Autonomic Neuroscience: Basic and Clinical xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Progressive cardiovascular autonomic dysfunction in rats with evolving metabolic syndrome

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ARTICLE INFO

Article history:
Received 13 September 2012
Received in revised form 22 January 2013
Accepted 17 February 2013
Available online xxxx

Keywords:
Autonomic nervous system
Cardiovascular system
Metabolic syndrome X
Insulin resistance

ABSTRACT

Metabolic syndrome is linked to increased cardiovascular mortality, which may be partially attributed to cardiac sympatho-vagal imbalance. However, autonomic changes were not evaluated during the metabolic syndrome development in a monosodium glutamate-induced animal model. We evaluate temporal changes in cardiovascular autonomic modulation in an animal model of metabolic syndrome. Eighteen neonate male spontaneously hypertensive rats (SHR) were treated with monosodium glutamate (MetS), and compared with Wistar–Kyoto (C) and saline-treated SHR (H). Lee index, insulin resistance and autonomic control (spectral analysis) were evaluated at 3 (3-mo), 6 (6-mo) and 9 (9-mo) months of age (compared by two-way ANOVA, $p<0.05$). Weight of visceral fat, Lee index and arterial pressure were higher in the MetS *vs.* C and H groups ($p<0.001$) at all ages. Heart rate variability (HRV) was decreased in the MetS and H groups at 3-mo and 9-mo *vs.* C. The LF component of HRV was reduced in the MetS group at 3-mo *vs.* C ($p=0.032$), and higher *vs.* C and H at 9-mo ($p<0.001$, all comparisons). H and MetS rats had a higher LF/HF index *vs.* C at 9-mo ($p=0.001$, all comparisons). The VLF component of systolic arterial pressure variability of the MetS was higher earlier (6-mo) than that of the H group. A reduction of 70%, 98% and 54% in α_{LF} index of H and MetS rats *vs.* C, was observed at 3, 6 and 9 months, respectively. Metabolic syndrome and hypertension in rats evolve with progressive autonomic dysfunction (worst at 9 months), with specific derangements occurring very early.

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1. Introduction

Autonomic dysfunction assessed by heart rate (Buccelletti et al., 2009) and arterial pressure (Shintani et al., 2007) variability is associated with high risk of incident cardiovascular events and death. Similarly, metabolic syndrome (Lakka et al., 2002) and insulin resistance (Zethelius et al., 2005) were linked to increased cardiovascular mortality. Whether the components of the metabolic syndrome (hypertension, diabetes, obesity, dyslipidemia) individually add to this risk, or a common causal factor for all abnormalities (insulin resistance, inflammation) is the main determinant for this high risk is yet unknown.

Acute infusions of insulin are associated with sympathetic neural activation, as was reported evaluating norepinephrine levels and muscle sympathetic nerve activity in healthy humans (Rowe et al., 1981). Healthy subjects with hyperinsulinemia present lower heart rate variability as compared to those with normal insulin levels, but these abnormalities were not maintained in long-term follow-up (Schroeder et al., 2005). Using spectral analysis, Quilliot et al. (2001) observed the association between changes in sympathetic nervous system modulation and insulin resistance (Quilliot et al., 2001) and one study in patients with metabolic syndrome reported an inverse association between insulin resistance and baroreflex sensitivity (Lindgren et al., 2006). However, the time-course of these changes was not followed over time.

Animal models allow multiple and detailed evaluations of many diseases under standardized conditions, and thus could help in determining how cardiovascular autonomic changes take place in metabolic syndrome as well. Fructose-fed rats, which are hypertensive and insulin-resistant, have vagal tonus reduction with no sympathetic tonus modification (Brito et al., 2008; Silva et al., 2011). However, these cardiovascular autonomic changes were not similar to those observed in humans and were not evaluated over the metabolic syndrome development in this model. As recently shown in our laboratory, spontaneously-hypertensive rats (SHR) with obesity induced by monosodium glutamate (MSG) present many characteristics (obesity, hyperglycemia, insulin resistance, hypertriglyceridemia, reduced HDL cholesterol and hypertension) of the metabolic syndrome (Leguisamo et al., 2012), as others showed (Grisk et al., 2007; Miesel et al., 2010; Sampey et al., 2011) and as it occurs in humans, thus they would be a useful animal model to evaluate autonomic changes in this condition over time.

