Effect of Salt Intake on Beat-to-Beat Blood Pressure Nonlinear Dynamics and Entropy in Salt Sensitive versus Protected Rats

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Abstract

Blood pressure exhibits substantial short and long term variability (**BPV**). We assessed the hypothesis that the complexity of beat-to-beat BPV will be differentially altered in salt sensitive (SS) versus protected from salt induced hypertension (SSBN13) Dahl rats maintained on high-salt versus low-salt diet. Beat-to-beat systolic and diastolic BP series from 9 SS and 6 SSBN13 rats (http://www.physionet.org) were analyzed following 9 weeks on low-salt and repeated after 2 weeks on high-salt. BP complexity was quantified by detrended fluctuation analysis (**DFA**) short- and long-range scaling exponents (α_S and α_L), sample entropy (**SampEn**) and traditional standard deviation (**SD**) and coefficient of variation (**CV(%)**). Mean systolic and diastolic BP increased on high-salt ($p<0.01$) particularly for SS rats. SD and CV(%) were similar across groups irrespective of diet. Salt sensitive and protected rats exhibited similar complexity indices on low-salt. On high-salt, 1) SS rats showed increased scaling exponents, or smoother, systolic $p=0.007$ (α_L)] and diastolic [$p=0.008$ (α_L)] BP series; 2) protected rats showed lower SampEn (less complex) systolic and diastolic BP ($p=0.046$); and 3) compared to protected SSBN13 rats, SS showed higher α_L for systolic ($p=0.01$) and diastolic ($p=0.005$) BP. Hypertensive SS rats are more susceptible to high salt with a greater rise in mean BP and reduced complexity. Comparable mean pressures in sensitive and protective rats when on low-salt diet coupled with similar BPV dynamics suggest a protective role of low salt intake in hypertensive rats. This effect likely reflects better coupling of biologic oscillators.

Keywords: Beat-to-beat variability, complexity, detrended fluctuation analysis

Introduction

Blood pressure (BP) exhibits substantial natural variability that ranges from beat-to-beat differences, within-the-day differences, and possibly across days or longer periods (26). BP variability (BPV) may be modified with processes such as aging, external stress, diet, as well as cardiovascular disease or hypertension. Multiple studies have shown that indices of BPV may independently predict organ damage and cardiovascular morbid and fatal events in certain patient groups (26). Relatedly, changes in BPV properties potentially have important consequences on treatment, yet these remain poorly understood.

Traditionally, the standard deviation (SD) and the coefficient of variation (CV) of the systolic, diastolic or mean BP time series have been used to assess BPV. However, other more sophisticated techniques such as complexity analysis (29) and detrended fluctuation analysis (DFA) (27) have been introduced for the analysis of beat-to-beat variability of physiologic data. These non-linear dynamical measures were shown to be effective in predicting organ damage and cardiovascular events in a manner complementary to the traditional measures and the mean BP (1, 16, 23). They also showed trends in the variability of biological signals that traditional variability measures failed to detect (41, 44).

Indices of signal complexity such as entropy measures (e.g. sample entropy (SampEn), approximate entropy (ApEn)) quantify the irregularity of biological signals; the more regular the signal, the lower the entropy value (29, 32). Alternatively, DFA quantifies the correlation

properties of the beat-to-beat time series via one or more scaling exponents (α) using the fractal property; more regular systems are characterized by higher values of α (13, 14). Healthy organisms are characterized by higher degrees of complexity (37) and therefore by higher entropies and lower scaling exponents. Complexity and DFA methods have been effectively used in analysis of heart and, to a lesser extent, respiratory rate variability (3, 8, 10, 17, 21, 42, 43, 45). Their use however in the analysis of blood pressure variability remains very limited.

This study aimed to explore the potential use of entropy (complexity) and fractal scaling exponents (correlation properties via DFA) for informative analysis of beat-to-beat blood pressure (BP) data from salt sensitive hypertensive Dahl rats (SS) and rats protected from salt induced hypertension (SSBN13). Towards this, we derived and compared the short and long term correlation properties (DFA scaling coefficients: α_S and α_L) and SampEn parameters under different conditions of hemodynamic stress modeled via exposure to high-salt versus low-salt diet. An implied hypothesis was that salt sensitive hypertensive rats will exhibit different non-linear BP dynamics compared to protected rats, irrespective of diet, as well as show a differential impact of salt intake on these metrics across the two strains of rats.

