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Baroreflex dysfunction in rats submitted to protein restriction

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Abstract

Earlier studies from the authors' laboratory showed that malnourishment induces alterations in the cardiovascular homeostasis increasing the basal mean arterial pressure and heart rate. In this study, the authors evaluated whether the sympathetic and parasympathetic efferent activities contribute to changes in the cardiovascular homeostasis through altered modulation of the arterial baroreflex of malnourished rats. After weaning, male Fischer rats were given 15% (Normal Protein—NP) or 6% (Low Protein—LP) protein diet for 35 d. The baroreflex gain and latency were evaluated before and after selective autonomic blockades in control and malnourished rats. It was observed that malnourishment affected the baroreflex gain in response to activation and deactivation of the arterial baroreflex. Moreover, malnourished rats showed increased baroreflex latency as compared to that of control rats. Regarding the autonomic efferent activity directed to the heart, the data showed increased sympathetic and decreased parasympathetic efferent activities in malnourished rats, and such alterations could be related to the observed changes in the arterial baroreflex gain as well as in the basal mean arterial pressure and heart rate.

Keywords: Autonomic dysfunction; Malnourishment; Baroreflex gain; Baroreflex latency; Methyl-atropine; Metoprolol

Introduction

Although the right to food and nutrition has been enshrined in the Universal Human Rights Declaration more than 50 years ago, malnourishment continues to be one of the most difficult problems of public health in developing countries (de Onis et al., 2000; Sawaya et al., 2003). Several studies suggest that low protein intake leads to biochemical, physiological, and cardiovascular alterations (Barker et al., 1989, 1990, 1993; Benabe et al., 1993a,b; Benabe and Martinez-Maldonado, 1993, 1998; Brawley et al., 2003; Mi et al., 2000). Blood pressure is under tonic autonomic control through sympathetic and parasympathetic branches of the autonomic nervous system whose efferent activities are adjusted every moment by integrating several neural cardiovascular reflexes to maintain the blood pressure within a narrow range of variation (Loewy, 1990). Among the cardiovascular neural mechanisms, the arterial baroreceptor reflex represents a homeostatic cardiovascular mechanism that maintains the mean arterial pressure within normal levels by coupling the cardiac output with the tissue demand (Machado et al., 1997; Machado, 2001). Baroreflex activation produces parasympathoexcitation and sympathoinhibition to "buffer" alterations produced by an increase in the arterial pressure (Machado et al., 1997; Machado, 2001). The sympathetic and parasympathetic contributions to the changes in the heart rate subsequent to baroreflex activation have distinct characteristics related to central pathways, latencies and abilities to increase or reduce the heart rate (Loewy, 1990). While the tachycardia observed in response to baroreflex deactivation seems to be determined by changes in both autonomic branches, including inhibition of parasympathetic activity and sympathetic activation (mainly), bradycardic responses during baroreflex activation seem to be dependent on rapid vagal discharges directed to

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the heart, because the sympathetic activity is slower to respond to sudden changes in mean arterial pressure (MAP) (Head and McCarty, 1987).

In earlier laboratory studies, the authors evidenced increased basal mean arterial pressure and heart rate (Oliveira et al., 2004), as well as increased baroreflex gain in rats subjected to a low protein diet (Tropia et al., 2001). The sympathetic vasomotor tonus in the experimental model also seems to have increased (Tropia et al., 2001). To further elucidate the cardiovascular autonomic changes associated with malnourishment, the present work was aimed at evaluating the involvement of the sympathetic and parasympathetic efferent activity directed to the heart in the modulation of the baroreflex function of malnourished animals.

Materials and methods

Animals

Male Fischer rats from the Experimental Nutrition Laboratory of the School of Nutrition at Federal University of Ouro Preto were used in this study. After birth, the offspring were randomly picked up and eight puppies were kept with each dam. The dams continued to receive commercial food (normal protein content) and water *ad libitum* and the pups were weaned after 28 d. After weaning, the male rats were divided into two groups: control group, receiving diet containing 15% protein (Normal Protein Diet—NP) and experimental malnourished group, receiving diet containing 6% protein (Low Protein Diet—LP) for the next 35 d. Thereafter, during the next 7 d, the rats underwent experimental protocols and continued receiving experimental rat chow (normal or low protein diet according to the experimental group). During all experimental protocols, the rats remained in controlled laboratory conditions (12/12 h light/darkness cycle, temperature: 23–25 °C).

