

## Leptin and Aldosterone in Sympathetic Activity in Resistant Hypertension with or without Type 2 Diabetes

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### Abstract

**Background:** The finding of adipocyte-derived hormone leptin as an overstimulator of sympathetic activity brought a new perspective to the pathophysiological mechanisms of obesity-hypertension.

**Objectives:** As aldosterone also increases sympathetic activity, we aimed to assess the relationship between sympathetic overactivity and plasma leptin and aldosterone levels in resistant hypertension (RHTN), comparing the groups with and without T2D.

**Methods:** Twenty-five RHTN patients underwent ambulatory electrocardiography to analyze heart rate variability (HRV) in time and frequency domains, which were stratified into two periods: 24 hours and daytime (DT), comprising the records between 2:00 p.m to 6:00 p.m (time domain) and one hour at 3:00 p.m (frequency domain).

**Results:** T2D group (n=10) had higher serum aldosterone and plasma leptin levels than the non-T2D (n=15) ( $26.0 \pm 11.5$  vs.  $16.9 \pm 7.0$  ng/dL –  $p=0.021$ ;  $81.368.7 \pm 47.086.1$  vs  $41.228.1 \pm 24.523.1$  pg/mL –  $p=0.048$ , respectively). Both groups had aldosterone correlated with HRV in frequency domain. Non-T2D had aldosterone correlated with DT low frequency in normalized units (LF nu) ( $r=0.6$  [0.12–0.85]  $p=0.018$ ) and DT high frequency in normalized units (HF nu) ( $r=-0.6$  [-0.85- -0.12]  $p=0.018$ ). Type-2-diabetes group had aldosterone correlated with DT LF nu ( $r=0.72$  [0.16–0.93]  $p=0.019$ ) and DT HF nu ( $r=-0.72$  [-0.93- -0.16]  $p=0.019$ ). However, despite of the importance of leptin in sympathetic overactivity in hypertension, leptin did not correlate with HRV.

**Conclusion:** Aldosterone seems to overdrive sympathetic activity in RHTN with and without T2D. This information combined with the clinical efficacy of mineralocorticoid receptor blocker in RHTN may reinforce that aldosterone is a major player to be a therapeutic target in RHTN. (Arq Bras Cardiol 2012;99(1):642-648)

**Keywords:** Hypertension; leptin; aldosterone; sympathetic nervous system; diabetes mellitus, type 2.

### Introduction

Hypertension, diabetes and obesity comprise a series of interactive physiologic disorders<sup>1</sup>. For instance, it is well known that obesity and diabetes mellitus are factors associated with resistance to antihypertensive drugs. Therefore, a better knowledge on the interactions among these pathophysiological pathways can aid treatment choice and thereby improving total cardiovascular risk management<sup>1</sup>.

The discovery of leptin brought new insights to the pathophysiological mechanisms of obesity and associated diseases<sup>2</sup>. Initial studies of leptin showed that it regulates appetite and enhances energy expenditure by activating sympathetic nerve activity to thermogenic brown adipose

tissue<sup>3</sup>. It was also demonstrated that leptin causes sympathetic excitation to the kidney that, in turn, increases arterial pressure<sup>3</sup>.

It is also well-known the high prevalence of hyperaldosteronism in resistant hypertension (RHTN)<sup>4</sup>. RHTN is defined as uncontrolled blood pressure (BP) despite the use of more than three medications or controlled BP, but that required four or more drugs to achieve blood pressure goals<sup>5</sup>. Moreover, primary aldosteronism patients showed significantly higher levels of leptin than essential hypertension (HTN) subjects<sup>6</sup>. However, a growing body of evidence suggests that aldosterone contributes broadly to the development and severity of HTN separately from the presence of classically defined primary aldosteronism<sup>7</sup>. In addition, aldosterone promotes RHTN by mediating maladaptive changes in the renal, cardiovascular and central nervous systems<sup>8</sup>. Sympathetic nervous system (SNS) activation seems to be a basic component of the adverse impact of aldosterone excess in the central nervous system<sup>9</sup>.