Materials and Methods

Animals and Experimental Protocol

We derived beat-to-beat BP series data from nine salt sensitive hypertensive (**SS**) Dahl rats and 6 rats protected from high salt induced hypertension (**SSBN13**) for whom raw sampled BP data epochs are available on Physionet (12). These data were previously analyzed to investigate the physiological origins of the baroreflex dysfunction in the SS rats (2). The original study was approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin (2). Briefly, the SSBN13 rats were derived by interbreeding two strains: the hypertensive SS rats and the normotensive Brown Norway rats (BN) as described previously (6). Rats were maintained on a low salt (0.4% salt) diet for nine weeks. After a one-week recovery period the diet was switched to high salt (8% salt) for two weeks. Blood Pressure was collected at each salt level using radiotelemetry for two minutes sampled at 100 Hz. Two-minute data epochs were available (12) for all rats at each salt level. We derived beat-to-beat systolic and

diastolic BP series for analysis using peak (valley) detection methods. The two-minute time series of the BP parameters varied between 600 and 900 beats in length (N).

Detrended Fluctuation Analysis (DFA)

DFA has been developed to detect the correlations of varying ranges (short- and long-range) embedded in a time series using the fractal property (27). First, the original time series of total length N is integrated, yielding

$$y(k) = \;\; = \;\; \sum_{i=1}^{k} \left(y(i) - y_m \right) \qquad (1)$$

where $y(i)$ is the ith beat-to-beat value and y_m is the mean value. The integrated time series is then divided into boxes of equal length n and $y(k)$ is detrended by removing the local trend, $y_n(k)$, consisting of the least square linear fit of each box. The root mean square fluctuation of the detrended time series F(n) is calculated over different box sizes (n), and hence providing a relationship between F(n) and n:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} \left(y(k) - y_n(k) \right)^2} \qquad (2)$$

The linear slope of the $\log_{10} F(n)$ to $\log_{10} n$ is the DFA scaling exponent α. As used previously (4 28, 47) DFA can be analyzed in two ways: a single slope α for the whole time series, and double slopes, one for short term correlations (α_S) and one for long term correlation (α_L) based on an *a priori* cutoff (n = 10).

For our SBP and DBP time series we used the double slope approach as it better reflected our data. To have a minimum of six windows averaged, we analyzed DFA up to a window size n = 100. The short-range slope α_S was computed for n = 4-10 and the long-range slope α_L for n=11-100. DFA scaling exponents α_S and α_L are measures of the smoothness of a time series, higher correlations indicate increased predictability or smoother series (27). α_S and α_L of SBP and DBP were computed for datasets of the two strains of rats on high and low-salt diets using a Matlab (MathWorks, Natick, MA, USA) code implemented in our laboratory.

Sample Entropy Analysis

Sample entropy (SampEn) is a measure of the self-similarity in a time series (32). It depends on two parameters: a dimension m which is the number of consecutive data points in a pattern and a tolerance r within which the m points are considered a self-match. SampEn is the negative natural logarithm of the conditional probability that sequences within r for the m consecutive data points remain within r for the next point. SampEn is related to ApEn (29), but unlike it, SampEn eliminates self-counting of matches and as a result minimizes the dependency of this complexity index on the length of the time series (32). Using SampEn will thus remove this bias introduced by ApEn. SampEn was calculated for $m = 2$ and $r = 0.2$. Low values of SampEn indicate loss of complexity and more predictability (more consistent with abnormal BP dynamics). SampEn of SBP and DBP was computed for all rats on both diets using a Matlab code available on Physionet (http://www.physionet.org/physiotools/sampen/c/).

Statistical analyses

In addition to scaling exponents (α_S, α_L) and SampEn, we derived the standard deviation (SD) and coefficient of variation (CV %) for SBP and DBP time-series. Continuous variables are presented as means ± standard error (SE) and were compared across groups and salt diets using the independent samples t test (between group) or the paired t test (within group) in case of normally distributed data. Alternatively, in case of non-normal data, the non-parametric Mann Whitney U test or the sign rank test were used. P-values < 0.05 were considered significant. Statistical analyses were performed using SigmaPlot version 11.2 for Windows.

