Title

Effects of Ambient Particulate Matter 2.5 (PM2.5) Exposure on Average Heart Rates and Mean Blood Pressures of Spontaneously Hypertensive Rats

Author

Vo, Michael H

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IRVINE

Effects of Ambient Particulate Matter 2.5 ($PM_{2.5}$) Exposure on Average Heart Rates and Mean Blood Pressures of Spontaneously Hypertensive Rats

THESIS

submitted in partial satisfaction of the requirements

for the degree of

MASTER OF SCIENCE

In Environmental Health Sciences

by

Michael H. Vo

Thesis Committee:
Associate Professor Andrea De Vizcaya Ruiz, Chair
Associate Professor Masashi Kitazawa
Professor Michael T. Kleinman

2023

Dedication

To

Martha Vo, my mother, my family, and all my friends.

Thank you for everything.

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List of Abbreviations

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ANOVA	Analysis of Variance
ANS	Autonomic nervous system
BP	Blood pressure
Ca_2^+	Calcium
CAPs	Concentrated ambient particles
CRP	C-reactive protein
ET	Endothelin
EPA	Environmental Protection Agency
HR	Heart rate
HRV	Heart rate variability
IL	Interleukin
LPM	Liters per minute
Na.	Sodium
NO_2	Nitrogen dioxide
O_2	Oxygen
O_3	Ozone
PM	Particulate matter
PM_{10}	Particulate matter with an aerodynamic diameter ≤ 10 μm
$PM_{2.5}$	Particulate matter with an aerodynamic diameter ≤ 2.5 μm
SHR	Spontaneously hypertensive rats
SO_4^{2-}	Sulphate
Tukey HSD	Tukey's honestly significant difference test
UCI	University of California, Irvine
VACES	Versatile aerosol concentration enrichment system

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Abstract of the Thesis

Effects of Ambient Particulate Matter 2.5 ($PM_{2.5}$)

Exposure on Average Heart Rates and Mean Blood Pressures of

Spontaneously Hypertensive Rats

By

Michael H. Vo

Master of Science In Environmental Health Sciences

Associate Professor Andrea De Vizcaya Ruiz, Chair

Air pollution is a major health risk. Chronic exposure to particulate matter (PM) has been associated with increased risks for several cardiovascular (CV) diseases. Exposure to fine particulate matter (aerodynamic diameter ≤ 2.5 μm; $PM_{2.5}$) is causally related to increases in sudden cardiac death, strokes, and myocardial infarction, which are coupled with erratic heart rates and blood pressure changes. The aim of this study is to assess changes in average heart rate (HR) and mean blood pressure (BP) in spontaneously hypertensive rats (SHRs) after 11-week exposure to ambient $PM_{2.5}$.

Six male SHRs were exposed 5 hours per day, 4 days per week (Mon-Thurs) for 11 weeks (June 10, 2008 to August 21, 2008) to concentrated ambient $PM_{2.5}$ (133 +/- 20 mcg/m^3). Six control male SHRs were simultaneously exposed to filtered, purified air for the same periods. Study rats were implanted with telemetry devices (DSI, model number C50-PXT), which recorded electrocardiograms (ECGs), heart rates (HR), and blood pressure (BP). Data were acquired continuously in 5-minute increments, transmitted, stored, and analyzed using the DataQuest Art ® software.

Mixed-effects ANOVA was used to test for the interaction between four groups (Control-Day, Exposed-Day, Control-Night, and Exposed-Night) and the respective changes in blood pressure and heart rate month-to-month and from the beginning to the end of the study. Marginal means with 95% confidence intervals were reported and interpreted for each analysis. Bivariate analyses were performed if a significant main effect was detected for an interaction. Statistical significance was assumed at an alpha value of 0.05, and all analyses were performed using SPSS Version 29 (Armonk, NY: IBM Corp.)

There was a statistically significant reduction (22%) in average heart rates and a statistically significant increase (19%) in mean blood pressures of PM2.5-exposed rats over the course of 11 weeks compared to Air-exposed rats. Possible explanations for the reduction of the average heart rates include the highly responsive parasympathetic nervous system of the exposed rats, which mitigates the elevation of blood pressure caused by activation of the sympathetic nervous system and possible development of an adaptation response by exposed rats to repeated $PM_{2.5}$ exposure by strengthening the parasympathetic nervous system, hence leading to continuous reduction of average heart rates.

In summary, following repeated $PM_{2.5}$ exposure, there was a statistically significant reduction in average heart rates and a statistically significant increase in mean blood pressures of $PM_{2.5}$-exposed rats. The reduction of the average HR of exposed rats compensates for the increase in the mean BP, thus allowing them to maintain a state of homeostasis. With prolonged $PM_{2.5}$ exposure, the elevation of blood pressure continues to compensate for further reduction of heart rate. The relentless increase in blood pressure leads to accelerated hypertension and, ultimately, death due to myocardial infarction, arrhythmia, and cardiac failure.

1. INTRODUCTION

Ambient air pollution is a global health problem. Constituents of air pollution are highly heterogeneous and are a mixture of particulates and gaseous compounds. Among them, particulate matter (PM) is most commonly found in air pollution. It comprises sulfate, nitrates, ammonia, sodium chloride, black carbon, mineral dust, and water suspended in the air we breathe. PM is subclassified according to their aerodynamic particle size into (a) coarse ($PM_{10-2.5}$, diameter 10-2.5 μm), (b) fine ($PM_{2.5}$, diameter < 2.5 μm), and (c) ultrafine ($PM_{0.1}$, diameter < 0.1 μm) (Figure 1). Estimated yearly global premature deaths attributable to ambient $PM_{2.5}$ exposure in 2019 amount to 2.89 million[1]. Exposure to air pollution is the largest environmental health risk. It ranks ninth among modifiable disease risk factors, above other common factors such as low physical activity, high cholesterol, and drug use[2]. Most of the excess deaths attributable to air pollution exposure are due to acute ischemic/thrombotic cardiovascular events. Exposure to $PM_{2.5}$ is significantly associated with an increased risk of cardiovascular admission, hypertension, stroke, and coronary heart disease hospitalizations[3]. Chronic exposure to $PM_{2.5}$ is associated with a higher risk of ischemic heart disease in both aluminum smelter and fabrication workers[4]. Chronic ambient $PM_{2.5}$ exposure enhances the risk of developing acute myocardial infarction[5] and other cardiovascular diseases, possibly including hypertension and systemic atherosclerosis[6,7]

