Biostatistical Analysis of Hypertension in Dahl Rat Models

by

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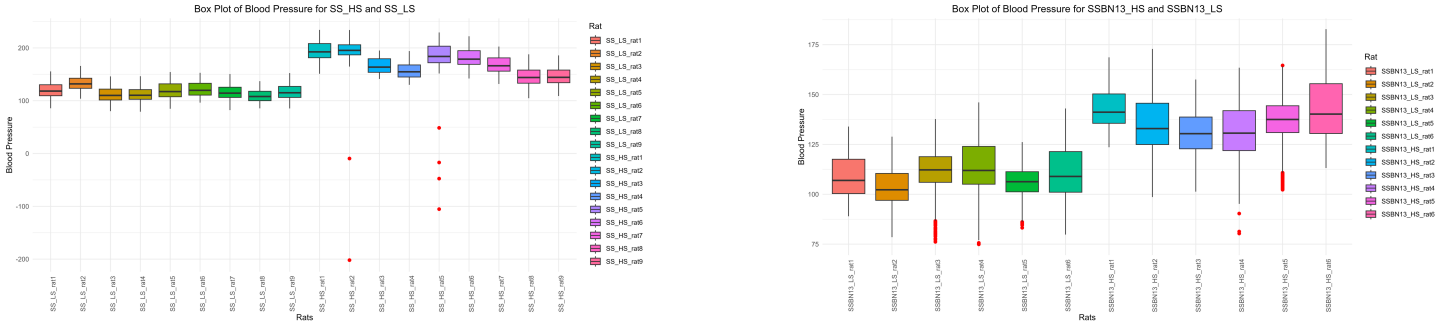
1 Overview

Hypertension is a disease condition affecting over 1.4 billion people worldwide, among which SS-HT is one of the most common forms of hypertension characterized by the elevation of BP in response to dietary salt intake. Despite extensive research during the last decades, mechanisms of SS-HT are multifactorial and not fully elucidated yet, including renal and extrarenal factors contributing to blood pressure regulation. The Dahl-SS rat model was one of the first models used to study SS-HT, and it demonstrated that these rats develop hypertension on a high-salt diet, whereas Dahl-SR rats maintain normal BP. Kidney transplant studies furthered the concept that "blood pressure follows the kidney," as SS-HT characteristics transferred between Dahl-SS and Dahl-SR rats based on the origin of the donor kidney. This suggested that the kidney has a central role in the development of SS-HT. However, conflicting findings emerged from these transplant experiments, indicating that extrarenal factors—such as the sympathetic nervous system, vascular abnormalities, and immune responses—also contribute to SS-HT. These findings highlight the complexity of salt sensitivity, suggesting that blood pressure regulation involves both renal and non-renal mechanisms.

With the development of newer methodologies in molecular biology and genomic techniques, newer ways are being sought to investigate interactions among these systems. This is a proposal for re-evaluation, by the use of modern methodologies, of the contribution of renal and extra-renal factors in SS-HT by comparing the Dahl-SS rat model with the SSBN13 rats, a congenic model resistant to salt-induced hypertension. This study, by elucidating the multifactorial nature of SS-HT, will help in pointing out newer and more specific treatments in hypertensive patients.