Arterial blood pressure and heart rate recordings

Under tribromoethanol anesthesia (250 mg/kg, ip; Aldrich Chemical Company, Inc., Milwaukee, WI, USA), a polyethylene catheter (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ, USA), filled with heparinized PBS (125 U/ml), was inserted into the abdominal aorta through the left femoral artery for measurement of pulsatile arterial pressure (PAP). Another catheter was inserted into the inferior vena cava through the left femoral vein for systemic administration of drugs. The free endings of both the catheters were tunneled subcutaneously and exteriorized through the back of the neck. The next day, at the time of cardiovascular recordings, the arterial catheters were connected to pressure transducer (Model MLT0699; ADInstruments Pty Ltd., Castle Hill, NSW Australia). The data acquisition was made through an analog-to-digital data acquisition system (Model PowerLab 400; ADInstruments Pty Ltd., Castle Hill, NSW Australia). The data were sampled at 12 bits using a 200-Hz sampling rate. Heart rate (HR) and mean arterial pressure (MAP) were derived off-line from PAP using the Chart for Windows software, version 4.1.2 (ADInstruments Pty Ltd., Castle Hill, NSW Australia). The baseline cardiovascular variables, MAP and HR, were recorded during the 15 min before each experimental trial. All trials were performed on conscious and unrestrained rats.

The baroreceptor reflex evaluation

Changes in MAP and HR were elicited by bolus injections of phenylephrine (0.25 to 4.0 μg/kg, i.v., Sigma, St. Louis, MO, USA) or sodium nitroprusside (0.7 to 10.0 μg/kg, i.v., Sigma, St. Louis, MO, USA). The difference between the baseline just before baroreflex activation or deactivation and the peak of the changes in the MAP and HR produced by baroreflex activation or deactivation were measured as ΔMAP and ΔHR, respectively.

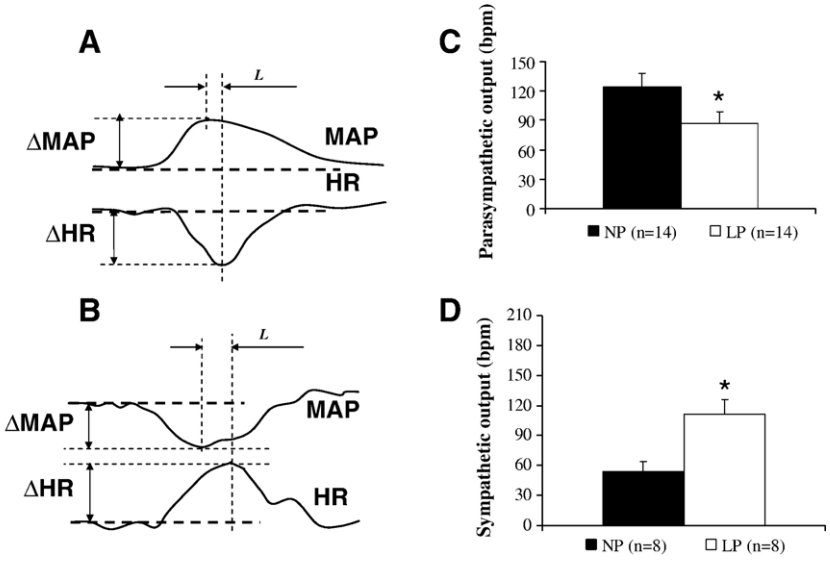

Fig. 1. The gain of the baroreflex was estimated by dividing the alteration in the heart rate (ΔHR, bpm) by the alteration in the mean arterial pressure (ΔMAP, mmHg) in the baroreflex activation (A) and deactivation (B). The latency (*L*) was calculated as the time lag between the peak of the change in the MAP and the peak of the change in the HR in response to baroreflex activation (A) and deactivation (B) in NP and LP rats. Parasympathetic (C) and sympathetic (D) tonus in the basal HR in malnourished (LP) and control (NP) groups (*=different from control group; $P<0.05$).