Heart rate variability (HRV) may be used to assess autonomic imbalances, diseases and mortality<sup>10</sup>. Measures of heart rate variability (HRV) in time and frequency domains

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have been used successfully to index sympathetic and vagal activity<sup>11</sup>. Nevertheless, while there are some differences among HRV parameters found in many studies, the consensus is that lower values of these indices of vagal function are prospectively associated with death and disability<sup>12</sup>.

Considering the significant influences that leptin and aldosterone exerts on the pathophysiology of RHTN<sup>9,13</sup>, we aimed to access the relationship between sympathetic activity and these two hormones in RHTN with and without type 2 diabetes (T2D).

## Methods

We included twenty-five (25) RHTN subjects<sup>14</sup>, 15 non-T2D and 10 T2D, regularly followed in the Ambulatory Service of Cardiovascular Clinical Pharmacology, complying with pharmacological prescription for HTN and T2D (taking 80% to 120% of the prescribed daily dose).

An accurate office blood pressure measurement technique and ambulatory blood pressure monitoring (ABPM) were applied to diagnosis resistant hypertension<sup>5</sup>. We excluded cases of pseudoresistance, including lack of blood pressure control secondary to poor medication adherence<sup>15</sup>. White coat hypertension (WCH) was excluded by ABPM<sup>5</sup>. Resistant hypertension included patients whose blood pressure was uncontrolled with the use of more than three medications or patients whose blood pressure was controlled, but required four or more drugs to achieve blood pressure goals<sup>5</sup>. All subjects provided written informed consent, and the study was approved by the local ethics committee.

The exclusion criteria comprised: acute or moderate-severe renal dysfunction (creatinine clearance < 40 mL/min/1,73 m<sup>2</sup>), non-complied pharmacological prescription, use of beta-blockers within the last six months, severe obesity (body mass index  $\geq$  35 kg/m<sup>2</sup>), heart failure (ejection fraction < 50%), valvular heart disease, cardiomyopathies, primary hyperaldosteronism [aldosterone: PRA ratio > 20 ng per 100 mL per ng.mL(-1)h(-1)], atrial fibrillation, sick sinus syndrome, supraventricular and ventricular tachycardias, aortic disease (Marfan's syndrome, coarctation of the aorta, aneurysms or aortic surgery, history of coronary artery disease or proven coronary artery disease by coronary angiography or noninvasive tests, familial hyperlipidemia, asthma or chronic obstructive lung disease, pregnancy or oral contraceptive use, connective tissue disorders, neurological problems, oncological malignancies, psychiatric diseases, other than T2D endocrinological diseases, smoking, alcohol use and drug abuse.

### Blood pressure measurements

Blood pressure was assessed by considering the orientations of the VI Brazilian Guidelines on Hypertension<sup>16</sup>. The blood pressure (SBP – systolic blood pressure/ DBP – diastolic blood pressure) of each subject was measured three times, using a digital sphygmomanometer (Omron HEM-711DLX) on the right upper arm, in the sitting position, after a 10-minute rest. The average of two consecutive measurements was used, with a variation lower than 5 mmHg.

### Laboratory analysis

All subjects underwent the following laboratory tests: hemogram, fasting serum glucose, glycolized hemoglobin (HbA1c), urea and creatinine, total cholesterol, LDL-cholesterol fraction, HDL-cholesterol fraction, triglycerides, uric acid, sodium and potassium, aldosterone (collected during DT) and plasma leptin levels (collected during DT) using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (Quantikine® Human Leptin Immunoassay, Catalog Number DLP00, R&D Systems, Inc., Minneapolis, USA).

### Heart rate variability

Heart rate variability (HRV) parameters were derived from the recording of 24-hour Holter monitoring and analyzed in time and frequency domains. Measures were stratified into two time periods per time domain: 24-hour period (24h), daytime period (DT), comprising the records from 1:00 p.m. to 5:00 p.m. Frequency domain measures were obtained from one-hour records at 3:00 p.m. (daytime period – DT). A three-channel, 24-hour Holter recording was obtained from each subject, using the CardioLight digital 24-hour recorder device and the CardioSmart Institutional CS 550 software (Cardio Sistema Comércio e Indústria Ltda, São Paulo, SP, Brazil).