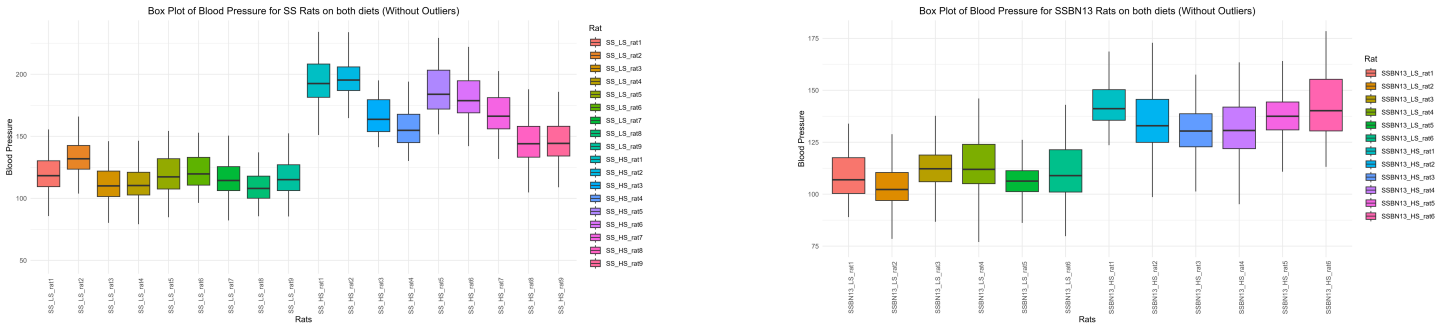
2 Data Visualization and Preprocessing

To prepare for statistical analysis, it was necessary to visualize the data. Box plots for *SS_LS & SS_HS* and *SSBN13_LS & SSBN13_HS*, as shown in Figures 1 and 2, were created to examine the data distribution and identify potential outliers. Outliers can significantly impact statistical results, particularly in sensitive physiological data like blood pressure. Therefore, detecting and addressing them was a crucial step. The Interquartile Range (IQR) method was used to detect outliers, which were subsequently removed to ensure the analysis remained accurate and reliable.



(a) *SS_LS & SS_HS* with Outliers (b) *SSBN13_LS & SSBN13_HS* with Outliers

Figure 1: Side-by-side comparison of *SS and SSBN13* Visualization with Outliers



(a) *SS_LS & SS_HS* without Outliers

(b) *SSBN13_LS & SSBN13_HS* without Outliers

Figure 2: Side-by-side comparison of *SS and SSBN13* Visualization without Outliers

2.1 Interquartile Range (IQR) and Outlier Removal Criteria

The Interquartile Range (IQR) is calculated as follows:

$$IQR = Q3 - Q1$$

Outliers are identified and filtered out using the following criteria:

$$\text{Lower Bound} = Q1 - 1.5 \times IQR$$

$$\text{Upper Bound} = Q3 + 1.5 \times IQR$$

Outliers are defined as any data points that fall below the lower bound or above the upper bound. This method is effective for detecting outliers by considering how far a data point is from the quartiles of the dataset.

3 Normality Test

The Shapiro-Wilk test was conducted to assess whether the blood pressure data in each group (SS_LS, SS_HS, SSBN13_LS, and SSBN13_HS) followed a normal distribution. This is a critical first step because the assumptions of normality are necessary for using parametric tests like t-tests and ANOVA, which are more powerful but assume that the data are normally distributed. The normality test was performed by taking the mean blood pressure value of each test subject and taking the confidence level of 95%. The results of the Shapiro-Wilk normality tests for the mean blood pressure of each group are summarized in Table 1. The mean Blood pressure of different dataset can be seen in the Figure 3

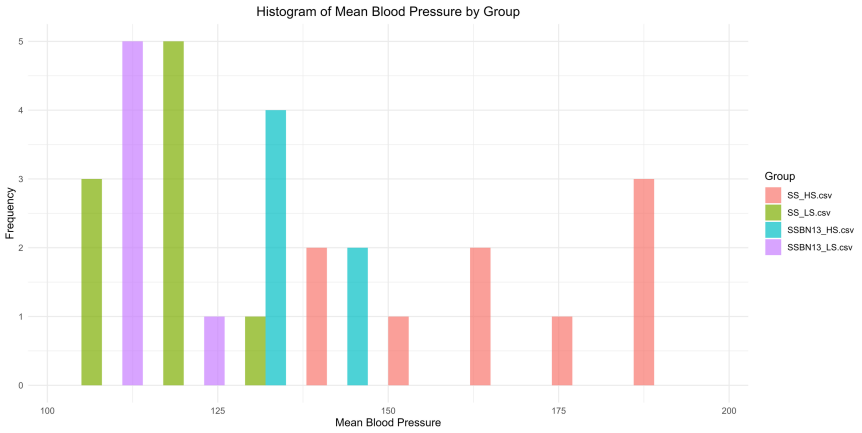


Figure 3: Mean values of all the data sets

Group	p-value
SS_LS	0.3016
SS_HS	0.3864
SSBN13_LS	0.8722
SSBN13_HS	0.3940

Table 1: Shapiro-Wilk Normality Test Results

4 Welch's t-Test

Based on the plots, the null and alternative hypotheses were formulated as follows:

(H_0): No significant difference in mean blood pressure between the two groups.