The aim of this study is to evaluate the temporal changes in cardiovascular autonomic modulation in an animal model of metabolic syndrome (SHR with obesity induced by MSG).

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1566-0702/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.autneu.2013.02.011

Please cite this article as: Lehnen, A.M., et al., Progressive cardiovascular autonomic dysfunction in rats with evolving metabolic syndrome, Auton. Neurosci. (2013), http://dx.doi.org/10.1016/j.autneu.2013.02.011

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2. Materials and methods

All animal experimentation procedures followed the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. The study was approved by the Research Ethics Committee of Instituto de Cardiologia do RS, protocol #4005/0. All animals were bred and kept under standard laboratory animal house conditions at the Animal Production and Research Unit of the Center for Scientific and Technological Development of Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul, Brazil. The animals received standard rat chow and water *ad libitum*, and were maintained under controlled 12-h light/12-h dark cycle (6 AM/6 PM) and 20–25 °C temperature conditions. Details of the experiment timeline are shown in Fig. 1.

2.1. Animal model

Eighteen neonate male SHR submitted to the subcutaneous administration of MSG (Sigma®) diluted in saline solution (NaCl, 0.9%), 5 mg/g/day, for 9 days comprised the MetS group (n = 6 for each evaluated period), starting at day one of life. We also evaluated 18 SHR (H group, n = 6 for each evaluated period) and 18 Wistar–Kyoto rats (C group, n = 6 for each evaluated period) treated with saline solution subcutaneously for the same period. At 21 days of life, the animals were weaned and placed in plastic boxes, at most 4 animals.

2.2. General characteristics evaluation

The animals were weighed and their naso-anal length was measured in dorsal decubitus on the day they were killed. The Lee index (cube root of the body weight by the nasoanal length) was also evaluated (Bernardis and Patterson, 1968).

2.3. Insulin tolerance test

The insulin tolerance test was performed as previously described (Mori et al., 2008) using commercial human insulin (Humulin, Eli Lilly, São Paulo, Brazil). After being deprived of food for 3 h, the animals were anesthetized (ketamine: 160 mg/kg body weight and xylazine: 10 mg/kg body weight). An insulin solution (0.75U/1000 g) was injected *via* the penile vein. A blood sample was collected from a caudal puncture to measure glycemia (reagent strips, Accu-check Advantage, Roche, Mannheim, Germany) before injecting the insulin and 4, 8, 12, 16 and 20 min after. These values were transformed into a neperian logarithm. A linear regression was performed from the graph was performed and the angular content of the straight line equation was analyzed. This, multiplied by 100, conferred on the test the final value in $\%\cdot min^{-1}$, which denoted the decay rate of glycemia after insulin infusion per minute.

2.4. Blood pressure recording

The animals were anesthetized (ketamine: 160 mg/kg body weight and xylazine: 10 mg/kg body weight) to place a polyethylene catheter (PE-10) inside the femoral artery. The cannula was filled with saline solution and positioned inside the abdominal aorta, through the left femoral artery, to record arterial pressure. After 24 h, the arterial cannula was connected to a pressure transducer, linked to the CODAS analog-digital board (2-kHz sample rate, Dataq instruments, Inc., Akron, OH). The arterial pressure data acquired were recorded for 20 min, in the conscious animal. The experimental protocol was started only after signal stabilization. The stabilization time was determined according to the conditions of the animal's attention. The protocol was initiated when the BP and HR signals were constant and the animal was definitively quiet and moving freely. The time between the system installation and the beginning of the records ranged from 10 to 30 min. For each pulse wave the same program calculated values for peak (systole), valley (diastole) and pulse interval (between one peak and the next).

2.5. Assessment of autonomic control — spectral analysis

Pulse intervals (PI, tachograms) and systolic arterial pressure (SAP, systograms) series were obtained from blood pressure records. Stationary segments (300 beats), coincident in tachogram and systogram, were selected and spectral analysis was performed using an autoregressive model which estimates the center frequency and power of each relevant oscillatory component. The spectral bands for rats (very low frequency, VLF: 0.0–0.2 Hz, low frequency, LF: 0.2–0.75 Hz, high frequency, HF: 0.75–3.0 Hz) were defined according to previous references (Malliani et al., 1991). Tachogram and systogram spectra were evaluated quantitatively and values of heart rate variability (HRV) and SAP variability (SAPV) were obtained. The spectral components were expressed in both absolute (ms^2 and $mm\ Hg^2$, respectively) and normalized units (NU). These NU were obtained by calculating the power of LF and HF and correlating them to the total power without the very low frequency component (Montano et al., 2009).