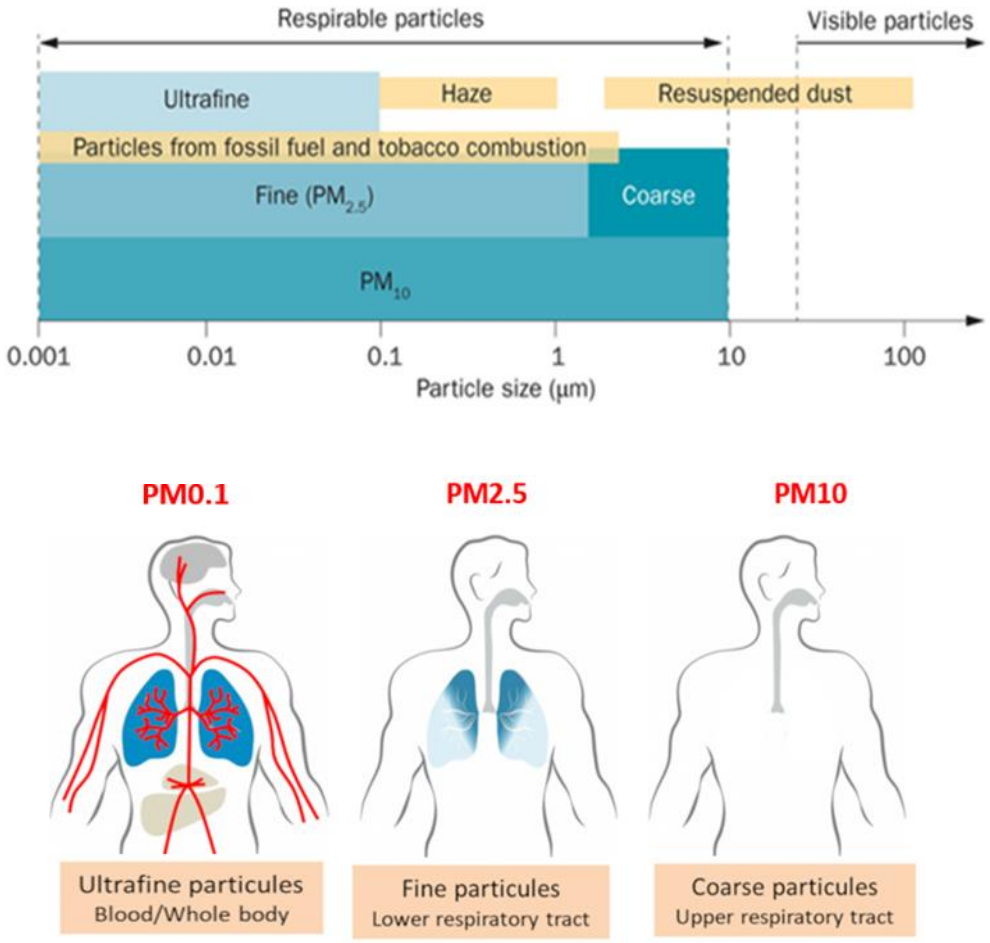


Figure 1. Size categorization of airborne pollutants. (Adapted from Cosselman et al., 2015)[8].

Figure 1 also shows that coarse particles tend to be suspended dust in the atmosphere, while fine particles can form a haze-like layer in the atmosphere. Coarse particles arise from frictional actions, such as brake wear and wind resuspension of soil, agriculture, plants, volcanoes, road dust, and surface mining operations. They generally do not penetrate beyond the upper bronchus and stay in the upper respiratory tract, thus less likely to lead to systemic inflammation. Fine particles generally arise from combustion sources, diesel, and gasoline

sources. They tend to penetrate into the lower respiratory tract. Ultrafine particles arise from gas-to-particle conversions, diesel, and gasoline combustion. They can penetrate the small airways and alveoli; some can translocate and enter the bloodstream[5], causing oxidative stress and systemic inflammation.

2. HYPOTHESIS

Repeated exposure to $PM_{2.5}$ will alter average heart rate and mean blood pressure in spontaneously hypertensive rats (SHRs).

3. OBJECTIVE

The aim of this study is to assess changes in average heart rate (HR) and mean blood pressure (BP) in spontaneously hypertensive rats (SHRs) after 11-week exposure to ambient $PM_{2.5}$.

4. LITERATURE REVIEW

Long-term exposures to ambient air pollution have been linked with cardiovascular morbidity and mortality[9, 10]. Hypertension is one important risk factor for cardiovascular diseases. It has been, therefore, hypothesized that exposure to air pollution could chronically raise blood pressure, thereby increasing hypertension[11]. Such a link has been investigated in a few studies. These studies showed that long-term exposure to ambient PM_{10} and NO_2 was significantly associated with increased hypertension[12,13]. However, the association between ambient $PM_{2.5}$ and hypertension has been inconclusive[14]. One recent meta-analysis pooling 5 studies[15] found a positive association, but the association was nonsignificant, indicating that more studies are warranted.

Exposure to PM leads to changes in disease biomarkers such as altering heart rate variability (HRV), changes in vascular tone, increased oxidative stress, induced vascular inflammation, and increased atherosclerotic plaque formation in animals[16-18] and humans[19-25]. Chronic exposure to PM has been associated with increased risks for cardiac arrhythmias, myocardial infarctions, cardiac hypertrophy, and heart failure, which can contribute to higher morbidity and mortality[26-28].