(H_A): A significant difference in mean blood pressure between the two groups.

Welch's t-test, a variation of the Student's t-test, was used to compare the means between two groups (e.g., SS_LS vs. SS_HS) under different dietary conditions. This test is particularly useful when variances and sample sizes are unequal, as is the case in the data.

Comparison	t	df	p-value	95% CI	Mean 1	Mean 2
SS_LS vs SS_HS	7.652	10.031	1.703×10^{-5}	[38.13, 69.43]	117.74	171.52
SSBN13_LS vs SSBN13_HS	10.323	9.234	2.244×10^{-6}	[21.46, 33.44]	109.46	136.91
SS_LS vs SSBN13_LS	2.896	12.689	0.0128	[2.09, 14.46]	117.74	109.46
SS_HS vs SSBN13_HS	4.979	9.586	0.00063	[19.03, 50.18]	171.52	136.91

Table 2: Welch's t-test Results for Group Comparisons of Mean Blood Pressure (Mean_BP). Mean 1 refers to the average blood pressure of the first group in the comparison, while Mean 2 refers to the average blood pressure of the second group.

4.1 Inference:

- **SS_LS vs. SS_HS:** The significant p-value ($p < 0.05$) indicates that the high-salt diet has a strong effect on increasing blood pressure in SS rats.
- **SSBN13_LS vs. SSBN13_HS:** Similarly, the high-salt diet significantly increased blood pressure in the SSBN13 group, though to a lesser extent compared to the SS rats.
- **SS_LS vs. SSBN13_LS:** The significant difference in blood pressure under low-salt conditions suggests that SS rats may have a genetic predisposition toward higher blood pressure.
- **SS_HS vs. SSBN13_HS:** High salt intake affects SS rats more than SSBN13 rats, reinforcing the greater sensitivity of SS rats to dietary salt.

5 Analysis of Variance (ANOVA)

Two-factor ANOVA is a statistical method used to assess the impact of two independent categorical variables on a continuous dependent variable. This research focuses on evaluating how diet and strain influence [specific response variable] blood pressure. a two-way ANOVA test was performed to assess the interaction between diet (high salt vs. low salt) and strain (SS vs. SSBN13). Using the Hypothesis:

(H_0): No effect of either Diet, Strain and Interation of Diet and Strain on the blood pressure of the rats.
(H_A): There is an effect of either Diet, Strain or Interation of Diet and Strain on the blood pressure of the rats.

The analysis was performed using R programming, with the following model employed:

$$\text{Response} \sim \text{Diet} + \text{Strain} + \text{Diet:Strain} \tag{1}$$

5.1 Inference

Source	Df	Sum Sq	Mean Sq	F value	Pr(¿F)
Diet	1	14037	14037	96.714	3.00e-10 ***
Strain	1	3303	3303	22.760	6.16e-05 ***
Diet:Strain	1	1248	1248	8.596	0.00694 **
Residuals	26	3774	145		

Table 3: ANOVA Summary Table

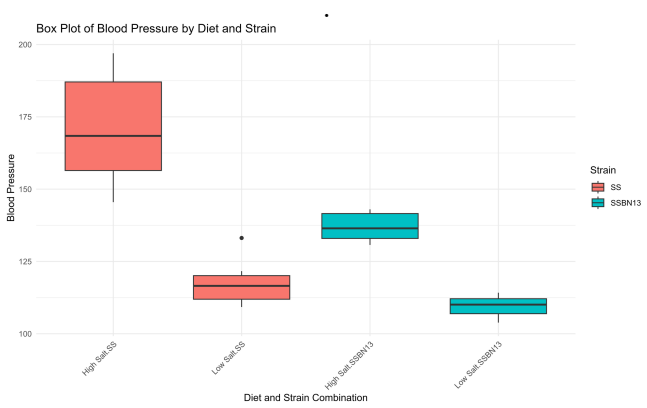


Figure 4: Box plot of blood pressure by diet (Low vs. High Salt) and strain (SS vs. SSBN13), showing median, variability, and outliers.