The baroreflex gain was evaluated through dividing the maximum ΔHR alteration by the maximum ΔMAP produced by pressor or depressor drugs. The index ΔHR/ΔMAP of each drug injection was calculated for all the groups in different conditions of autonomic blockades, that is, sympathetic and/or parasympathetic antagonism (using metoprolol or methyl-atropine, respectively). The latency of the baroreflex was accepted as the time (expressed in seconds) between the peak of the pressure response and the peak of its respective chronotropic (bradycardic or tachycardic) response (Fig. 1A and B, respectively).

Autonomic cardiac antagonisms

To perform vagal blockade in this study, atropine methyl-sulfate (4 mg/kg, i.v., in bolus, Sigma Chemical Co., St. Louis, MO, USA) was used. β1-adrenergic blockade was performed by the β1-adrenergic receptors antagonist metoprolol (10 mg/kg, i.v. in bolus, Sigma Chemical Co., St. Louis, MO, USA). The parasympathetic tonus to the heart was calculated by the difference between the maximum HR after systemic administration of atropine and the basal level of HR. The sympathetic tonus to the heart was obtained by calculating the difference between the basal HR and the minimum HR after the systemic administration of metoprolol.

Statistical analysis

All the data were expressed as mean±S.E.M. Unpaired or paired Student *t*-tests were used when comparing two groups. Significance level was fixed at $P<0.05$.

Results

Protein restriction, during the 35 d of experimental protocol, induced in the animals significant reduction in body weight of the LP group as compared to that of the NP group (67±1 g *vs.* 207±2 g, respectively), and significant increase in both basal MAP and HR (124±3 mmHg and 435±9 bpm; $n=26$ *vs.* 114±2 mmHg and 388±6 bpm, respectively).

Brief note regarding the animals' health

But for the low weight gain, none of the malnourished animals used in this study presented any signs of sickness of any kind. The