Time domain HRV parameters included the following measures<sup>10,17</sup>:

- rMSSD (ms): Square root of the mean squared differences between successive RR intervals.
- SDNN (ms): Standard deviation of all normal RR intervals in a 24-hour Holter recording.
- SDANN (ms): Standard deviation of mean RR interval in all 5-minute segments of a 24-hour recording.
- pNN50 (%): Percentage of differences between successive RR intervals greater than 50 ms.

Frequency domain measures were calculated using the fast Fourier transform (FFT) to break down the time series to its underlying periodic function. Frequency domain HRV parameters included the following measures<sup>10,17</sup>:

Low frequency (LF) and high frequency (HF) measured in normalized units, which represent the relative value of each power component in proportion to the total power minus the very low frequency (VLF) component. Normalized LF (LF nu) was calculated as LF power in normalized units LF/(total power-VLF)  $\times$  100, and normalized HF (HF nu) as HF power in normalized units HF/(total power-VLF)  $\times$  100. Low frequency (LF) and high frequency (HF), LF nu and HF nu denote the energy in the heart period power spectrum between 0.04 and 0.15 Hz (which is due to the joint action of the vagal and sympathetic components on the heart, with a predominance of the sympathetic ones) and 0.15 and 0.40 Hz (which corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart), respectively. "Daytime" was established at 3:00 p.m. in order to collect HRV data during wake.

The LF nu/HF nu ratio reflects the global sympatho-vagal balance and can be used as a measure of this balance. In a normal adult under resting conditions, the ratio is generally between 1 and 2.

### Statistical analysis

Data were expressed as mean ( $\mu$ ) and standard deviation (SD) or mean ( $\mu$ ) and standard error of the mean (SEM) for HRV measures according its correct use. Unpaired groups were compared using Mann-Whitney U test, while correlation analysis was performed using Spearman's rank test. Fisher's exact test was used to determine whether a certain group had significantly different proportion of a particular characteristic. The level of statistical significance accepted was less than 0.05. All data were entered into a spreadsheet program (MS Excel Microsoft Corp, Phoenix, Arizona, USA) for statistical analysis. Analytical statistics were performed by Analyse-it version 2.21 Excel 12+ (Analyse-it Software Ltd., Leeds, UK), a statistical add-in program for Excel (MS Excel Microsoft Corp, Phoenix, Arizona, USA).

### Results

We found no differences in age and gender between the non-T2D and T2D subgroups (table 1). Both groups demonstrated similar characteristics despite of laboratory analysis concerning T2D diagnosis like fasting and HbA1c levels (table 1). However, the T2D group showed a greater body mass index (BMI) and higher serum triglyceride values than the non-T2D group (table 1). Similarly, plasma leptin ( $81,368.7 \pm 47,086.1$  vs  $41,228.1 \pm 24,523.1$  pg/mL –  $p=0.048$ ) and serum aldosterone ( $26.0 \pm 11.5$  vs  $16.9 \pm 7.0$  ng/dL –  $p=0.021$ ) were increased in the T2D group compared with the non-T2D.

Regarding the anti-hypertensive drugs distribution, the T2D group was taking more antihypertensive drugs than the non-T2D group ( $4.1 \pm 0.7$  vs  $3.3 \pm 0.5$  –  $p=0.02$ ).