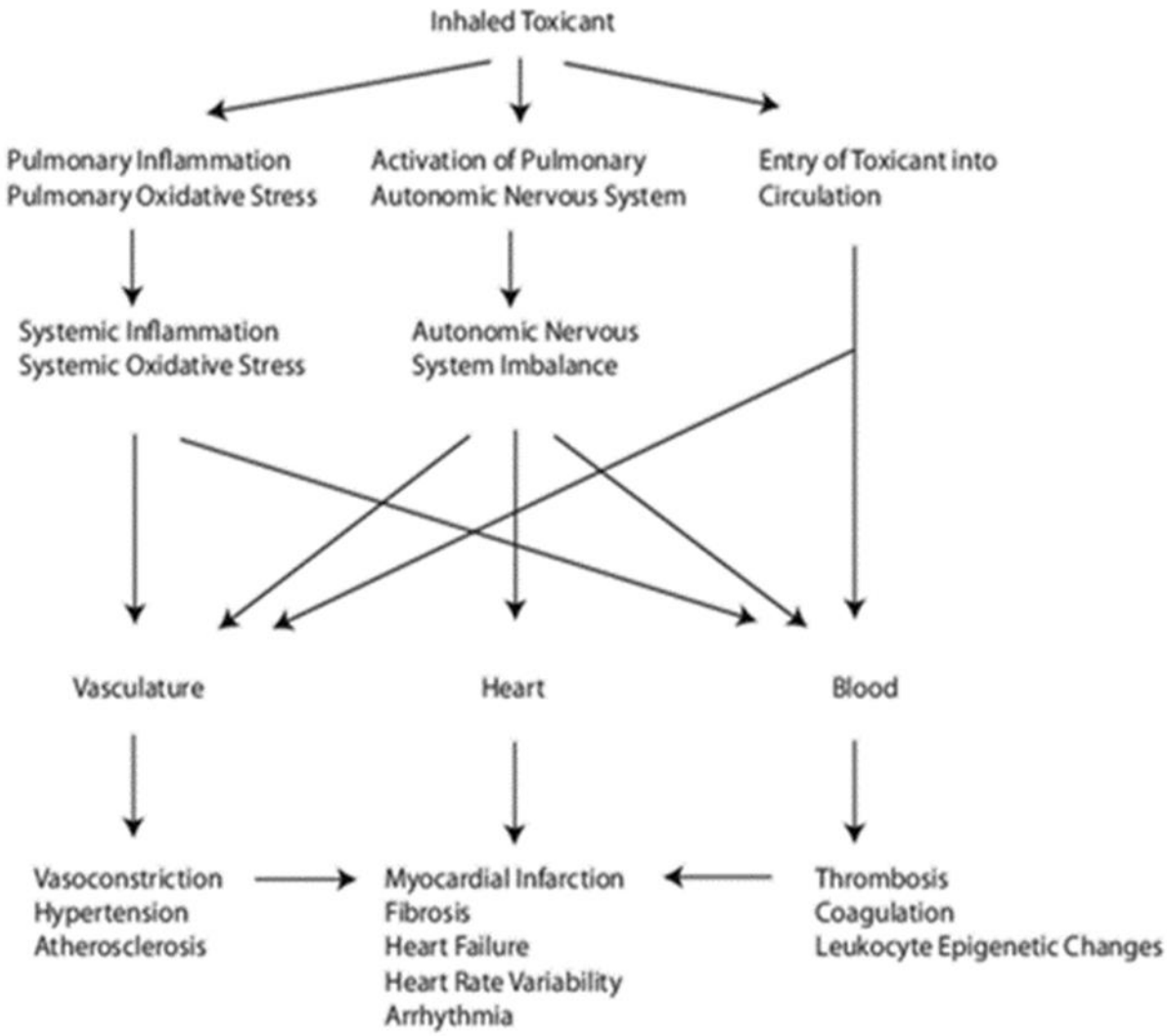


Figure 2. Working model of how air pollution exposure promotes adverse cardiovascular effects (adapted from Chin, 2014)[29].

There are three distinct hypotheses to explain the association between PM exposure and cardiovascular disease with varying degrees of evidence and consensus[29, 30]. The first hypothesis is best supported and asserts that PM entering the lungs provokes an inflammatory response that promotes oxidative stress and is sufficient to promote systemic oxidative stress and

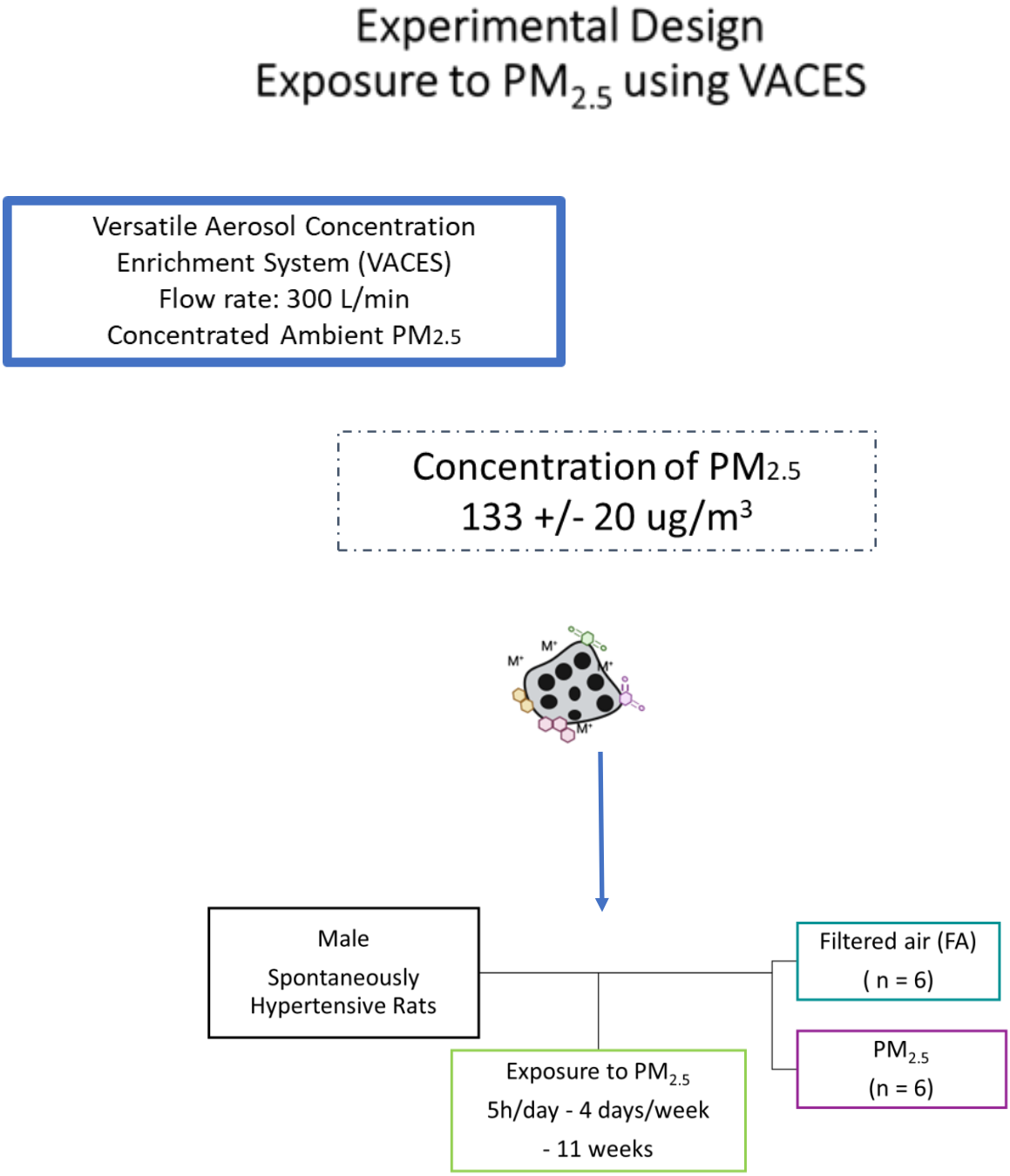
inflammation. This pro-inflammatory state is then thought to promote a variety of pathological processes related to cardiovascular disease, such as increased thrombosis, hypercoagulability, endothelial dysfunction, atherosclerosis progression, and insulin resistance. The second hypothesis is somewhat supported and asserts that pulmonary exposure leads to activation of lung autonomic nervous system (ANS) arcs mediated by transient receptor potential (TRP) channels that then cause ANS imbalance, leading to pathological alterations in vasoconstriction, endothelial dysfunction, hypertension, platelet aggregation, tachycardia, increased heart rate variability, and increased arrhythmia potential. Only a few studies support the third hypothesis and assert that airborne particulates and/or their constituents inhaled through the lungs directly enter the circulation, where they may directly interact with tissue components to promote vasoconstriction, endothelial dysfunction, atherosclerosis, hypertension, platelet aggregation, systemic oxidative stress, and inflammation.

5. EXPERIMENTAL DESIGN

Animals and exposure

Spontaneously hypertensive rats (SHRs) were chosen as exposed and control subjects in this experiment. There are desirable characteristics of spontaneously hypertensive rats (SHRs) which may be ideal for this experiment. These include: 1. SHR mimics a specific subtype of human primary hypertension, which is inherited in a Mendelian fashion (dominant versus recessive)[31], 2. SHRs can develop more severe end-organ damage (such as heart failure, stroke, and kidney failure) in addition to cardiac hypertrophy and impaired endothelium-dependent relaxations[32], 3. SHRs demonstrate coping mechanisms to adapt to elevated blood pressure[33], and 4. Blood pressure in SHRs can effectively be lowered by inhibition of the renin–angiotensin system, calcium antagonists, and vasodilators[34].