Results

Beat-to-beat systolic (SBP) and diastolic (DBP) time series for example rats belonging to the two groups on both diets are shown in Figure 1. On low salt diet, hypertensive salt sensitive rats exhibited higher mean systolic pressure compared to the protected rats (137±2 vs 125±2, p = 0.006) while the mean diastolic pressures were similar (Table 1). As expected, mean systolic and diastolic blood pressure increased significantly with high salt but more so in the hypertensive salt sensitive rats; SBP (salt sensitive versus protected): $\Delta_{H-L} = 57\pm5$ v $\Delta_{H-L} = 30\pm2$, p = 0.002 ; DBP

(salt sensitive versus protected): $\Delta_{H-L} = 51\pm6$ v $\Delta_{H-L} = 26\pm3$, p = 0.003 (Table 1). The standard deviation and coefficient of variation were similar for systolic and diastolic blood pressure series across groups and irrespective of diet (Table 2).

Detrended fluctuation analysis

Examples of DFA log–log plot for SBP and DBP in the short-term and long-term beat ranges are shown in Figure 2. Table 3 summarizes the results of DFA scaling exponents α_S and α_L. On low salt diet, both strains of rats exhibited similar systolic and diastolic scaling exponents. High salt diet altered the blood pressure scaling exponents in both the salt sensitive and salt protected rats (Table 3 and Figure 3): salt sensitive rats showed increased α_L (smoother) for systolic BP (p = 0.012, p = 0.007 respectively.) and diastolic BP (p = 0.013and p = 0.008 respectively.); protected rats showed a trend towards an increased α_S for systolic BP (p = 0.072). On high salt, salt sensitive rats showed higher α_L for systolic and diastolic BP (p= 0.01and p = 0.005 respectively) and lower α_S for systolic BP (p = 0.001) compared to the protected rats.

Sample entropy

Table 3 summarizes the results of SampEn. On low salt diet, both strains of rats exhibited similar systolic and diastolic complexity. High salt diet altered the blood pressure complexity in both strains (Table 3 and Figure 3): salt sensitive rats showed a trend towards a decreased SampEn (less complex) for diastolic BP (p = 0.064); protected rats showed a decreased SampEn for systolic and diastolic BP (p = 0.047 and p = 0.037 respectively).On high salt diet, salt sensitive rats showed a trend towards a lower SampEn for diastolic BP (p = 0.087) compared to the protected rats (Table 3).

Discussion

We found that on low salt diet, both groups of rats had similarly irregular or complex BP time series. However, when exposed to a high salt diet, both groups showed a loss of complexity

with the salt sensitive rats showing a greater loss of complexity (Table 3). Whereas, standard measures of variability, i.e, standard deviation and coefficient of variation (Table 2) did not differ. Our results are in line with the study by Vandendriessche et al. where two mouse models were compared in terms of SD of the beat-to-beat diastolic blood pressure series after induced shock (44), and another study by Subramaniam et al. where the SD of the systolic, diastolic and pulse pressure series of twenty subjects with preoperative major adverse events during surgery were compared to those of twenty matched controls (41). The SD's were unchanged between cases and controls in both studies. Older studies that showed the effect of the SD and CV on prognosis and cardiovascular outcomes, such as stroke, myocardial infraction, and death, have analyzed BPV using ambulatory blood pressure monitoring over twenty four hours at fixed intervals such as every 5-15 minutes, one hour, or between visits (15, 24, 35, 36, 40). Recently however, beat-to-beat BPV became of greater interest due to the advancements in non-invasive monitoring, which allows blood pressure data to be measured continuously (beat-to-beat) over epochs of 5-30 minutes or longer.

As first proposed about 30 years ago (11), physiological rhythms, such as BP, are under the control of coupled biological oscillators, which are affected by neural, humoral, and cytokine components. Changes in any of these components would then lead to changes in physiologic rhythms, which can be measured and quantitated.   Salt loading increases both BP and microvascular permeability (34). Salt loading acts on the V_{1a} receptors in the paraventricular nucleus of the hypothalamus to modulate the salt-induced sympathoexcitation (31). Furthermore, salt intake differentially effects muscle sympathetic nerve activity and catecholamine levels in salt sensitive and salt resistant persons (22). The renin- angiotensin - aldosterone system is partially effected by pulsatile secretion of these hormones and help to modulate BPV (9). These effects mediate the changes in BPV that we found from salt as measured by DFA and SampEn.

Our study also showed that beat-to-beat blood pressure dynamics, measured by SampEn and scale exponents α_S and α_L were altered with high salt diet and the resultant increased systolic and diastolic blood pressures. As α_L and α_S measure "roughness" or complexity of the BP pattern over different time scales, we suggest that there are at least two controllers acting over different time scales of BP variation in these rats. α_S probably represents respiratory

oscillations (27) and α_L hormonal influences although much further study is needed to evaluate this. Other studies have shown that entropy measures of hormone levels differentiate between health and disease (18, 25, 33)Our results suggest the potential utility of DFA and SampEn of BP time series to not only detect pathological BP states but suggest the time scales over which the controllers act.