Concerning HRV parameters, the following evaluations were reduced in T2D when comparing with non-T2D: 24-hour-SDNN ( $89.1 \pm 19.9$  vs  $122.9 \pm 39.5$  ms;  $p=0.0009$ ), daytime SDNN ( $58.2 \pm 13.6$  vs  $78.5 \pm 24.9$  ms;  $p=0.03$ ), 24-hour-SDANN ( $79.8 \pm 17.1$  vs  $122.9 \pm 39.5$  ms;  $p=0.0012$ ), daytime SDANN ( $47.8 \pm 3.5$  vs  $65.5 \pm 6.5$  ms;  $p=0.03$ ), daytime rMSSD ( $13.8 \pm 1.9$  vs  $19.8 \pm 2.2$ ;  $p=0.05$ ), 24-hour-pNN50 ( $1.8 \pm 2.1$  vs  $5.3 \pm 6.4$  %;  $p=0.047$ ), daytime pNN50 ( $0.5 \pm 0.5$  vs

$2.6 \pm 2.9$  %;  $p=0.035$ ) (Figure 1). Although the remaining HRV parameters in time domain tended to lower values in T2D group, they did not achieve statistically significance. There were no differences between non-T2D and T2D groups in frequency domain parameters (data no shown).

Considering total patients (non-T2D and T2D groups), BMI correlated positively with aldosterone ( $r=0.47$  [0.09 – 0.73]  $p=0.018$ ; Figure 2a) and leptin ( $r=0.58$  [0.24 – 0.8]  $p=0.002$ ; Figure 2b). In addition, total patients had HRV in frequency domain correlated with serum aldosterone. Aldosterone also correlated with LF nu/HF nu ratio ( $r=0.60$  [0.28 – 0.81]  $p=0.001$ ), LF nu ( $r=0.60$  [0.28 – 0.81]  $p=0.001$ ; Figure 2c) and HF nu ( $r= -0.60$  [-0.81 – -0.28]  $p=0.001$ ; Figure 2d). However, we found no correlation with plasma leptin (daytime LF nu/HF nu:  $r=-0.09$  [-0.48 – 0.33]  $p=0.68$ ; daytime LF nu:  $r=0.01$  [-0.39 – 0.41]  $p=0.95$ ; daytime HF nu:  $r=-0.01$  [-0.41 – 0.39]  $p=0.95$ ).

Both groups (non-T2D and T2D) separately also had aldosterone correlated with HRV in frequency domain. Non-T2D had aldosterone correlated with daytime LF nu ( $r=0.60$  [0.12 – 0.85]  $p=0.018$ ; Figure 2e) and daytime HF nu ( $r=-0.60$  [-0.85 – -0.12]  $p=0.018$ ; Figure 2f). In addition, T2D group had aldosterone correlated with daytime LF nu ( $r=0.72$  [0.16 – 0.93]  $p=0.019$ ; Figure 2g) and daytime HF nu ( $r=-0.72$  [-0.93 – -0.16]  $p=0.019$ ; Figure 2h).