Six male spontaneously hypertensive rats (SHRs) were exposed 5 hours per day, 4 days per week (Monday to Thursday) for 11 weeks (June 10, 2008 to August 21, 2008) to concentrated ambient $PM_{2.5}$ (133 +/- 20 mcg/m3); and six control male SHRs were exposed to filtered, purified air for the same periods. All exposures to $PM_{2.5}$ occurred at the University of California, Riverside (UCR) while non-exposure periods were collected at UC, Irvine. Non-exposure day and all the night measurements were made at UCI while rats breathed purified air.



A Versatile Aerosol Concentration Enrichment System (VACES)[35]allowed for the concentration of local ambient PM, known as CAPs, and has been adapted for animal exposures

in real-world environments and can enrich the concentration of ambient particles in the diameter range of 0.02 to 10 μm by a factor of 10[36]. In this system, ambient $PM_{2.5}$ is pulled in through a size-selective inlet with air saturated with water vapor. The particle-containing air is passed through a condensation tower and chilled, causing water to condense on the ultrafine and fine particles and allowing them to grow large enough in size to be separated by inertia. The schematic of the VACES[37] used to study the seasonal effects of PM is shown in Figure 3.

Study rats were implanted with electrocardiogram (ECG) telemetry devices (DSI, model number C50-PXT) to measure heart rates (HR) and mean blood pressure (BP). Data were acquired continuously in 5-minute increments and stored in DataQuest Art ® software.

Husbandry and Housing

Animals were housed at the University of California, Irvine in a vivarium accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The vivarium was maintained in temperature-controlled rooms with a 12-hr light/dark cycle, and rats were housed in ventilated cages supplied with purified air. All rats received a standard chow diet (Teklad Envigo, Indianapolis, IN, USA) and water *ad libitum* while in housing.

Statistical Analysis

Mixed-effects ANOVA was used to test for the interaction between groups (Control-Day, Exposed-Day, Control-Night, and Exposed-Night) and the respective change in blood pressure and heart rate month-to-month and from the beginning to end of the study. Marginal means with 95% confidence intervals were reported and interpreted for each analysis. If a significant main effect was detected for an interaction, bivariate analyses were performed, and simple main effects were established using Tukey's HSD tests in a post hoc fashion. Statistical significance

was assumed at an alpha value of 0.05, and all analyses were performed using SPSS Version 29 (Armonk, NY: IBM Corp.).

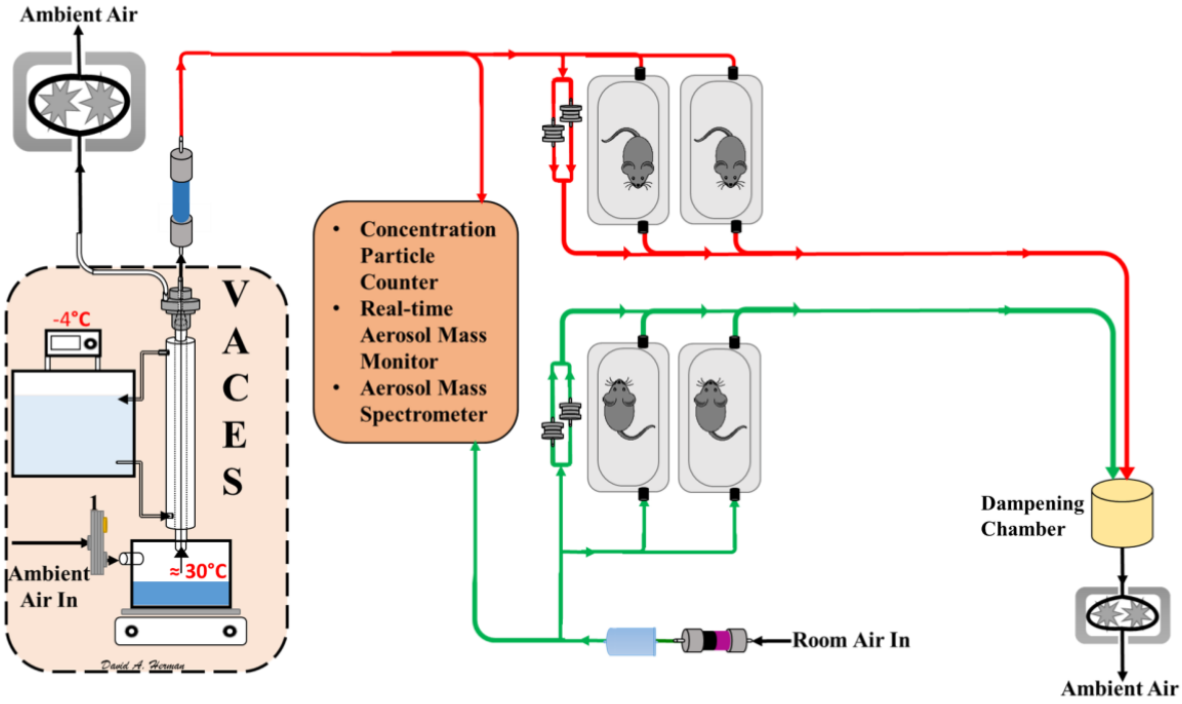


Figure 3. Schematic diagram of VACES and Exposure. (Taken from Herman et al., 2020)[37].

6. RESULTS

A statistically significant interaction effect was detected for the monthly analysis of exposure average heart rate: $F(9,60) = 4.21$, $p = 0.003$, partial eta-squared = 0.39, power = 0.95. There were significant differences between the groups at baseline, $F(3,20) = 17.41$, $p < 0.001$, and post hoc tests showed significant differences between Control-Day and Exposed-Day, $p < 0.001$, and Exposed-Day and Exposed-Night, $p = 0.001$. At the end of the first month, there was a significant main effect, $F(3,20) = 50.94$, $p < 0.001$, and post hoc differences were found between Control-Day and Control-Night, $p < 0.001$ and Exposed-Day and Exposed-Night, $p < 0.001$. At the end of the second month, there was a significant main effect, $F(3,20) = 4.34$, $p = 0.017$, and post hoc tests showed differences between Control-Day and Control-Night, $p = 0.007$, and Control-Day and Exposed-Night, $p = 0.005$. At the end of month 3, there was a significant main effect, $F(3,20) = 13.00$, $p < 0.001$, and post hoc tests showed significant differences between Control-Day and Control-Night, $p < 0.001$ and Exposed-Day and Exposed-Night, $p = 0.007$. See Table 1 for the marginal means and 95% confidence intervals and Figure 4 for a visual depiction of the interaction.

For month-to-month weekday analysis, there were statistically significant differences in heart rates of exposed rats, daytime *vs.* nighttime ($n = 6$, $p < 0.007$) and control rats daytime *vs.* nighttime ($n=6$, $p<0.001$) with lower heart rates of exposed and control rats in the daytime.

Table 1. Month-to-month marginal mean heart rates and 95% confidence intervals of exposed and control rats weekday – Interaction and Simple Effects Analysis.