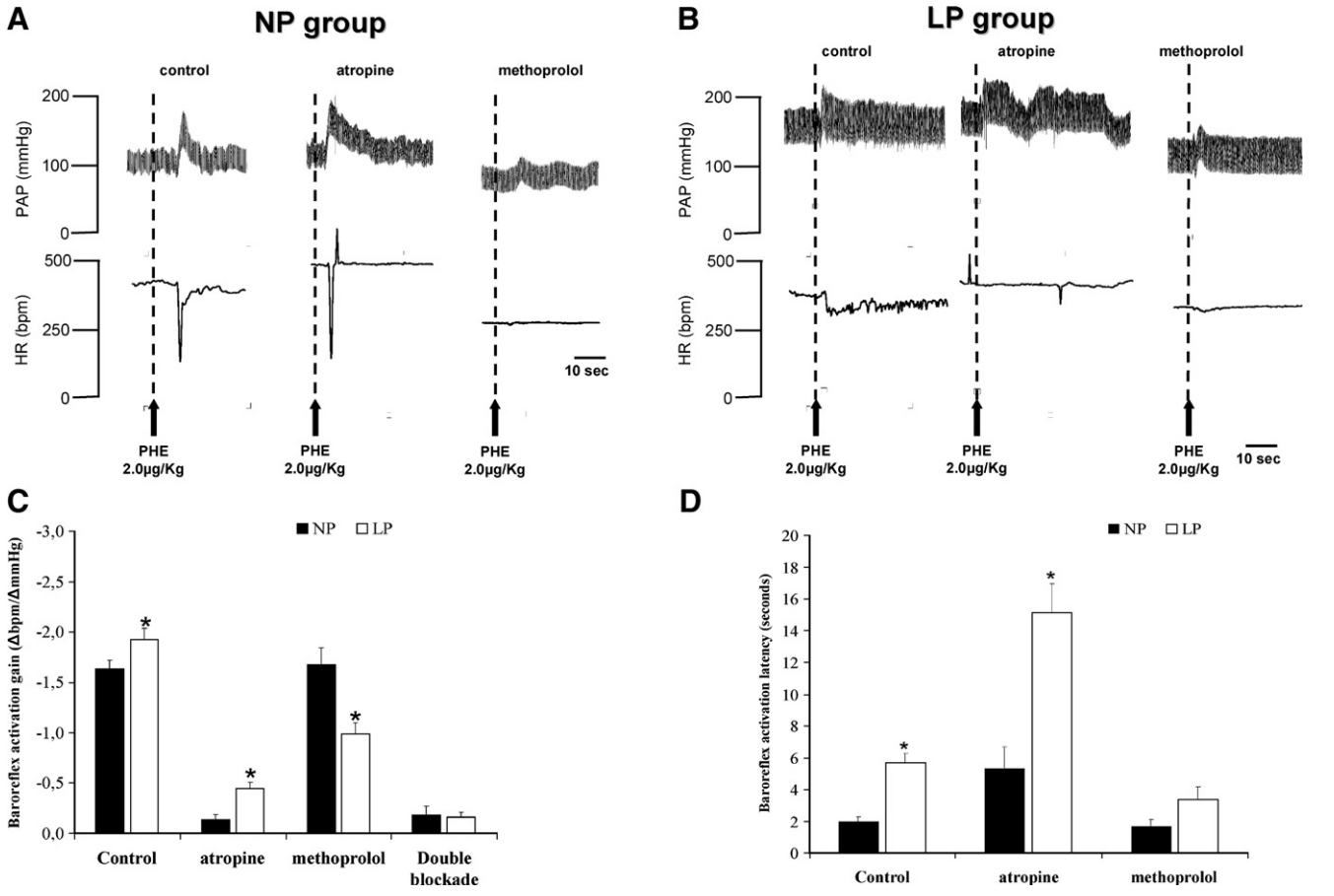

Fig. 2. Representative tracings of rats from the NP (A) and LP (B) groups, showing the changes in the baseline pulsatile arterial pressure (PAP, mmHg) and in the HR (bpm) in response to baroreflex activation. C—Baroreflex gain (bradycardic responses) in malnourished (LP—white bars) or control (NP—black bars) rats. Baroreflex bradycardic indexes were determined by ΔHR/ΔMAP ratio. The baroreflex was elicited by L-phenylephrine i.v., in basal (control) condition ($n=21$), after cholinergic blockade ($n=13$), sympathetic blockade ($n=7$), or double blockade ($n=5$). (*=different from the control group; $P<0.05$). D—Baroreflex latency in response to baroreflex activation in NP (black bars) and LP (white bars) rats, in basal (control) condition ($n=21$), after cholinergic blockade ($n=13$), sympathetic blockade ($n=7$).

evaluation of the autonomic tonus directed to the heart, illustrated in Fig. 1C, shows that the parasympathetic tonus, observed after administering atropine, diminished in LP rats as compared to that in NP animals (+87±11 *vs.* +124±14 bpm, respectively), while Fig. 1D shows that sympathetic efferent activity increased in the LP group as compared to that of the NP group (+111±15 *vs.* +54±11 bpm, respectively).

Fig. 2, showing representative tracings from representative rats of the groups, illustrates the cardiovascular changes observed in the MAP and HR subsequent to baroreceptor activation of NP (2A) and LP (2B) groups. In the panel C of Fig. 2 are depicted the data concerning the baroreflex gain in control (NP) and malnourished rats (LP) before and after systemic administration of atropine and/or metoprolol. The LP group showed more baroreflex gain in response to phenylephrine bolus injection, before (control) (−1.93±0.11 bpm/mmHg *vs.* −1.64± 0.08 bpm/mmHg) and after atropine administration (−0.44±0.07 *vs.* −0.14±0.05 bpm/mmHg, respectively) than that shown by NP animals. After β1-adrenergic blockade, the baroreflex gain was higher in NP rats than in LP rats (−1.68±0.17 bpm/mmHg *vs.* −0.99±0.11 bpm/mmHg, respectively) and after the double blockade (atropine+metoprolol), no difference was found in baroreflex gain between LP and NP groups (−0.31±0.41 *vs.* −0.33±0.17 bpm/mmHg, respectively). Also, the latency of the baroreflex response (Fig. 2D) to phenylephrine injection increased in LP group before (5.70±0.58 *vs.* 1.99±0.29 s, respectively) and after systemic administration of atropine (15.13±1.81 *vs.* 5.31±1.37 s, respectively). No significant differences were found between LP and NP animals after the systemic administration of metoprolol (5.08±2 *vs.* 3.14±3.57 , respectively).