### Discussion

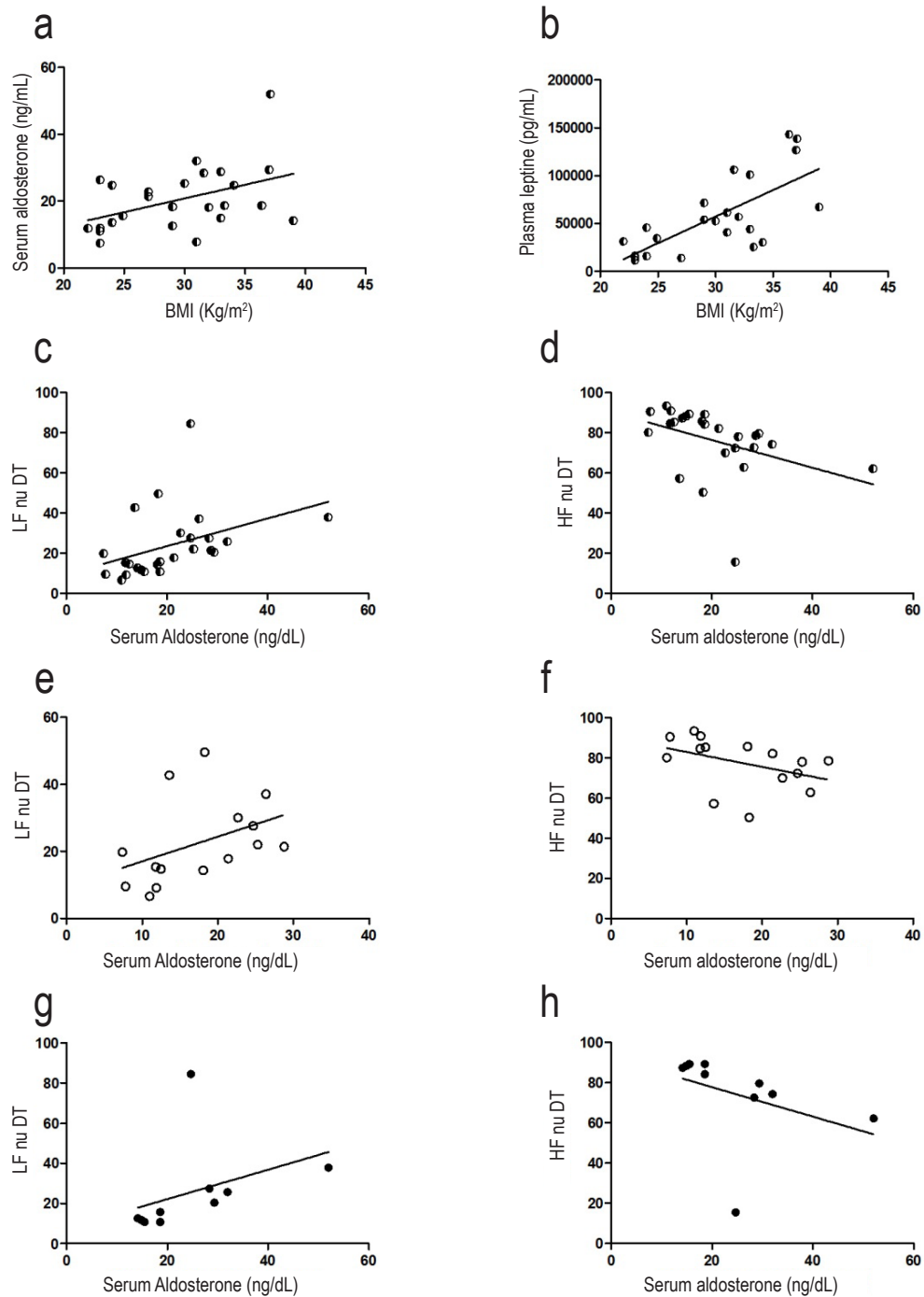
The main finding of our study was that serum aldosterone levels correlated positively with sympathetic activity in RHTN with and without T2D. Moreover, in spite of the importance of leptin in sympathetic overactivity in hypertension, we found no correlation between leptin and HRV.

We also found that the HRV is lower in T2D RHTN patients than in the non-T2D group. In addition, the group T2D showed higher correlations between aldosterone and HRV than the non-T2D. These results are in accordance with previous studies showing that aldosterone exerts a major influence on both sympathetic overactivity and disturbances of hepatic gluconeogenesis<sup>8,18-21</sup>.

**Table 1 - General characteristics of study groups**

Characteristic/ Variable	Non-T2D group (n=15)	T2D group (n=10)	p-value
Gender – male (%)	40	40	1.00
Age (year)	54.7 ± 10.0	54.9 ± 8.7	0.89
Body mass index (kg/m <sup>2</sup> ) *	26.6 ± 3.7	33.7 ± 4.0	<b>0.00</b>
Fasting glucose (mg/dL) *	92.9 ± 9.2	167.8 ± 64.0	<b>0.00</b>
HbA1c (%) *	5.8 ± 0.3	9.3 ± 2.1	<b>0.00</b>
Serum creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.1	0.93
Serum triglycerides (mg/dL) *	108.6 ± 48.7	254.8 ± 226.4	<b>0.04</b>
Office SBP (mmHg)	154.3 ± 21.6	156.5 ± 30.6	0.93
Office DBP (mmHg)	92.9 ± 10.8	90.1 ± 18.2	0.33

The values are expressed as means ± standard deviation; (\*) Statistical significance ( $p<0.05$ ); Non-T2D: non-type 2 diabetes resistant hypertension; T2D: type 2 diabetes resistant hypertension.