Exposure – Heart Rate		Group	Month 0	Month 1	Month 2	Month 3	Main Effect p value
	Monthly						
		Control-Day	304.1 (295.5 – 312.7)	263.0 (252.9 – 273.2)	273.8 (259.1 – 288.6)	278.5 (265.3 – 291.8)	
		Exposed-Day	313.1 (304.5 – 321.7)	274.3 (264.1 – 284.4)	289.4 (274.6 – 304.1)	286.8 (273.5 – 300.0)	
		Control-Night	339.6 (331.1 – 348.2)	328.8 (318.6 – 339.0)	303.9 (289.2 – 318.7)	327.7 (314.4 – 340.9)	
		Exposed-Night	335.3 (326.7 – 343.9)	328.0 (317.8 – 338.1)	305.3 (290.5 – 320.0)	313.5 (300.3 – 326.8)	0.003
	Simple effects p-value		< 0.001	< 0.001	0.017	< 0.001	

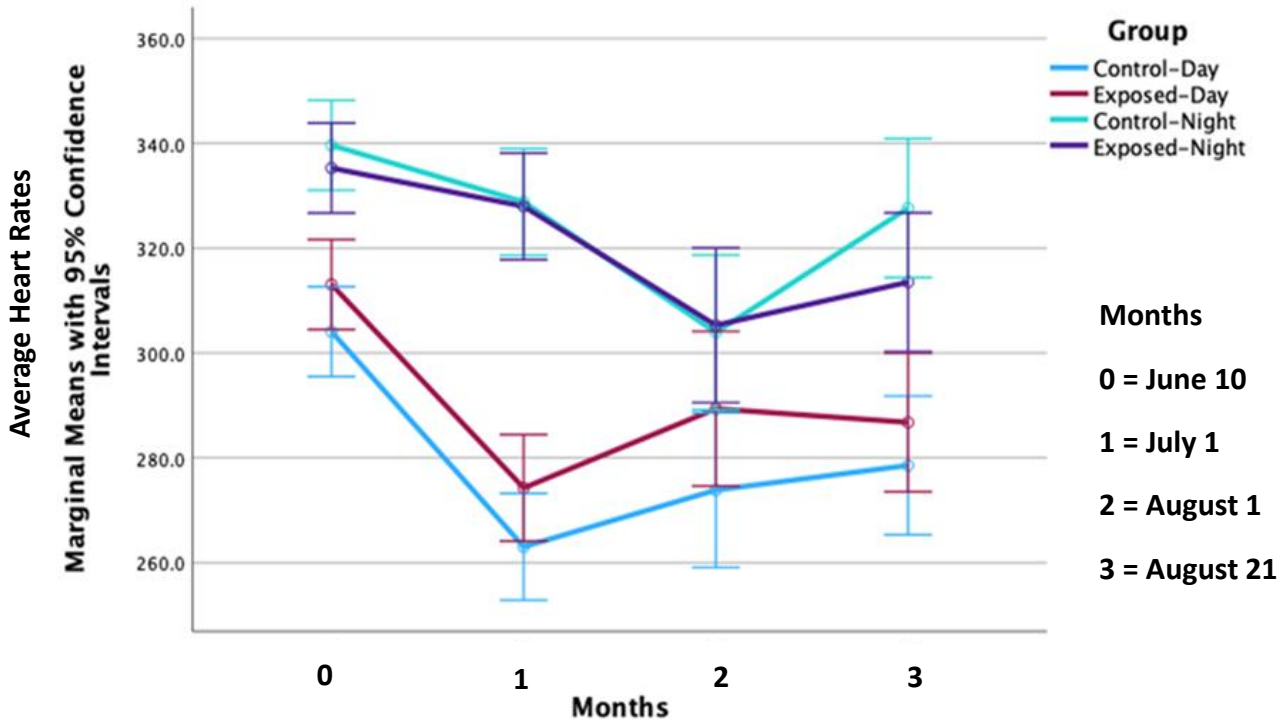


Figure 4. Month to Month Average Heart Rates of Exposed and Control Rats Weekday.

A statistically significant interaction effect was detected for the monthly analysis of weekend heart rate: $F(9,60) = 2.82$, $p = 0.017$, partial eta-squared = 0.30, power = 0.86. There were significant differences between the groups at baseline, $F(3,20) = 6.55$, $p = 0.003$, with post hoc differences between Control-Day and Control-Night, $p = 0.003$, Control-Day and Exposed-Night, $p = 0.004$, and Exposed-Day and Exposed-Night, $p = 0.01$. At the end of Month 1, there was a statistically significant main effect, $F(3,20) = 27.06$, $p < 0.001$, with post hoc tests showing significant differences between Control-Day and Control-Night, $p < 0.001$ and Exposed-Day and Exposed-Night, $p < 0.001$. At the end of Month 2, there was a significant main effect, $F(3,20) =$

19.00, $p < 0.001$, and post hoc tests showed differences between Control-Day and Control-Night, $p < 0.001$, Control-Day and Exposed-Night, $p < 0.001$, and Exposed-Day and Exposed-Night, $p < 0.001$. At the end of Month 3, there was a significant main effect, $F(3,20) = 17.01$, $p < 0.001$, with significant post hoc differences detected between Control-Day and Control-Night, $p = 0.014$ and Exposed-Day and Exposed-Night, $p < 0.001$. See Table 2 for the marginal means with 95% confidence intervals and Figure 5 for a visual depiction of the interaction.

Table 2. Month-to-month marginal mean heart rates and 95% confidence intervals of exposed and control rats weekend - Interaction and Simple Effects Analysis.

Weekend – Heart Rate		Group	Month 0	Month 1	Month 2	Month 3	Main effect p value
	Monthly						
		Control-Day	307.8 (296.9 – 318.6)	293.1 (284.3 – 301.8)	295.7 (286.5 – 304.9)	301.2 (291.6 – 310.8)	
		Exposed-Day	310.8 (300.0 - 321.7)	293.7 (284.9 – 302.4)	293.7 (284.5 – 302.9)	288.6 (279.0 – 298.2)	
		Control-Night	332.9 (322.0 – 343.8)	332.6 (323.8 – 341.3)	326.9 (317.7 – 336.2)	318.8 (309.2 – 328.5)	
		Exposed-Night	331.7 (320.8 – 342.5)	329.6 (320.9 – 338.4)	329.0 (319.8 – 338.2)	331.7 (322.1 – 341.3)	0.017
	Simple effects p-value		0.003	< 0.001	< 0.001	< 0.001	