Among the parameters obtained by spectral analysis, the LF and HF components are distinguished for their physiological significance. They are mainly related to sympathetic and parasympathetic cardiac modulations, respectively, their relationship with each other – the LF/HF index – or sympathetic–vagal balance (Montano et al., 2009), and the absolute powers of the LF and VLF components of SAPV, related predominantly to vascular sympathetic modulation and to renin–angiotensin system modulation on SAP (Stauss, 2007). Moreover, a relationship expressed by the root of the ratio between the absolute powers of LF components of HRV and VPAS – the α_{LF} index – is known to express spontaneous baroreflex sensitivity (Fazan et al., 2005).

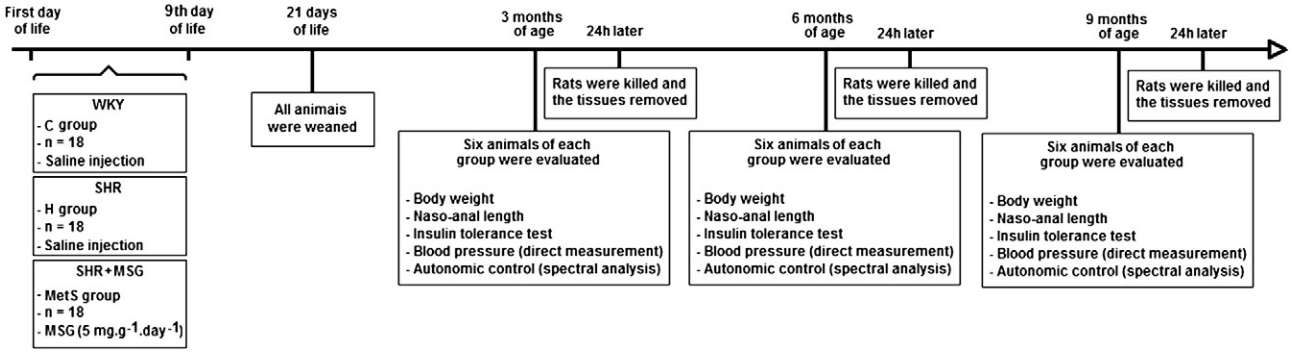

Fig. 1. Experimental design. C: WKY (Wistar–Kyoto rats) that received saline solution during the neonatal period; H: SHR (spontaneously hypertensive rats) that received saline solution during the neonatal period; MetS: spontaneously hypertensive rats that received MSG (monosodium glutamate) during the neonatal period.

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2.6. Statistical analysis

The results are presented as mean ± standard deviation and compared by two-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test. The homogeneity test (Levene's Test of Equality of Error Variances) was used and the level of significance was 5% for all tests performed. All analyses were performed using the SPSS (version 17.0).

3. Results

General characteristics of the animals are shown in Fig. 2. Rats from the MetS groups weighed less than C and H at 6 and 9 months of age ($p<0.001$ for both comparisons). However, the weight of epididymal white fat was greater in the MetS group *vs.* C and H ($p<0.001$) at all ages studied. When compared temporally within the same group, the body weight of C and H groups, but not of the MetS group, increased at 9 months of age as compared to 3-month ones (Fig. 2A). Likewise, the weight of epididymal white fat increased at 9 months of age as compared to 3-month ones in all groups studied (Fig. 2B). The Lee index of MetS animals was higher when compared to the C group at 3 months ($p<0.001$), 6 months ($p=0.001$) and 9 months of age ($p=0.012$), revealing the obesity of MetS rats — Fig. 2C.