Decreased BP complexity indicative of more regular patterns has been previously observed in diseased populations. The study by Subramaniam et al. on patients with preoperative major adverse events during surgery and the study by Vandendriessche et al. on mice after induced shock, measured complexity by multiscale entropy. Both studies showed a loss of complexity as measured by lower multiscale entropy in the diseased groups (41, 44). In another study, Cerutti et al. studied 20 male patients admitted to the hospital for programmed electrical cardioversion for persistent atrial fibrillation, at rest and during tilt table testing. They compared the SampEn and ApEn of the systolic and diastolic blood pressure series and showed that both complexity measures increased during tilt in patients in whom the SBP appropriately increased (5). Shin et al. compared the ApEn of the daytime blood pressures in patients with complicated and uncomplicated hypertension to normotensive patients and showed that ApEn was lower in hypertensive patients compared to controls (38). A study by Soehle et al. assessed the complexity of the intracranial pressure in patients with traumatic brain injury during periods of intracranial hypertension and showed loss of complexity in that the SampEn and multiscale entropy of the intracranial pressure time series decreased with intracranial hypertension (39).   Nevertheless, not all studies show decreased complexity with pathology, e.g., Zhang et al found increased Cross SampEn in a rat model of hemorrhage (46)

The main limitation of our study is that only 15 SS Dahl rats were studied. Other rat strains, breeds, or models of salt sensitive hypertension may have different physiologic controllers of blood pressure and produce different DFA and SampEn values. Also, with only 15 animals our chance of finding a spurious finding is increased. Further study is needed with a greater variety of breeds and more animals. Finally, DFA can be biased at short data lengths, particularly 64 or 128 points (7).While our study used 626-953 points, decreasing the risk of bias, it does not eliminate it and additional studies should be done using longer time series.

One of the strengths of our study is that we measured BPV by two complementary, but not redundant, techniques. DFA has the advantage of being able to estimate the "roughness" of the pattern over different timescales, while SampEn assesses the predictability of the next member of the series across the whole time series. As neural, hormonal, and other controllers of BP have different oscillatory or pulsatile frequencies, DFA may provide a useful tool for determining the interactions between salt or other inputs and the neural, hormonal, and other controllers of BP by matching the DFA scale exponents to the frequency of the controller. Previous studies also showed increased scale exponents coefficients in unhealthy individuals. However, while most of these studies were focused on heart rate time series and not blood pressure time series BP and heart rate are intricately, although not perfectly, linked. Changes in heart rate rhythm lead to beat-to-beat changes in stroke volume, which is proportional to BP through vascular compliance. However, over different time scales other factors may contribute to a loss of coupling (30). A study by Zhang et al examined BPV using DFA scale exponents in preterm infants with and without intraventricular hemorrhage. The study included thirty infants and showed a significant increase in the short-term correlation coefficient α_S in infants who developed intraventricular hemorrhage while α_L was unchanged (47). Two studies by Lee et al. on EEG time series data also showed lower scaling exponents in patients with sleep apnea compared to healthy subjects and in unmedicated unipolar depressed subjects compared to non-depressed controls (19, 20). While DFA exponents clearly discriminate between healthy and unhealthy subjects, they do so in varied fashions and to different extents, which indicates that more research on DFA and BPV is needed.

The significance of the current studies is the quantitative demonstration of the role of salt –induced hypertension, a result of intravascular volume expansion, in modifying the characteristics of BPV, as quantitated by two measures of beat-to-beat complexity - SampEn and DFA scaling exponents. Averaged over years or decades, individuals on high salt diets would be expected to spend a significantly greater proportion of time under conditions of increased vascular stress and higher intracapillary pressures and wall tension. Clinical monitoring of BPV by non-traditional indices such as those described here may provide earlier indication of subtle changes in intravascular filling and pressure many years before the onset of hypertension and its resultant vascular injury and end-organ damage. This hypothesis, however, requires prospective

validation including the design of long-term studies on large populations of susceptible individuals.