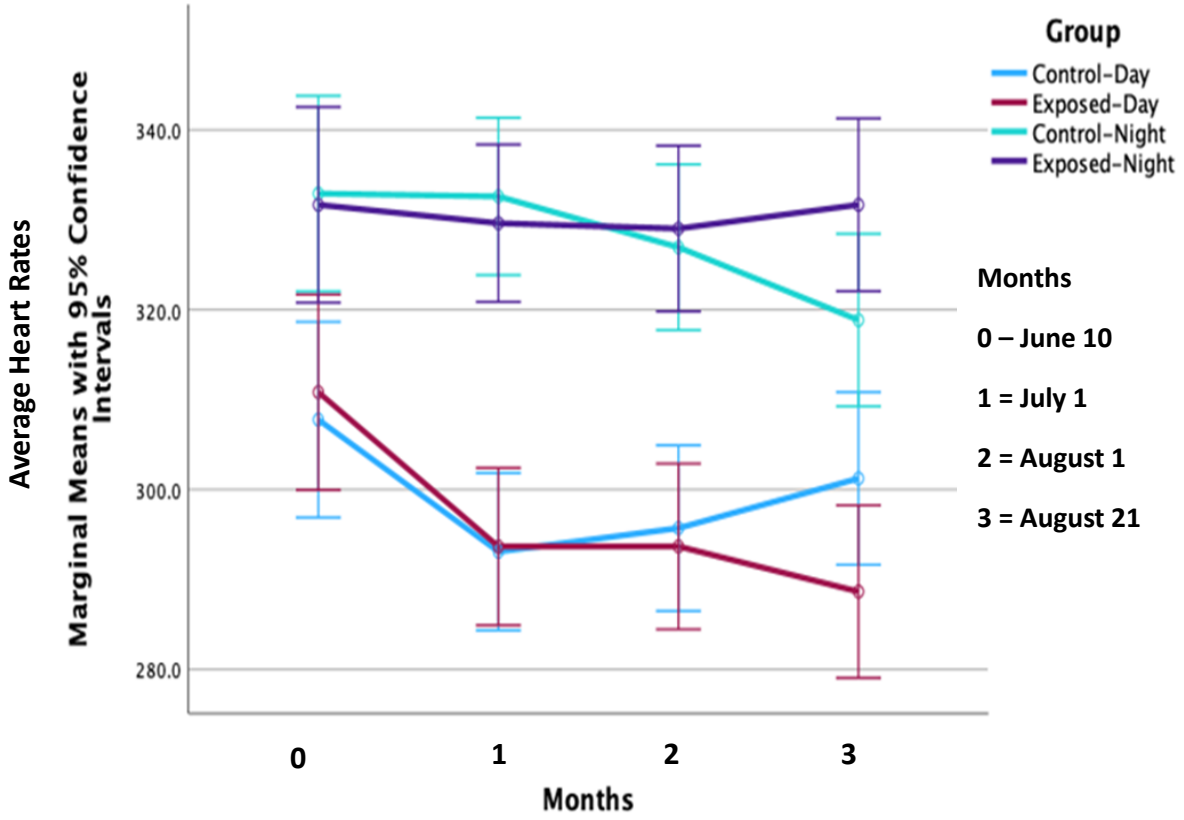


Figure 5. Month to Month Average Heart Rates of Exposed and Control Rats Weekend.

For month-to-month weekend analysis, there were statistically significant differences in heart rates of exposed rats, daytime vs. nighttime (n=6, p<0.001), and control rats daytime *vs.* nighttime (n=6, p<0.014) with lower heart rate of exposed rats daytime.

For beginning to end analysis of exposure heart rate, there was not a statistically significant interaction effect, $F(3,20) = 0.78$, $p = 0.52$, partial eta-squared = 0.10, power = 0.19. There was a significant main effect detected at beginning of the study, $F(3,20) = 17.41$, $p < 0.001$, and post hoc differences were found between Control-Day and Control-Night, $p < 0.001$, Control-Day and Exposed-Night, $p < 0.001$, Exposed-Day and Control-Night, $p < 0.001$, and

Exposed-Day and Exposed-Night, $p = 0.001$. At the end of the study, there was a significant main effect, $F(3,20) = 13.00$, $p < 0.001$, with post hoc differences found between Control-Day and Exposed-Day, $p < 0.001$, Control-Day and Exposed-Night, $p < 0.001$ and Exposed-Day and Exposed-Night, $p = 0.007$. See Table 3 for the marginal means with 95% confidence intervals for this analysis, and the interaction effect is depicted visually in Figure 6.

Table 3. Beginning to End marginal mean heart rates and 95% confidence intervals of exposed and control rats weekday - Interaction and Simple Effects Analysis.

Exposure – Heart Rate	**Start to End**	**Group**	**Start**			**End**	**Main Effect p value**
	Baseline to Post-intervention						
		Control-Day	304.1 (295.5 – 312.7)			278.5 (265.3 – 291.8)	
		Exposed-Day	313.1 (304.5 – 321.7)			286.8 (273.5 – 300.0)	
		Control-Night	339.6 (331.1 – 348.2)			327.6 (314.4 – 340.9)	
		Exposed-Night	335.3 (326.7 – 343.9)			313.5 (300.3 – 326.8)	0.52
	Simple effects p-value		< 0.001			< 0.001	

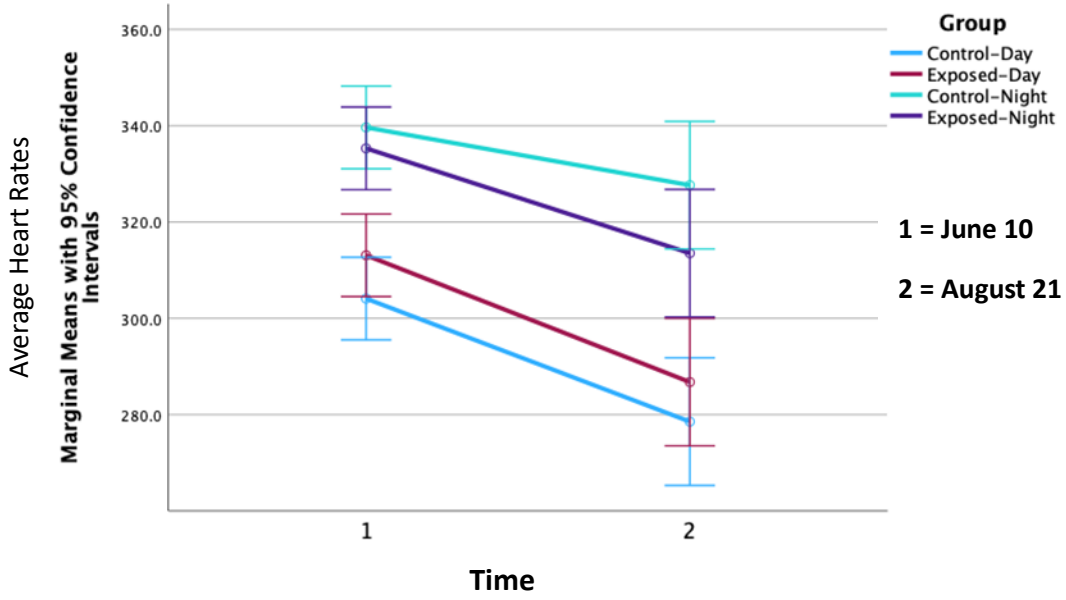


Figure 6. Beginning to End Average Heart Rates of Control and Exposed Rats Weekday.

For the beginning to the end of the study weekday analysis, there were statistically significant differences of average heart rates of exposed rats, daytime *vs.* nighttime (n =6, p = 0.007).

Analysis from beginning to end of weekend heart rate, showed no significant interaction effect, $F(3,20) = 3.07$, $p = 0.052$. There were significant main effects observed at the beginning of the study, $F(3,20) = 6.55$, $p = 0.003$, with post hoc differences found between Control-Day and Control-Night, $p = 0.003$, Control-Day and Exposed-Night, $p = 0.004$, Exposed-Day and Control-Night, $p = 0.007$, and Exposed-Day and Exposed-Night, $p = 0.01$. At the end of the study, a significant main effect was detected, $F(3,20) = 17.01$, $p < 0.001$, and post hoc differences were found between Control-Day and Control-Night, $p = 0.014$, Control-Day and Exposed-Night, $p < 0.001$, Exposed-Day and Control-Night, $p < 0.001$, and Exposed-Day and Exposed-

Night, $p < 0.001$. See Table 4 for the marginal means with 95% confidence intervals and see Figure 7 for a depiction of the interaction.