Fig. 3A and B depict tracings of representative rats from NP and LP groups (respectively) showing the changes in the baseline MAP and HR in response to baroreflex deactivation. Also, Fig. 3 shows the changes in the gain of the tachycardic response to baroreflex deactivation (3C) and latency (3D) through sodium nitroprusside bolus injection, before and after systemic administration of atropine and/or metoprolol in LP and NP animals. Before autonomic blockade (control), the baroreflex gain in LP rats was not different from that observed in NP rats (−2.84±0.10 bpm/mmHg *vs.* −3.06±0.12 bpm/mmHg,

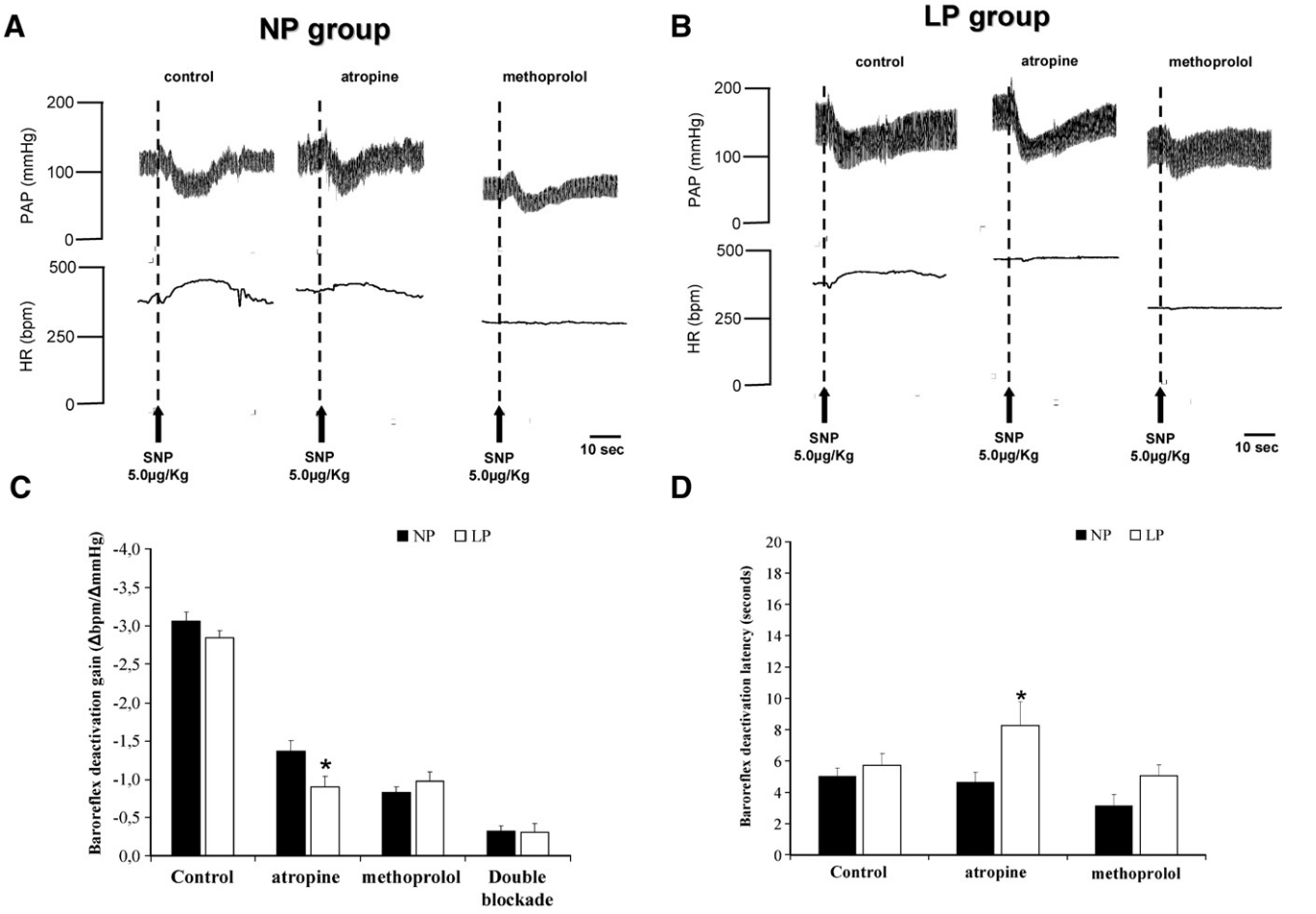

Fig. 3. Representative tracings of rats from the NP (A) and LP (B) groups, showing the changes in the baseline PAP (mmHg) and in the HR (bpm) in response to baroreflex deactivation. C—Baroreflex gain (tachycardic responses) in malnourished (LP—white bars) or control (NP—black bars) rats. Baroreflex tachycardic indexes were determined by ΔHR/ΔMAP ratio. The baroreflex deactivation was elicited by sodium nitroprusside i.v., in basal (control) condition (n=21), after cholinergic blockade (n=13), sympathetic blockade (n=7), or double blockade (n=5). (*=different from the control group; P<0.05). D—Baroreflex latency in response to baroreflex deactivation in NP (black bars) and LP (white bars) rats, in basal (control) condition (n=21), after cholinergic blockade (n=13) or sympathetic blockade (n=7).

respectively). On the other hand, atropine significantly reduced the baroreflex gain in LP rats as compared to that in NP rats, (−0.90±0.13 *vs.* −1.37±0.13 bpm/mmHg, respectively). No differences were found after β1-adrenergic (−0.98±0.56 *vs.* −0.83±0.33 bpm/mmHg, respectively) or double autonomic blockade (−0.31±0.41 *vs.* −0.33±0.17 bpm/mmHg, respectively). Similar results were obtained for the latency of the tachycardic response to baroreflex deactivation (Fig. 3D): In the control condition (before autonomic blockades), the latency of tachycardic baroreflex responses in LP rats was not different from that of the NP group (5.70±0.76 *vs.* 5.02±0.53 s, respectively) and no significant changes were found after metoprolol (5.08±3.35 *vs.* 3.14±3.57 s, respectively). After cholinergic antagonism, however, the latency of the baroreflex response seems to have increased in LP rats in comparison to that in NP rats (8.25±6.81 *vs.* 4.64±2.84 s, respectively).