Conclusion

Using an animal model, our study is the first study to look mutually at entropy and DFA parameters in hypertensive and non-hypertensive subjects switching from a low- to a high-salt diet. In summary we showed that hypertensive rats are more susceptible to high salt diet with both a greater rise in blood pressure and more reduced beat-to-beat BP complexity. Entropy and DFA scaling exponents detected inherent differences in BP variability with hypertension and salt diet that are not observed with the simpler traditional variability measures (SD, CV). Both salt sensitive (SS) and protected (SSBN13) rats showed altered BP dynamics with high salt versus low salt diet, albeit in varied fashion and to different extents. Comparable mean pressures in sensitive and protective rats when on low-salt diet coupled suggest a protective role of low salt intake in hypertensive rats. Moreover, the finding that a low-salt diet potentially equalizes equalizes complexity (entropy and DFA scaling exponents) of beat-to-beat BPV in hypertensive and non-hypertensive rats provides evidence that its salutary effects likely act through better coupling of biologic oscillators.

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Table 1 – Comparison of salt intake effects on BP in salt sensitive and protected rats

Study Group / Treatment	Salt-sensitive			Salt-protected		
	Low Salt	High Salt	$\Delta_{\text{HS-LS}}$	Low Salt	High Salt	$\Delta_{\text{HS-LS}}$
Systolic BP (mmHg)	137±2[a b]	195±7[a b]	57±5[b]	125±2[a b]	155±3[a b]	30±2[b]
Diastolic BP (mmHg)	102±3[a]	153±7[a b]	51±6[b]	97±1[a]	123±3[a b]	26±3[b]

Number of beats: 626-953; data shown as mean±standard error averaged across rats within a group; [a] = P<0.05 Low salt vs High salt (within-group); [b] = P<0.05 Salt-sensitive vs Salt-protected (between-groups; same treatment).

$\Delta_{\text{HS-LS}}$: changes calculated as (High salt - Low salt) values.

Table 2 – Comparison of traditional BP variability metrics in salt sensitive and protected rats

Study Group / Treatment	Salt-sensitive			Salt-protected		
	Low Salt	High Salt	Δ_{HS-LS}	Low Salt	High Salt	Δ_{HS-LS}
Systolic BP						
SD (mmHg)	4.40±0.30	5.73±0.46	1.33±0.57	4.30±1.30	5.19±0.49	0.89±0.59
CV(%)	3.2±0.2	2.9±0.2	-0.30±0.34	3.5±0.4	3.4±0.4	-0.10±0.48
Diastolic BP						
SD (mmHg)	4.03±0.31	4.70±0.48	0.67±0.59	3.80±0.50	4.65±0.56	0.85±0.58
CV(%)	3.97±0.25	3.13±0.29	0.85±0.41	3.89±0.47	3.82±0.47	-0.08±0.48

Number of beats: 626-953; data shown as mean±standard error averaged across rats within a group; Δ_{HS-LS}: changes calculated as (High salt - Low salt) values.

Table 3 - Comparison of entropy and detrended fluctuation analysis indices in salt sensitive and protected rats

Study Group / Treatment	Salt-sensitive			Salt-protected		
	Low Salt	High Salt	Δ_{HS-LS}	Low Salt	High salt	Δ_{HS-LS}
Systolic BP						
Scaling exponents						

α_S	1.24±0.05	1.12±0.05[b]	-0.13±0.07[b]	1.16±0.12[aᴵ]	1.43±0.06[aᴵ b]	0.27±0.06[b]
α_L	1.01±0.04[a]	1.21±0.05[a b]	0.20±0.06[bᴵ]	1.01±0.06	0.99±0.05[b]	-0.02±0.09[bᴵ]
Entropy						
SampEn	1.43±0.06	1.26±0.06	-0.16±0.10	1.51±0.09[a]	1.24±0.08[a]	-0.28±0.09
Diastolic BP						
Scaling exponents						
α_S	1.46±0.04	1.37±0.07	-0.09±0.08	1.47±0.10	1.51±0.06	0.04±0.10
α_L	1.04±0.04[a]	1.21±0.05[a b]	0.17±0.05[b]	1.02±0.05	0.97±0.05[b]	-0.05±0.08[b]
Entropy						
SampEn	1.27±0.04[aᴵ]	1.03±0.09[aᴵ bᴵ]	-0.24±0.14	1.37±0.06[a]	1.21±0.02[a bᴵ]	-0.16±0.06