Animals from the MetS group had lower glucose decay constants in the insulin tolerance test as compared to C at 3 (C: 3.92 ± 0.19 *vs.* MetS: 2.79 ± 0.18%·min^{-1}, $p=0.038$; ~29%), 6 (C: 4.18 ± 0.18 *vs.* MetS: 2.08 ± 0.20%·min^{-1}, $p=0.003$; ~50%) and 9 months (C: 4.20 ± 0.19 *vs.* MetS: 2.26 ± 0.18%·min^{-1}, $p=0.021$; ~46%). The same was observed when comparing the MetS group with the H group at 3 (H: 4.10 ± 0.19 *vs.* MetS: 2.79 ± 0.18%·min^{-1}, $p=0.029$; ~32%), 6 (H: 3.58 ± 0.18 *vs.* MetS: 2.08 ± 0.20%·min^{-1}, $p=0.042$; ~42%) and 9 months (H: 3.90 ± 0.19 *vs.* MetS: 2.26 ± 0.18%·min^{-1}, $p=0.036$; ~43%). Rats from the H group had lower glucose decay constants in the insulin tolerance test at 6 ($p=0.040$) and 9 months ($p=0.042$) of age as compared to rats of C groups. When compared temporally within the same group, animals from MetS groups at 6 and 9 months showed lower glucose decay constants in the insulin tolerance test than 3 month ones ($p=0.001$ and $p=0.020$, respectively).

3.1. Spectral analysis of heart rate variability

As shown in Fig. 3, the heart rate (HR) of MetS was ~33%, ~11% and ~14% higher, as compared to C group at 3 months ($p<0.001$), 6 months ($p=0.044$) and 9 months of age ($p=0.045$), respectively. When compared to the H group, the HR of animals from the MetS groups was similar at all ages. Heart rate variability was decreased in H and MetS groups at 3 months of age, as compared to their controls at the same age (C *vs.* H: ~57% and C *vs.* MetS: ~66%, respectively; $p<0.001$ for both comparisons). At 6 months of age, all groups had similar HRV, and at 9 months, H and MetS groups again showed lower HRV as compared to C rats (C *vs.* H: ~48%, $p=0.021$ and C *vs.* MetS: ~41%; $p=0.014$). No differences were observed at all ages between H and MetS groups.

The spectral analysis approach on HRV is shown in Table 1. The LF component, in absolute units (ms^2), was reduced in the MetS group at 3 months of age, as compared to C rats ($p=0.032$), with no difference observed between them at 6 months. However, the LF component of MetS rats at 9 months of age was higher as compared to both C and H groups at the same age ($p=0.005$ and $p=0.019$, respectively). The evaluation of the same variable in normalized units (nu), showed that the LF component was reduced in the MetS group at 3 months of age, as compared to C rats ($p<0.001$), and was higher in the MetS group as compared to the C group at both 6 and 9 months ($p=0.007$ and $p<0.001$, respectively). H and MetS groups showed lower HF component values than the C group, in both absolute (ms^2) and normalized (nu) units, regardless of age ($p<0.001$ for all comparisons). No change was observed between H and MetS groups.

The H group showed a lower HF frequency peak than the MetS and C groups, at 6 months ($p=0.012$ and $p=0.026$, respectively) — Table 1. However, at 9 months, no change was observed between H and C groups, but the HF peak of the MetS group was lower than the C group ($p=0.041$).

No differences were observed among the groups regarding the LF/HF index at 3 months of age (Fig. 3). At 6 months, only H animals showed a higher LF/HF index as compared to the C group of the same age (C *vs.* H; $p=0.025$). At 9 months of age, both H and MetS rats had a higher LF/HF index than C rats (C *vs.* H, $p=0.001$ and C *vs.* MetS; $p<0.001$), which represents a sympathetic predominance in the autonomic cardiovascular modulation of these groups.

3.2. Spectral analysis of systolic arterial pressure variability

Fig. 4 shows the SAPV parameters. The SAP was similarly higher in H and MetS groups, as compared to the C group at 3 months of age ($p<0.001$). At 6 months of age SAP was lower in the animals from MetS group as compared to H rats ($p<0.001$) and similar to the control group ($p=0.742$). However, at 9 months of age, the SAP of MetS rats returned to the same levels observed in the H group and both levels were higher than those presented by the C group ($p<0.001$). No changes were observed in the SAPV evaluated in absolute units (ms^2) at 3 months of age. However, at 6 and 9 months, H and MetS groups showed 6 to 8-fold increments in this parameter, as compared to the C group. Interestingly, at 6 months, MetS rats had increased SAPV as compared to H animals (~56%; $p=0.011$).