Discussion

Reduced nutrient intake is a serious problem of public health in underdeveloped countries (de Onis et al., 2000). Malnourishment often produces marked impacts leading to multiple organ lesions, including cardiovascular disturbances (Fernandez-Repollet et al., 1987, 1989; Hawkins et al., 2000; Oliveira et al., 2004). It also produces disturbances in the baroreflex sensitivity (Tropia et al., 2001).

Reduced protein intake was used in this study and in others (Agarwal et al., 1981; Benabe et al., 1993a,b; Benabe and Martinez-Maldonado, 1993, 1998; Ferreira et al., 2003; Tropia et al., 2001) as a model of malnourishment. In this study, it was demonstrated that in the rats, subjected to protein restriction, the mean body weight loss was approximately one-third of the mean body weight of their respective controls. In the literature, the deficit in body weight was considered as an index of protein restriction and malnourishment in distinct experimental protocols (Kim et al., 1994; Oliveira et al., 2004; Zucoloto et al., 1975).

The present study comprised experiments regarding the autonomic modulation of the baroreflex chronotropic responses. Initially, the evaluation of the autonomic tonus to the basal HR in malnourished rats showed an increased basal sympathetic efferent activity and a reduced parasympathetic activity. The chronic sympathetic activation of the heart leads to the development and/or maintenance of heart failure status, thereby increasing the risk of sudden death both in rats and humans (Fletcher, 2001; Judy et al., 1976; Schultz et al., 2007; Schultz and Li, 2007). Moreover, several researchers related the development of cardiovascular dysfunction to malnourishment in individuals (Sawaya et al., 2003; Young et al., 1985), thereby underscoring the importance of studies concerning the genesis of autonomic imbalance in individuals subjected to chronic low protein diet.