Number of beats: 626-953; data shown as mean±standard error averaged across rats within a group; [a] = P<0.05 Low salt vs High salt (within-group); [b] = P<0.05 Salt-sensitive vs Salt-protected (between-groups; same treatment); [aᴵ] = 0.05≤P<0.1 Low salt vs High salt (within-group); [bᴵ] = 0.05≤P<0.1 Salt-sensitive vs Salt-protected (between-groups; same treatment). α_S for Nbeats=4-10; α_L for Nbeats=11-100. $\Delta_{HS\text{-}LS}$: changes calculated as (High salt - Low salt) values.
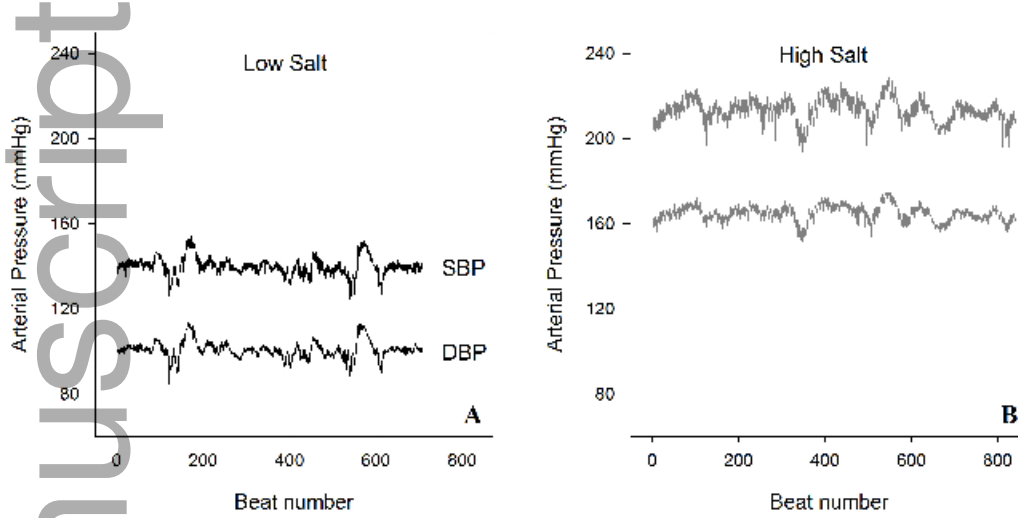
Figure legend:

Figure 1. Example beat-to-beat systolic (SBP) and diastolic (DBP) blood pressure time series derived from 2-min data epoch recordings: **(A)** salt sensitive rat on low salt; **(B)** Same salt sensitive rat on high salt **(C)** Protected rat on low salt; and **(D)** same protected rat on high salt.
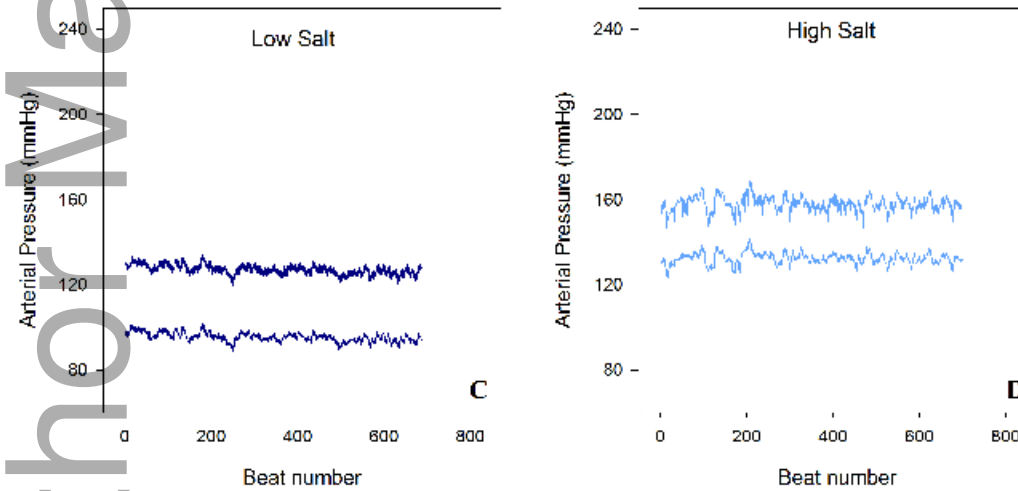
Figure 2. Log-Log Plot of DFA derived F(n) versus n derived for systolic and diastolic BP time series data sets shown in Figure 1 for example salt sensitive and protected rats while on low salt diet compared to high salt diet:**(A)** SBP, salt sensitive rat **(B)** DBP, same salt sensitive rat; **(C)** SBP, protected rat; **(D)** DBP, same protected rat. Log-Log plots of all rats are shown in supplement figures S1-S4.