Spectral analysis of SAP is shown in Table 1. The VLF component of SAP was higher in the H and MetS groups at 6 and 9 months, as compared to their controls. The MetS group had an increased VLF component of ~145% as compared to the H group ($p<0.001$); MetS and H groups showed the higher LF component of SAPV in absolute units (mm Hg^2) than C animals ($p<0.001$), regardless of the age of

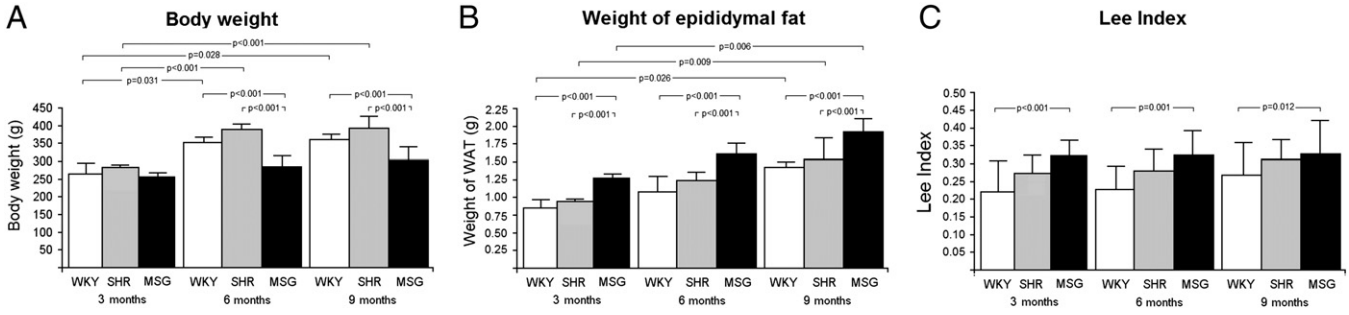

Fig. 2. General characteristics of the animals studied. Panel A: Body weight. Panel B: Weight of epididymal fat. Panel C: Lee index. C: WKY (Wistar–Kyoto rats) that received saline solution during the neonatal period; H: SHR (spontaneously hypertensive rats) that received saline solution during the neonatal period; MetS: spontaneously hypertensive rats that received MSG (monosodium glutamate) during the neonatal period. WAT: white adipose tissue. Two-way ANOVA followed by Bonferroni's post hoc test.

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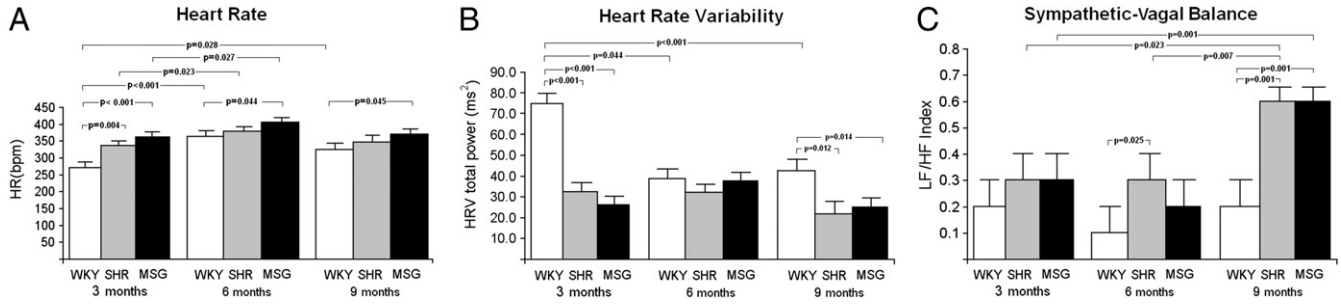

Fig. 3. Heart rate variability. Panel A: heart rate. Panel B: heart rate variability. Panel C: sympathetic–vagal balance. C: Wistar–Kyoto rats that did not receive any treatment; H: spontaneously hypertensive rats that did not receive any treatment; MetS: spontaneously hypertensive rats that received MSG during the neonatal period. HR: heart rate; HRV: heart rate variability; LF: low frequency; HF: high frequency. Two-way ANOVA followed by Bonferroni's post hoc test.

the animals. The HF component of SAPV, in absolute units (mm Hg^2), was similarly raised in H and MetS rats, when compared to 6-month old C animals ($p<0.001$ for both comparisons).