The box plot 4 visually summarizes blood pressure distributions across different diets (Low Salt vs. High Salt) and strains (SS vs. SSBN13). Larger boxes indicate greater variability. Whiskers extend to the overall range, and points outside this range are outliers. By comparing medians and examining the presence of outliers, the plot highlights significant differences in blood pressure between groups, illustrating the effects of dietary salt on blood pressure across genetic strains.

6 Tukey's Honest Significant Difference

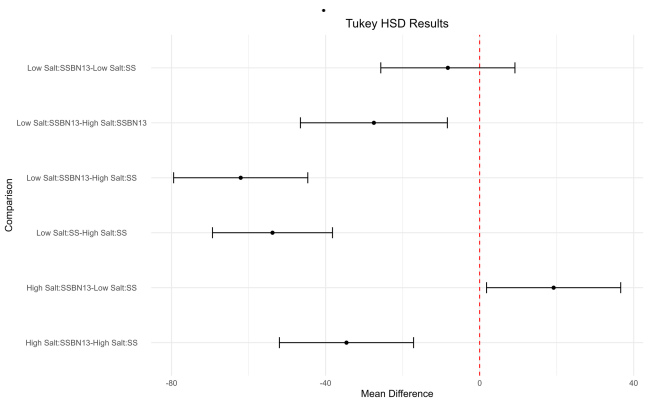


Figure 5: Tukey HSD plot of mean differences in blood pressure between diet and strain combinations, with 95% confidence intervals and a reference line at zero.

The Tukey HSD analysis reveals significant differences in blood pressure across various diet and strain combinations. Specifically, the Low Salt diet significantly reduces blood pressure compared to the High Salt diet within both strains. Furthermore, SSBN13 rats consistently exhibit lower blood pressure than SS rats across both dietary conditions. These findings suggest that dietary salt intake significantly influences blood pressure levels, with strain-specific responses highlighting the genetic factors involved in hypertension. Overall, the analysis underscores the importance of diet in managing blood pressure and provides insights into the physiological differences between the strains studied.

Fit: `aov(formula = BloodPressure ~ Diet * Strain, data = combined_data)`

Diet:Strain

Diet:Strain	diff	lwr	upr	p adj
Low Salt:SS-High Salt:SS	-53.791819	-69.371427	-38.212210	0.0000000
High Salt:SSBN13-High Salt:SS	-34.582512	-52.001043	-17.163980	0.0000584
Low Salt:SSBN13-High Salt:SS	-62.048235	-79.466767	-44.629704	0.0000000
High Salt:SSBN13-Low Salt:SS	19.209307	1.790776	36.627838	0.0266439
Low Salt:SSBN13-Low Salt:SS	-8.256417	-25.674948	9.162115	0.5709348
Low Salt:SSBN13-High Salt:SSBN13	-27.465724	-46.546769	-8.384679	0.0028243

7 Conclusion

In this study, I explored how dietary salt impacts blood pressure in Dahl salt-sensitive (SS) and consomic (SSBN13) rats, with the goal of gaining a deeper understanding of the mechanisms behind salt-sensitive hypertension (SS-HT). The results were clear, high-salt diets led to a significant rise in blood pressure in SS rats, which supported the hypothesis that increased salt intake worsens hypertension in those genetically predisposed to be salt-sensitive. Interestingly, even the SSBN13 rats showed some elevation in blood pressure in response to high salt, just significantly lower than what was demonstrated in SS. This would then imply that at the intact animal level, SSBN13 are resistant to the hypertensive effect of salt.

In general, the findings strongly support the critical and well-established role that dietary salt plays in regulating blood pressure, showing that increased salt intake can lead to a significant increase in blood pressure, especially in populations sensitive to salt. It also shows how this effect can vary among genetic strains, where some strains, such as SS rats, would have a greater response to high salt intake than others, such as SSBN13 rats. The variation thus points toward the complexity and multifactorial aspects of SS-HT influenced both by genetic and environmental variables. These findings are of great significance in light of the complex mechanisms underlying SS-HT and offer new insights into how genetic variations can modulate the organism's response to dietary salt. These findings not only advance the knowledge of hypertension but also open the door for further research that can explore how genetic factors interact with other physiological processes to influence salt sensitivity, ultimately informing better strategies for prevention and treatment.

Link to dataset: Blood Pressure in Salt-Sensitive Dahl Rats