Figure 3. Effect of high salt (HS) diet following low salt (LS) diets on entropy and detrended fluctuation properties of the systolic and diastolic BP time series shown for all study salt sensitive (n=9) and protected (n=6) rats individually: **(A)** α_S of SBP; **(B)** α_L of SBP; **(C)** SampEn of SBP; **(D)** α_S of DBP; **(E)** α_L of DBP; and **(F)** SampEn of DBP. Comparisons were done using the paired t test and the sign-rank test as appropriate.
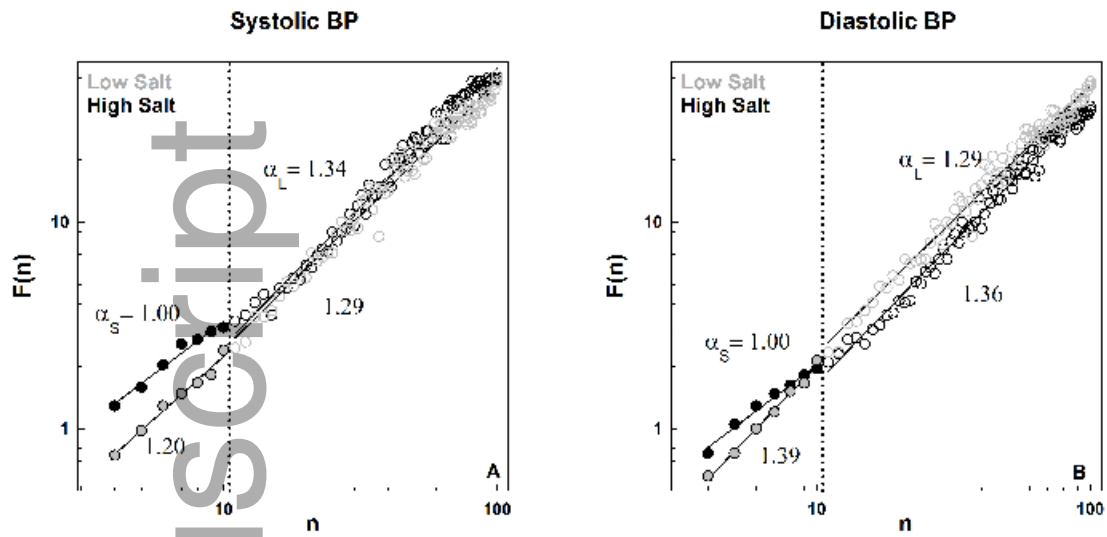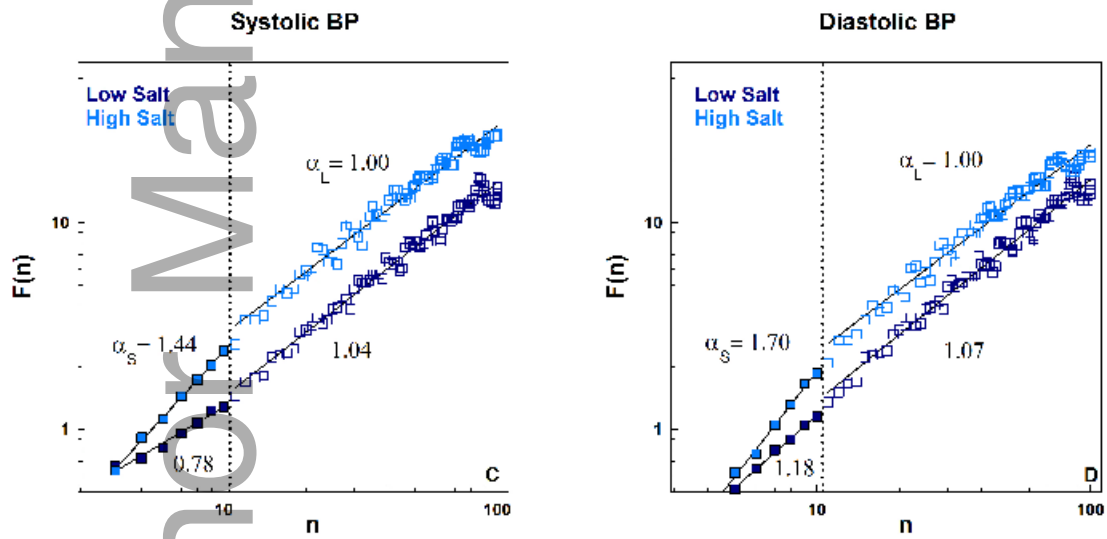
Salt-sensitive Rat #1