The spontaneous baroreflex sensitivity (α_{LF} index), was similar between H and MetS groups at all ages and both were lower when compared to the C group ($p=0.025$) — Fig. 4. A reduction of 70%, 98% and 54% in the α_{LF} index of MetS rats, as compared to the C group, was observed at 3, 6 and 9 months of age, respectively.

4. Discussion

Our data provide a unique insight into the cardiovascular autonomic temporal changes in an animal model of metabolic syndrome – SHR with obesity induced by MSG – which presents many characteristics similar to those observed in humans.

The novel findings in this study are that changes in cardiovascular autonomic modulation in this animal model, evaluated up to 9 months, are similar to those observed in hypertensive rats. Although multiple components of the metabolic syndrome (insulin resistance, visceral obesity, dyslipidemia, and hypertension) were present in rats of the MetS group, as occurs in humans, the determinant of the cardiovascular autonomic modulation was high arterial pressure levels.

On the other hand, the finding that many characteristics similar to those observed in humans were seen in metabolic syndrome rats favors the use of this animal model (Leguisamo et al., 2012). Other metabolic syndrome animal models proposed in literature do not present sustained hypertension and activation of the renin–angiotensin and sympathetic nervous system (Grassi et al., 2009; Lambert et al., 2010). Ideally, besides the features of human obesity, the sequential cardiovascular changes that occur with weight gain should be observed. Zucker rats, for example, have decreased plasma renin levels (Alonso-Galicia et al., 1996), on the contrary of what is observed in humans (Engeli et al., 2005; Krug and Ehrhart-Bornstein, 2008; Licata et al., 1994). Diet-induced animal models of obesity present predominantly characteristics related to human obesity, and few of them also have high blood pressure levels (Dobrian et al., 2000). In particular, fructose-fed rats, which are hypertensive and insulin-resistant, have reduced vagal tonus, with no sympathetic tonus modification (Brito et al., 2008; Silva et al., 2011). The similarity of the present animal model to metabolic syndrome in humans represents successful translational research, allowing it to be used in future work.

Beyond that, in the present study, at 9 months the MetS group presented reduced HRV and spontaneous baroreflex sensibility, and increased cardiac sympatho-vagal balance, SAP and SAPV, all similar to the H group, indicating an impairment of cardiovascular nervous control. Some of these results were shown previously in humans, for example a decrease of vagal cardiac modulation, with or without an increase in sympathetic modulation (Gao et al., 1996; Quilliot et al., 2001). Sympathetic activation plays an important role in the pathogenesis of insulin resistance (Jamerson et al., 1993; Pollare et al.,

Table 1
Cardiovascular autonomic control analysis: heart rate variability (HRV) and systolic arterial pressure variability (SAPV).