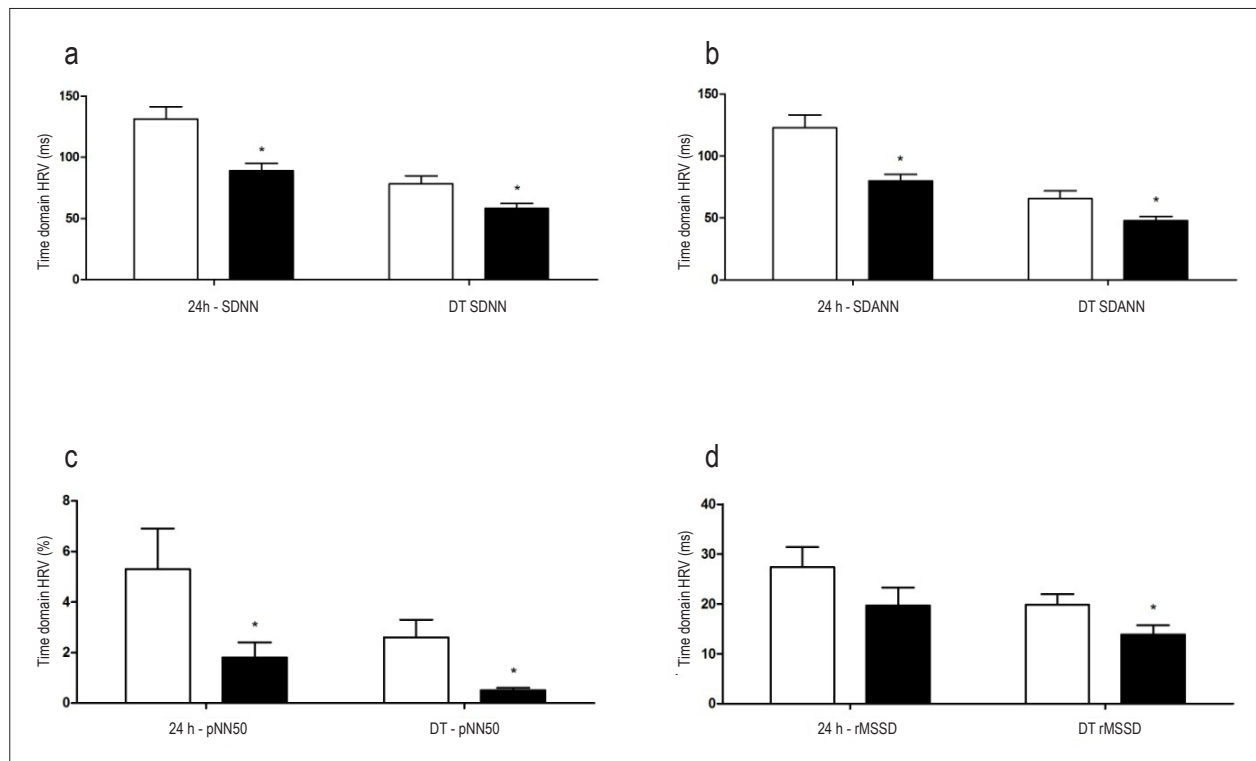
**Figure 1** – A and B: Correlations between BMI and the hormones aldosterone and leptin, respectively, in the all group of patients (black-white circle). C-H: Correlations between HRV (sympathetic and parasympathetic activities) and aldosterone in the all group of patients (black-white circle), non-T2D (white circle), and T2D (black circle). C: LF nu DT and aldosterone in all patients; D: HF nu DT and aldosterone in all patients; E: LF nu DT and aldosterone in non-T2D; F: HF nu DT and aldosterone in non-T2D; G: LF nu DT and aldosterone in T2D; H: HF nu DT and aldosterone in T2D. BMI: body mass index; HRV: heart rate variability; T2D: type 2 diabetes; LF: low frequency; DT: daytime; HF: high frequency.