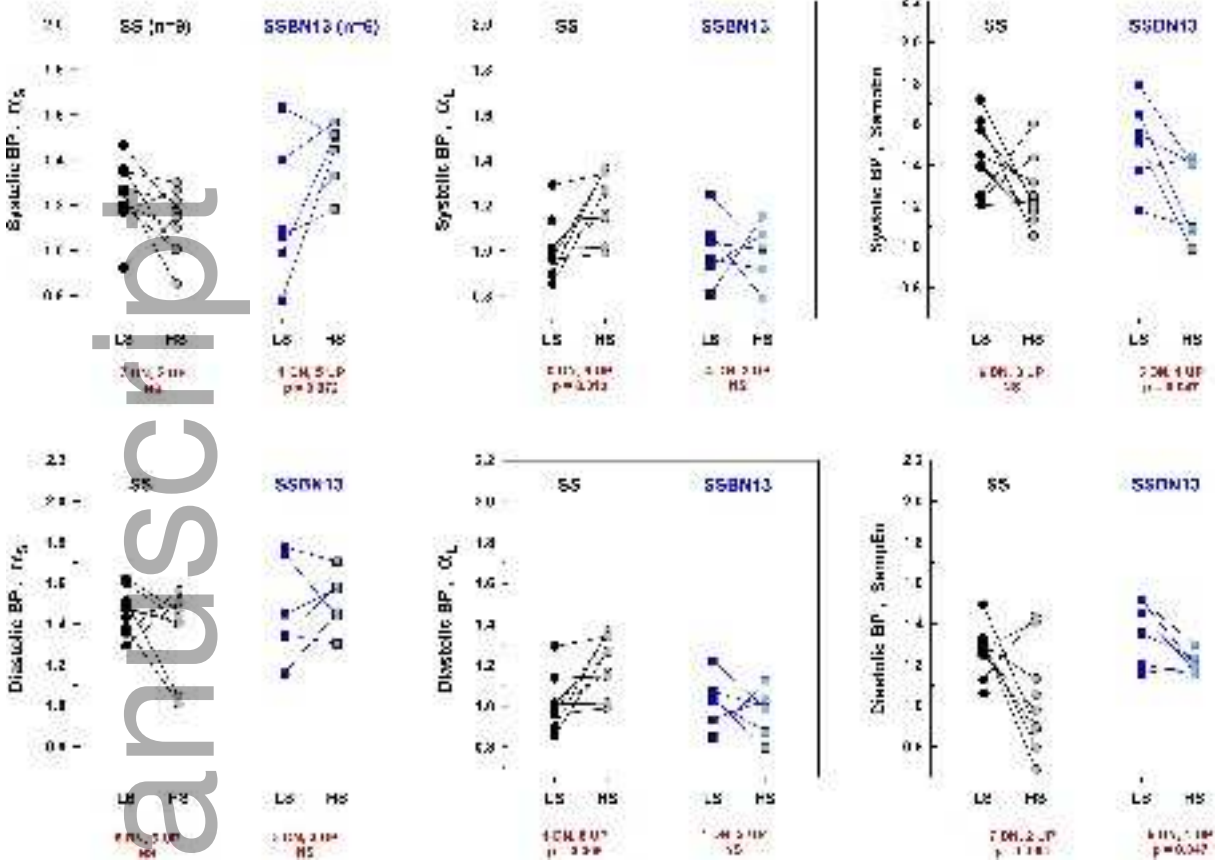**Salt-protected Rat #1**

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Salt-sensitive Rat #1

Systolic BP

Low Salt
High Salt

$\alpha_L = 1.34$

$\alpha_S - 1.00$

1.29

1.20

F(n)

n

A

Diastolic BP

Low Salt
High Salt

$\alpha_L = 1.29$

$\alpha_S = 1.00$

1.36

1.39

F(n)

n

B

Salt-protected Rat #1

Systolic BP

Low Salt
High Salt

$\alpha_L = 1.00$

$\alpha_S - 1.44$

1.04

0.78

F(n)

n

C

Diastolic BP

Low Salt
High Salt

$\alpha_L - 1.00$

$\alpha_S = 1.70$

1.07

1.18

F(n)

n

D

phy2_12823_f2.jpg

phy2_12823_f3.jpg