during pregnancy and lactation and the development of arterial hypertension in offspring during adult life has been previously described (6, 7, 29, 30), its underlying mechanisms are poorly understood. We recently reported that rat offspring subjected to protein undernutrition during pregnancy and lactation exhibited reduced ponderal gain and higher baseline arterial pressure at 90 d but not at 30 d of age. Despite the lack of augmented baseline arterial pressure, 30-d-old rats subjected to a perinatal LP diet exhibited an increased baseline respiratory frequency and enhanced respiratory responses to hypoxia and hypercapnia, suggesting that changes in the mechanisms controlling respiratory motor activity and/or increased oxygen/carbon dioxide chemosensitivity occur before the development of hypertension (7). Accordingly, in the present study, juvenile offspring from protein-restricted dams showed increased Rf in both the conscious state and in the in situ preparation. It was clearly shown that protein-restricted rats exhibited higher PN burst frequency and amplitude, indicating an increased central inspiratory activity at rest. Therefore, our data strongly support the notion that an LP diet during gestation and lactation elicits changes in the functioning of the central respiratory pattern and rhythm generator that precede the development of arterial hypertension.

Although arterial pressures were similar between the NP and LP groups at 30 d old, our data indicate that the sympathetic vasoconstrictor tonus of LP rats is enhanced. In unanesthetized rats, we verified that the variability at an LF range of SAP and pulse inteval, which are correlated with sympathetic drive to blood vessels and to the heart (23, 31), was significantly enhanced in the LP group. These observations suggest that maternal protein restriction can lead to augmented sympathetic modulation of the cardiovascular system in juvenile offspring. We also verified that in situ preparation of LP rats presented

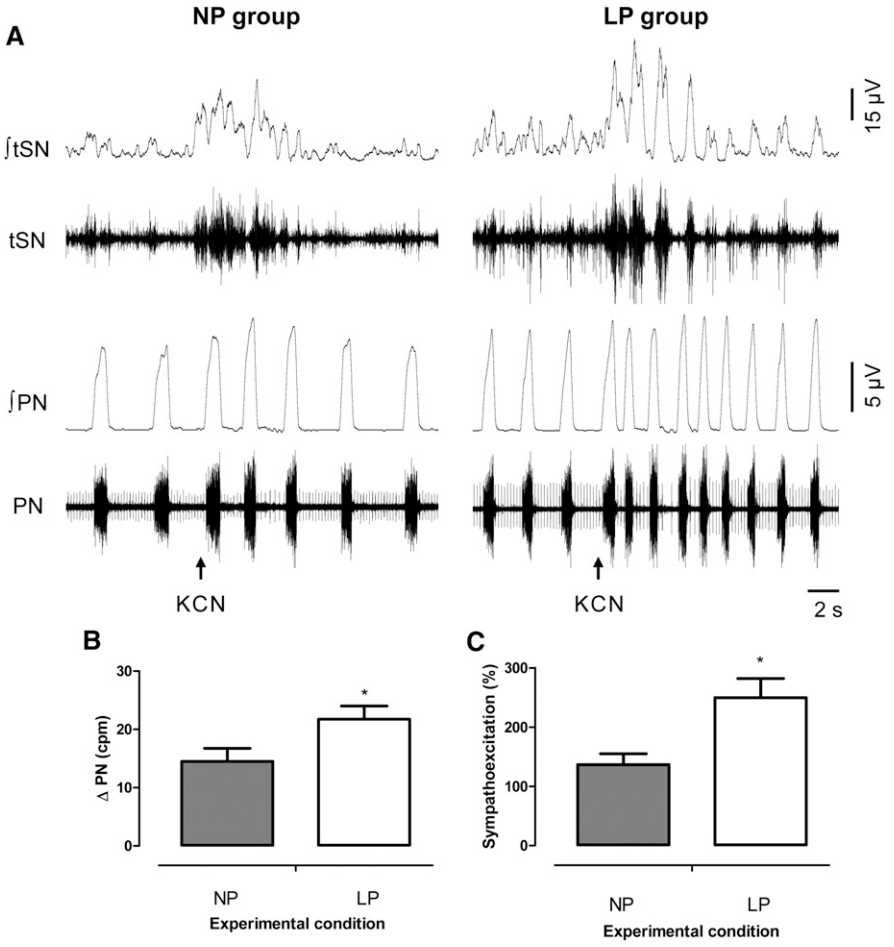

FIGURE 4 Representative tracings showing raw and integrated PN and tSN activities during peripheral chemoreflex activation (A) and averages of percentage tSN (B) and PN (C) during peripheral chemoreflex activation in 30-d-old male rat pups of dams fed an NP or an LP diet during pregnancy and lactation. Values are means ± SEMs, $n = 6$–8. *Different from NP, $P < 0.05$ (unpaired Student's t test). cpm, cycles per minute; KCN, potassium cyanide; LP, offspring of experimental rat dams fed a low-protein diet (8% protein); NP, offspring of control rat dams fed a normoproteic diet (17% protein); PN, phrenic nerve; tSN, thoracic sympathetic nerve; ∫, integrated activity.

increased levels of sympathetic nerve activity, supporting the notion that baseline sympathetic activity is indeed elevated in rats subjected to an LP diet during the perinatal period. This higher level of sympathetic activity observed before the onset of hypertension in LP rats suggests that dysfunction of the sympathetic nervous system, culminating in enhanced baseline sympathetic activity, contributes to the development of hypertension in these rats. These findings are in agreement with those observed in spontaneously hypertensive rats, which exhibit an elevated sympathetic activity in early life, just before the development of arterial hypertension (32, 33).