	3 months			6 months			9 months			p (group)	p (time)	p (interaction)
	C (n=7)	H (n=7)	MetS (n=8)	C (n=7)	H (n=10)	MetS (n=9)	C (n=5)	H (n=5)	MetS (n=8)			
HRV												
LF peak (Hz)	0.6±0.04	0.5±0.04	0.6±0.05	0.6±0.04	0.6±0.04	0.6±0.03	0.6±0.05	0.5±0.06	0.5±0.04	0.435	0.512	0.618
LF power (ms^2)	6.9±1.2	4.3±1.2	3.4±1.4*	1.9±1.2	4.5±1.0	3.6±1.0	3.7±1.4	2.2±1.6	6.0±1.1*,†	0.765	0.239	0.019
LF power (nu)	18.5±3.3	21.5±3.3	19.9±4.0	8.1±3.3	20.9±2.8*	16.1±4.4*	14.8±3.1	32.2±2.9*	34.9±3.1*	<0.001	<0.001	0.048
HF peak (Hz)	1.5±0.1	1.9±0.1	1.8±0.1	1.8±0.1	1.4±0.1*	1.7±0.1†	1.8±0.1	1.7±0.1	1.5±0.1*	0.956	0.157	<0.001
HF power (ms^2)	31.0±3.9	13.4±3.9	12.2±3.7	22.5±3.9	16.0±3.3	18.0±2.5	20.0±3.7	5.1±3.5	10.1±3.7	<0.001	0.063	0.366
HF power (nu)	81.5±3.7	77.8±3.7	78.9±4.3	89.9±3.7	77.5±3.1	74.3±4.9	84.5±3.4	67.9±3.2	61.7±3.4	<0.001	0.009	0.099
SAPV												
VLF power ($mm\ Hg^2$)	5.9±2.6	6.6±6.6	3.5±1.8	2.4±6.6	14.7±5.5*	36.1±5.8*,†	3.2±1.2	38.8±8.7*	31.4±6.2*	<0.001	0.021	0.005
LF peak (Hz)	0.6±0.03	0.5±0.03	0.4±0.03*	0.4±0.03	0.3±0.03	0.5±0.03†	0.4±0.04	0.4±0.04	0.5±0.03	0.024	0.236	0.004
LF power ($mm\ Hg^2$)	2.0±0.8	9.9±0.8	6.3±0.6	1.3±0.8	13.4±2.3	14.7±3.7	2.5 ±0.6	14.0±2.5	17.4±2.6	<0.001	0.076	0.351
HF peak (Hz)	1.5±0.1	1.5±0.1	1.6±0.1	1.6±0.1	1.3±0.1*	1.3±0.1*	1.5±0.1	1.6±0.1	1.4±0.1	0.502	0.216	0.008
HF power ($mm\ Hg^2$)	2.9±1.2	2.8±1.2	4.9±2.0	2.6±1.2	14.3±1.8*	15.2±1.9*	3.1±1.0	3.4±0.9	7.6±2.0	0.001	<0.001	0.024

C: Wistar–Kyoto rats that did not receive any treatment; H: spontaneously hypertensive rats that did not receive any treatment; MetS: spontaneously hypertensive rats that received MSG during the neonatal period. n = indicates the number of animals. LF: low frequency; HF: high frequency; VLF: very low frequency. The differences showed are within the same time. Two-way ANOVA followed by Bonferroni's post hoc test.

* $p<0.05$ *vs.* C.

† $p<0.05$ *vs.* H.

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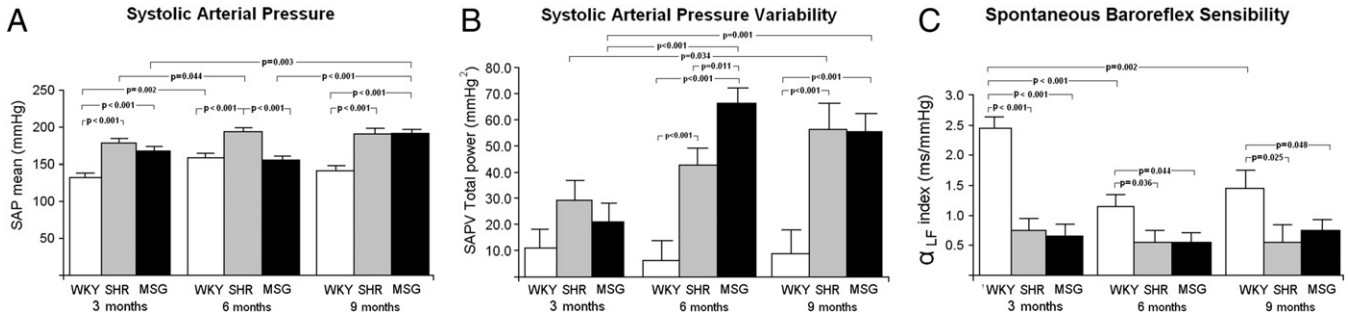

Fig. 4. Systolic arterial pressure variability. Panel A: systolic arterial pressure. Panel B: systolic arterial pressure variability. Panel C: spontaneous baroreflex sensibility. C: Wistar–Kyoto rats that did not receive any treatment; H: spontaneously hypertensive rats that did not receive any treatment; MetS: spontaneously hypertensive rats that received MSG during the neonatal period. SAP: systolic arterial pressure; SAPV: systolic arterial pressure variability; LF: low frequency. Two-way ANOVA followed by Bonferroni's post hoc test.