The role of aldosterone in hypertension

Aldosterone mediates several maladaptive changes in the nervous and cardiovascular systems that promote hypertension in addition to cardiovascular disease (CVD) and chronic kidney disease (CKD)<sup>22</sup>. Elevated levels of aldosterone, in association with obesity and insulin resistance, promote nongenomic inflammation and oxidative stress pathways that advance the development of resistant hypertension through a number of mechanisms<sup>8</sup>. These actions potentiate the elevation of blood pressure that occurs from the classic effects of aldosterone to promote salt retention and volume expansion, causing severe hypertension resistant to treatment, unless a mineralocorticoid receptor (MR) antagonist such as spironolactone or epleronone is used as part of the therapeutic regimen<sup>8,23</sup>. Similarly to the heart, vasculature, pancreas, skeletal muscle and fat, high levels of circulating aldosterone can also overactivate local renin-angiotensin-aldosterone system in brain regions that contribute to increased sympathetic tone in hypertension<sup>8,20,21</sup>. Additionally, aldosterone also causes disturbances in hepatic insulin metabolic signaling, contributing, in part, to increased hepatic gluconeogenesis<sup>18,19</sup>. Our results are aligned with this information due the correlation between serum aldosterone and sympathetic and parasympathetic activity during daytime.

The role of leptin in hypertension

The evidence that increasing plasma leptin to levels similar to those found in obesity raises arterial pressure in non-obese rats is consistent with the hypothesis that leptin is an important link between obesity, sympathetic activity and hypertension<sup>24</sup>. Since obesity plays a major role in contributing to human essential hypertension, it is not surprising that plasma leptin concentrations are often elevated in hypertensive patients, or that leptin and blood pressure are correlated<sup>24</sup>. Illustrating this statement, it has been found that serum leptin levels were highly correlated with mean arterial pressure and BMI in male Japanese adolescents. Moreover, heart rate was also correlated with serum leptin even after adjustment for age and BMI<sup>25</sup>. It was also observed that systolic blood pressure correlated with plasma leptin after adjustment for BMI in hypertensive women and in non-hypertensive men, but not in hypertensive men<sup>26</sup>. Most of the data suggest that the correlation between leptin and blood pressure in hypertensive men is related mainly to the correlation between adiposity and blood pressure. However, not all studies have demonstrated a close relationship between leptin and hypertension. For example, leptin gene polymorphisms were not linked to hypertension in African Americans<sup>27</sup>.



**Figure 2 – Autonomic imbalance.** Heart rate variability (HRV) in time domain between non-type 2 diabetes (non-T2D) (white column) and T2D (black column) RHTN patients. A: SDNN (ms): Standard deviation of all normal RR intervals in 24-hour Holter recording; B: SDANN (ms): Standard deviation of mean RR interval in all 5-minute segments of 24-hour recording; C: rMSSD (ms): Square root of the mean squared differences between successive RR intervals; D: pNN50 (%): Percentage of differences between successive RR intervals that are greater than 50 ms; DT: Daytime (1 p.m. – 5 p.m.); 24h: 24 hours; (\*) Statistical significance (p < 0.05).



Regarding our findings, although leptin correlated with BMI, we have not found a correlation between leptin and sympathetic activity in this group of resistant hypertensive patients.

### Limitations of the study

Our main limitation was the recruitment of “true” RHTN patients and, subsequently, the small sample size. Despite of the small sample size, it was possible to identify lower HRV in the T2D subgroup. We assumed that the small sample size of the T2D group is a result of the prevalent exclusion criteria of moderate to severe renal dysfunction, history of coronary artery disease and the widespread  $\beta$ -blocker prescribing in this population in Brazil.

### Conclusion

Aldosterone correlated positively with sympathetic activity in RHTN. This information summed with the recent clinical trial evidences, may reinforce the effectiveness of MR blockers in resistant hypertension and points to aldosterone as one possible player in pharmacologic therapeutic.

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### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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