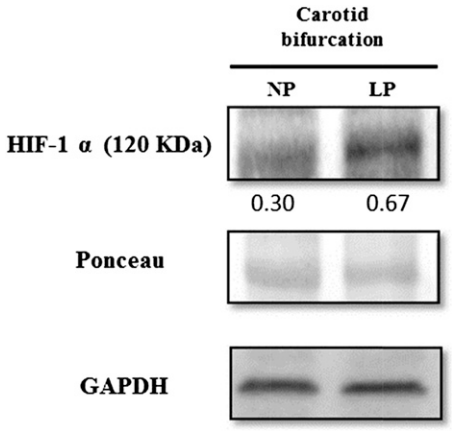

FIGURE 5 Western blot assay for expression of HIF-1α showing results presented to confirm equal loading of the protein samples. Relative densities of HIF-1α were normalized by the respective amounts of Ponceau S red in 30-d-old male rat pups of dams fed an NP or an LP diet during pregnancy and lactation. HIF-1α, hypoxia-inducible factor 1α; LP, offspring of experimental rat dams fed a low-protein diet (8% protein); NP, offspring of control rat dams fed a normoproteic diet (17% protein).

The role of the sympathetic nervous system in the generation of neurogenic hypertension has been convincingly supported with different experimental models (22, 32, 34–36). Because high levels of tSN activity in protein-restricted rats were associated with elevated Rf and increased PN burst frequency and amplitude, we hypothesize that the increased sympathetic drive of LP rats is linked to the increased baseline inspiratory activity. It is well established that the respiratory system markedly modulates sympathetic nerve discharge at rest (27, 34, 35, 37), introducing phasic bursts in sympathetic activity, mainly during the inspiratory/postinspiratory phases (27, 28, 35, 38). This respiratory modulation of sympathetic activity is consequent to synaptic interactions between respiratory and sympathetic neurons of the brainstem (37, 39–41). Presympathetic neurons that exhibit an inspiratory-modulated pattern of activity with increased frequency of discharge during inspiration have been identified within the rostral ventrolateral medulla (41).

Therefore, because baseline central inspiratory activity is enhanced in protein-restricted rats, we theorize that perinatal protein restriction increases the activity of inspiratory neurons that, in turn, send excitatory inputs to presympathetic neurons of the rostral ventrolateral medulla, thereby enhancing sympathetic activity primarily during inspiration (41). However, this hypothesis still requires further experimental verification.

In addition to baseline cardiorespiratory changes, the sympathetic and inspiratory reflex responses to peripheral chemoreceptor stimulation were also amplified in LP rats. These data are in agreement with our previous observation that the ventilatory responses to hypoxia are enhanced in unanesthetized LP rats at the same age (7), suggesting that peripheral chemoreflex is sensitized in rats subjected to perinatal protein restriction. In agreement with this hypothesis, we verified that the expression of HIF-1α is enhanced in the carotid bodies of these rats, revealing that this transcriptional factor, which is related to the response of cell to reduced oxygen and energy availabilities (42–44), may be involved in the adaptive responses elicited by protein restriction during the perinatal period (45) and mediates, at least in part, the cardiorespiratory changes observed in protein-restricted rats.

Recent experimental and clinical evidence indicates that dysfunction of carotid body chemoreceptors plays a critical role in the progression of cardiorespiratory morbidities associated with baseline sympathetic overactivity, including sleep disorders, congestive heart failure, chronic pulmonary obstructive disease, and hypertension (9, 12, 13, 27, 46–48). In agreement with this notion, we hypothesize that the enhanced baseline sympathetic and inspiratory motor activities of juvenile offspring from dams subjected to protein restriction during pregnancy and lactation, before the development of hypertension, are dependent, at least in part, on the sensitization of the peripheral chemoreflex. These represent new insights into the mechanisms underlying the arterial blood pressure control of rats that have undergone perinatal protein restriction.

In conclusion, the present study suggests that juvenile offspring from protein-restricted dams exhibit enhanced baseline sympathetic and inspiratory motor activities combined with amplified ventilatory and autonomic responses to peripheral chemoreflex activation before the establishment of hypertension. These changes are apparently associated with a high HIF-1α concentration in carotid body peripheral chemoreceptors. These findings can aid in understanding why blood pressure increases in individuals subjected to protein undernutrition during a critical period of life.

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JLdBA, CGL, DBZ, and JHC-S designed the research; JLdBA, VON, MPCN, DBZ, and JHC-S conducted the research; JLdBA, VON, MPCN, AML, CC, DSAC, EC, AGW, CGL, DBZ, and JHC-S analyzed the data and performed the statistical analysis; and JLdBA, DBZ, and JHC-S had primary responsibility for the final content. All authors read and approved the final manuscript.

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