Table 4. Beginning to End marginal mean heart rates and 95% confidence intervals of exposed and control rats weekend - Interaction and Simple Effects Analysis.

Weekend – Heart Rate		Group	Beginning			End	Main effect p value
	Start to End						
		Control-Day	307.8 (296.9 – 318.6)			301.2 (291.6 – 310.8)	
		Exposed-Day	310.8 (300.0 – 321.7)			288.6 (279.0 - 298.2)	
		Control-Night	332.9 (322.0 – 343.8)			318.8 (309.2 – 328.5)	
		Exposed-Night	331.7 (320.8 – 342.5)			331.7 (322.1 – 341.3)	0.052
	Simple effects p-value		0.003			< 0.001	

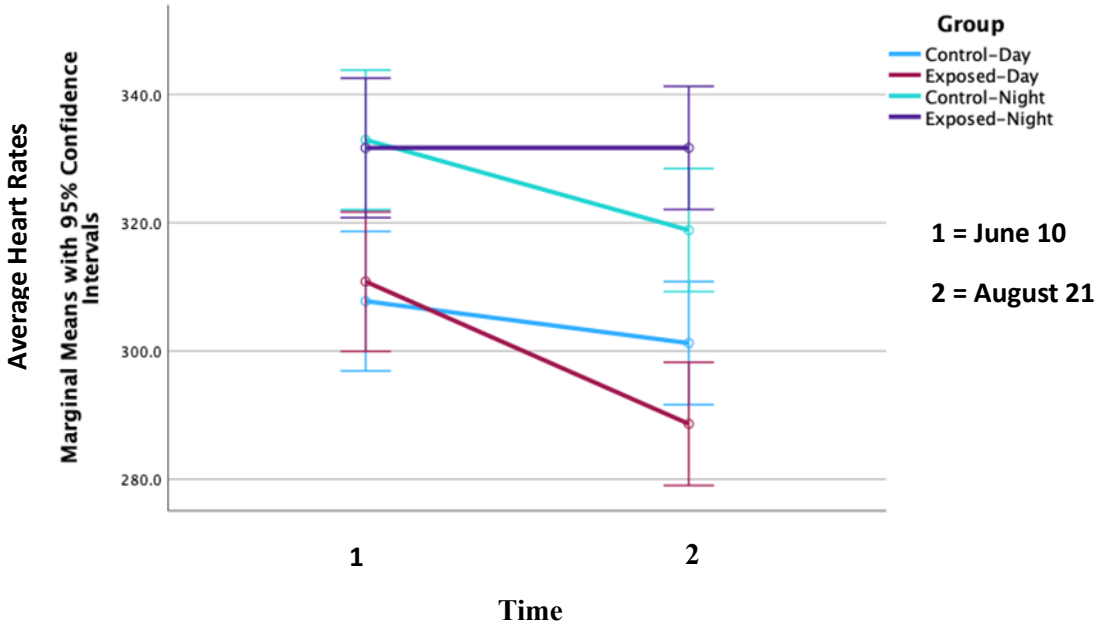


Figure 7. Beginning to End Average Heart Rates of Control and Exposed Rats Weekend.

For the beginning to end of study weekend analysis, there was statistically significant differences of average heart rates of exposed rats, daytime versus nighttime (n =6, p = 0.001),

For the monthly analysis of exposure mean pressure, a statistically significant interaction effect was detected, $F(9,60) = 3.23$, $p = 0.003$, partial eta-squared = 0.33, power = 0.91. There were significant differences between the groups at baseline, $F(3,20) = 4.12$, $p = 0.02$, and post hoc tests showed significant differences between Control-Day and Exposed-Day, $p = 0.04$, and Control-Night and Exposed-Day, $p = 0.002$. At the end of the first month, there was a significant main effect, $F(3,20) = 9.56$, $p < 0.001$, and post hoc differences were found between Control-Day and Control-Night, $p < 0.001$, Control-Day and Exposed-Night, $p = 0.003$, Exposed-Day and Exposed-Night, $p = 0.004$, and Control-Night and Exposed-Day, $p < 0.001$. At the end of Month

2, a significant main effect was detected, $F(3,20) = 4.63$, $p = 0.013$, with post hoc differences detected between Exposed-Day and Control-Night, $p = 0.001$. At the end of Month 3, there was also a significant main effect detected, $F(3,20) = 11.03$, $p < 0.001$, with post hoc differences found between Control-Day and Control-Night, $p = 0.001$, Exposed-Day and Control-Night, $p < 0.001$, Exposed-Day and Exposed-Night, $p = 0.007$, and Exposed-Night and Control-Night, $p = 0.02$. See Table 5 for the marginal means and 95% confidence intervals for these findings and see Figure 8 for a visual depiction of the interaction.

Table 5. Month-to-Month marginal mean blood pressures and 95% confidence intervals of Exposed and Control Rats Weekday- Interaction and Simple Effects Analysis.

Exposure-Mean Pressure		Group	Month 0	Month 1	Month2	Month 3	Main effect p value
	Monthly						
		Control-Day	141.1 (136.5 – 145.6)	136.4 (130.9 – 141.9)	146.4 (140.1 – 152.7)	149.9 (144.6 – 155.2)	
		Exposed-Day	134.3 (129.7 – 138.8)	136.9 (131.4 – 142.4)	137.6 (131.3 – 144.0)	143.3 (138.0 – 148.7)	
		Control-Night	145.1 (140.5 – 149.6)	152.1 (146.6 – 157.6)	153.6 (147.2 – 159.9)	163.6 (158.3 – 169.0)	
		Exposed-Night	140.1 (135.5 – 144.7)	149.0 (143.5 – 154.5)	145.3 (139.0 – 151.6)	154.1 (148.8 – 159.5)	0.008
	Simple effects p-value		0.02	< 0.001	0.013	< 0.001	