Regarding baroreflex activation, it was observed that the increase in baroreflex gain, following baroreflex activation in the control period and after methyl-atropine systemic administration, was higher in LP rats than in NP rats. This suggests an increase in the sympathoinhibition in LP animals, which is probably related to an increased basal sympathetic activity. The baroreflex activation after metoprolol i.v. injection showed a decreased baroreflex gain, suggesting a decreased parasympathoexcitation. Moreover, the latency to baroreflex activation appears to have increased in LP animals, especially after cholinergic blockade, pointing to an impaired sympathoinhibitory pathway. The gain and latency related to baroreflex deactivation decreased in LP animals only after cholinergic blockade, suggesting an impaired modulation of the sympathetic activity. Taken together, the data suggest that protein restriction disrupts cardiovascular homeostasis. These alterations in the modulation of the efferent autonomic activity might be responsible for the increased levels of baseline MAP and HR in LP rats. These observations are in tune with the findings of earlier workers on rats (Kim et al., 1994; Langley-Evans et al., 1996a,b, 2003; Tropia et al., 2001; Young et al., 1985; Zucoloto et al., 1975) and in humans (Cruz Angeles and Ortiz-Hernandez, 2006; Fernandes et al., 2003; Florencio et al., 2004; Franco et al., 2006; Sawaya et al., 2003, 2005; Sesso et al., 2004; Tropia et al., 2001).

Several possible mechanisms have been proposed to account for these observations. Highlighting a few important possibilities of some of these mechanisms is worthwhile. Malnourishment during the critical period of development can lead to autonomic imbalance through morphological changes in several areas of the central nervous system (CNS) involved in the generation and/or modulation of the sympathetic activity, such as hypothalamus (Plagemann et al., 2000), or through changes in the neurotransmitters content and/or release into the CNS (Agarwal et al., 1981; Kim et al., 1994). Agarwal et al. (1981) observed disturbances in cerebral glutamate metabolism in malnutrition state. As glutamate seems to be the main neurotransmitter in most cases of synapses of the central baroreflex pathway (Talman et al., 1980), the changes in baroreflex parameters could possibly be ascribed to glutamate metabolism disturbance associated with the malnutrition state. Moreover, malnourishment induces direct and/or indirect changes in neurohumoral systems that affect blood pressure regulation (Bell and Slotkin, 1988; Benabe et al., 1993a,b; Benabe and Martinez-Maldonado, 1993; Fernandez-Repollet et al., 1987; Ferreira et al., 2003; Hawkins et al., 2000; Langley-Evans et al., 1996a; Mi et al., 2000). Another important mechanism is related to the production of insulin growth factors (IGF): IGFs are produced under growth hormone (GH) stimulation of the hepatic cells (Conchillo et al., 2007) and are found to be vasodilator via NO production (Begum et al., 1998; Cruzado et al., 2005; Nguyen et al., 2006; Vickers et al., 2001). In malnourished animals, the production of GH may be impaired because of high levels of corticosterone (Langley-Evans et al., 1996a). Therefore, the lack of IGFs in malnourished animals (Lauterio and Scanes, 1987) could lead to high blood pressure levels. It was also mentioned that malnourishment affects kidney function and hydro-electrolyte homeostasis (Benabe et al., 1993a,b; Benabe and Martinez-Maldonado, 1998; Fernandez-Repollet et al., 1987, 1989; Langley-Evans et al., 1996b, 2003). Taken together, these evidences suggest that malnourishment affects several systems in parallel. On the other hand, how several changes observed in malnourished animals have an impact on their pathophysiology remains to be elucidated. The knowledge

concerning these pathophysiological mechanisms could provide crucial information for improving the treatment of homeostatic disturbances in people subjected to low protein intake. It also helps the governments of underdeveloped countries in adopting suitable policies for public health management. Moreover, this kind of study could be helpful in unraveling the cross-talk between the cardiovascular and the energetic homeostasis, and in improving the knowledge regarding autonomic modulation of the cardiovascular system.

In summary, the study showed that malnourished male Fischer rats present autonomic disturbances (e.g. increased sympathetic and decreased parasympathetic outflows) directed to their heart and blood vessels, and this accounts for the cardiovascular disturbances observed in them.

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