1988; Snitker et al., 2000), hypertension, and activation of the renin–angiotensin system (Sowers et al., 1982). This was also confirmed in our study, since rats from MetS group became more insulin-resistant and have more visceral fat (epididymal) as compared to their controls, accompanied by increased activation of the sympathetic system. In female Wistar rats treated with oral fructose, insulin resistance, increased systolic and diastolic, as well as sympathetic hyperactivity were previously shown (Silva et al., 2011). These characteristics were also found in the MetS group at 6 and 9 months of age, in our study.

Interestingly, H and MetS rats have similar cardiovascular derangements, although those with metabolic syndrome were also obese. However, two important cardiovascular parameters, obtained by spectral analysis, were different between H and MetS: the VLF component of SAPV of the MetS group was higher than that of the H group, and the HF peak in the H group was lower than that of the MetS group. In rats, the VLF band is related to the renin–angiotensin system modulation of blood pressure (Stauss, 2007). In obese humans, circulating angiotensinogen, renin, aldosterone, and angiotensin-converting enzyme activity have been reported to be elevated, and down-regulated during weight loss (Engeli et al., 2005; Krug and Ehrhart-Bornstein, 2008; Licata et al., 1994). The reduced HF peak value represents a higher respiratory frequency in H than in C and MetS groups, which is first described here.

It was previously shown in other metabolic syndrome animal models that the blood pressure changes are mediated by the activation of the sympathetic nervous system (Farah et al., 2006), impairment of the cardiac parasympathetic tonus (Brito et al., 2008) and dysfunction in the renin–angiotensin system (Farah et al., 2006). In humans with type 2 diabetes there is a positive association between cardiovascular autonomic dysfunction (parasympathetic, sympathetic, and baroreceptor functions) and metabolic syndrome characteristics (Garruti et al., 2012). In particular, it was shown that type 2 diabetes plus metabolic syndrome is more strongly associated with cardiovascular autonomic dysfunction than isolated type 2 diabetes, highlighting the association of metabolic disorders with cardiovascular dysfunction. Taken together, these changes are related to the results here presented, as the animal model showed insulin resistance, obesity, hypertension, accompanied by high SAPV and low spontaneous baroreflex sensibility, as well as increased activation of the sympathetic system. Furthermore, a possible relationship between obesity of MSG-induced rats and sympatho-vagal activity may be derived from a low metabolic rate (Djazayery et al., 1979), related to decreased thermogenesis (Duloo and Miller, 1984), which may be attributed to cardiac sympatho-vagal imbalance, also described here in the rats from MetS group.

In summary, autonomic dysfunction changed over time, as we observed 1. increased sympathetic–vagal imbalance in MetS group at 9 months in relation to 3 and 6 months and C group; 2. higher systolic arterial pressure in MetS group at 9 months *vs.* 3 and 6 months of age, and 3. changes in systolic arterial pressure variability. The autonomic dysfunction accompanied increased visceral fat and insulin resistance at 9 months of age as compared to 3 and 6 months of age. Thus, we think that, although 3 and 6-month derangements were similar, what was observed at 9 months characterizes a late temporal worsening of the components of autonomic control in the animal model studied. We provide information that shows that cardiovascular autonomic modulation dysfunction evolves over time in an MSG-induced animal model, in particular, sympathetic–vagal balance and systolic arterial pressure variability, associated with a higher amount of visceral fat and insulin resistance, as observed in humans. Since cardiovascular autonomic dysfunction is associated with high risk of incident cardiovascular events and death, these data support the use of this animal model in investigations into the pathophysiology of metabolic syndrome, especially those related to the cardiovascular system and its neural control.

5. Conflicts of interest

There are no conflicts of interest.

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Please cite this article as: Lehnen, A.M., et al., Progressive cardiovascular autonomic dysfunction in rats with evolving metabolic syndrome, Auton. Neurosci. (2013), http://dx.doi.org/10.1016/j.autneu.2013.02.011

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Please cite this article as: Lehnen, A.M., et al., Progressive cardiovascular autonomic dysfunction in rats with evolving metabolic syndrome, Auton. Neurosci. (2013), http://dx.doi.org/10.1016/j.autneu.2013.02.011