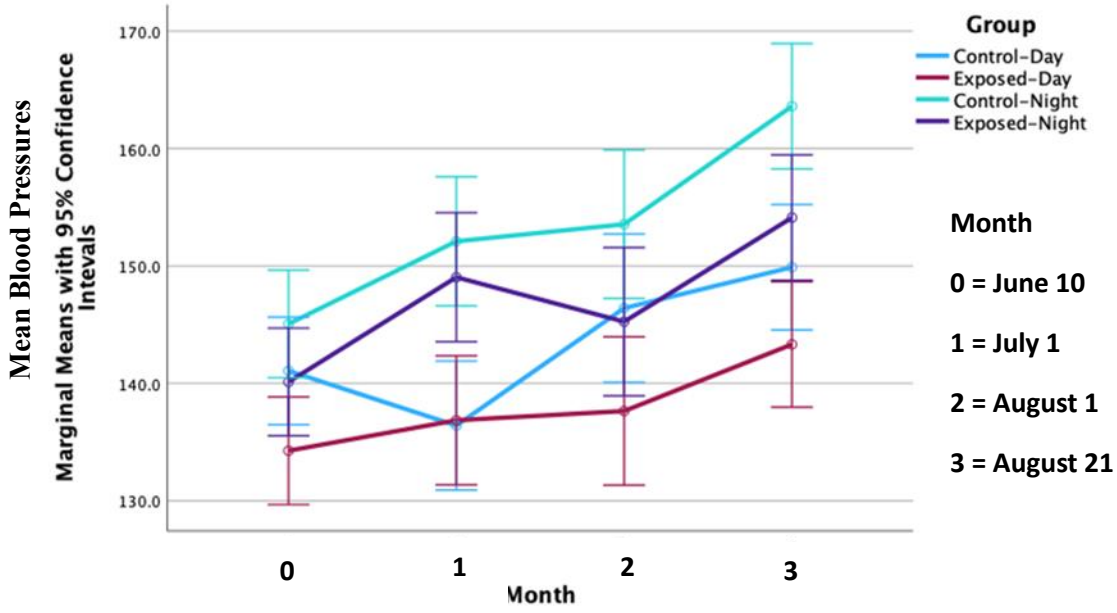


Figure 8. Month-to-Month Mean Blood Pressures of Exposed and Control Rats Weekday.

For month-to-month weekday analysis, there were statistically significant mean blood pressure differences, daytime vs nighttime of exposed rats (n =6, p = 0.007) and daytime vs nighttime of control rats (n = 6, p = 0.001).

For the monthly analysis of weekend mean pressure, there was not a statistically significant interaction effect, $F(9,60) = 1.24$, $p = 0.31$. There were no simple main effects detected at baseline, $F(3,20) = 0.38$, $p = 0.77$, the end of the first month, $F(3,20) = 1.35$, $p = 0.29$, the end of the second month, $F(3,20) = 0.87$, $p = 0.48$, or at the end of the third month, $F(3,20) = 1.51$, $p = 0.24$. The marginal means with 95% confidence intervals for these findings are presented in Table 6 and depicted visually in Figure 9.

Table 6. Month-to-month marginal mean blood pressures and 95% confidence intervals of Exposed and Control Rats Weekend- Interaction and Simple Effects Analysis.

Weekend – Mean Pressure		Group	Month 0	Month 1	Month 2	Month 3	Main effect p value
	Monthly						
		Control-Day	143.0 (138.4 – 147.7)	146.5 (140.4 – 152.5)	153.0 (145.7 – 160.4)	155.8 (150.5 – 161.2)	
		Exposed-Day	142.9 (138.3 – 147.6)	151.3 (145.3 – 157.4)	155.7 (148.4 – 163.0)	154.9 (149.5 – 160.2)	
		Control-Night	145.5 (140.8 – 150.1)	150.5 (144.4 – 156.5)	155.8 (148.4 – 163.1)	158.6 (153.2 – 164.0)	
		Exposed-Night	145.2 (140.5 – 149.8)	154.7 (148.6 – 160.8)	160.9 (153.5 – 168.2)	161.9 (156.5 – 167.3)	0.31
	Simple effects p-value		0.77	0.29	0.48	0.24	

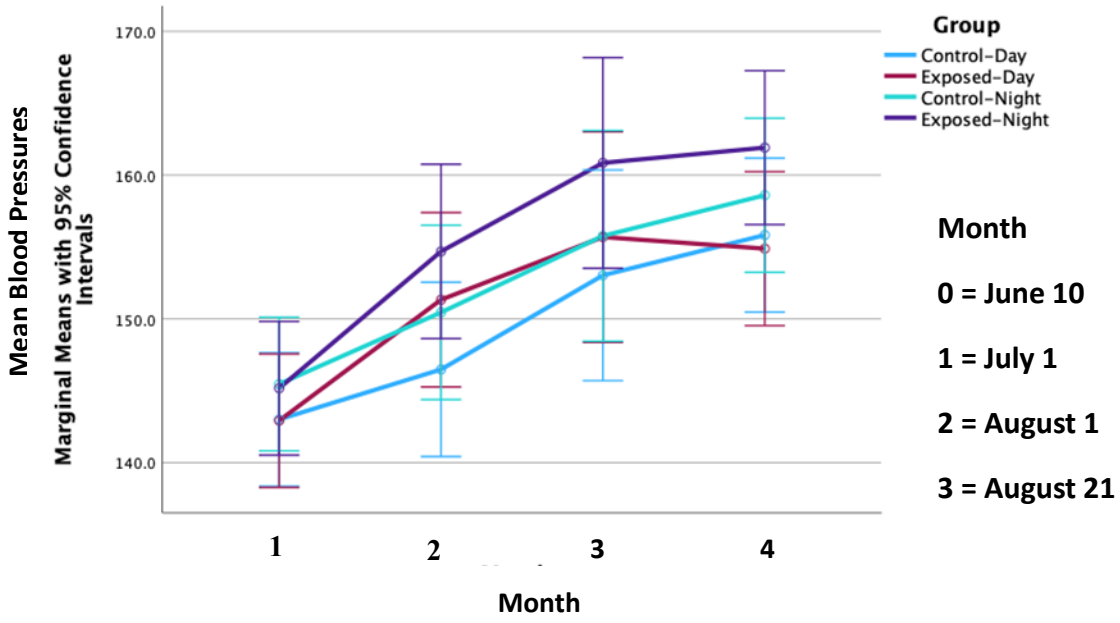


Figure 9. Month-to-Month Mean Blood Pressures of Exposed and Control Rats Weekend.

There were no statistically significant differences of mean blood pressures of exposed rats and control rats in the weekend.

For the beginning to end of study analysis of exposure mean pressure, a statistically significant interaction effect was detected, $F(3,20) = 3.12$, $p = 0.049$, partial eta-squared = 0.32, power = 0.64. There was a significant main effect at the beginning of the study, $F(3,20) = 0.02$, with post hoc differences between Control-Day and Exposed Day, $p = 0.05$, and Exposed-Day and Control-Night, $p = 0.002$. A significant main effect was also detected at the end of the study, $AF(3,20) = 11.03$, $p < 0.001$, with post hoc differences found between Control-Day and Control-Night, $p = 0.001$, Exposed-Day and Control-Night, $p < 0.001$, Exposed-Day and Exposed-Night, $p = 0.007$, and Control-Night and Exposed-Night, $p = 0.016$. The marginal means with 95%

confidence intervals can be found in Table 7, and the interaction is depicted visually in Figure 10.

Table 7. Beginning to End marginal mean blood pressures and 95% confidence intervals of Exposed and Control Rats Weekday- Interaction and Simple Effects Analysis.

Mean Blood Pressure Weekday	Beginning to End	Group	Starting			End	Main effect p value
		Control-Day	141.1 (136.5 – 145.6)			149.89 (144.6 – 155.2)	
		Exposed-Day	134.3 (129.7 – 138.8)			143.3 (138.0 – 148.7)	
		Control-Night	145.1 (140.5 – 149.6)			163.6 (158.3 – 169.0)	
		Exposed-Night	140.1 (135.5 – 144.7)			154.1 (148.8 – 159.5)	0.049
	Simple effects p-value		0.02			< 0.001	

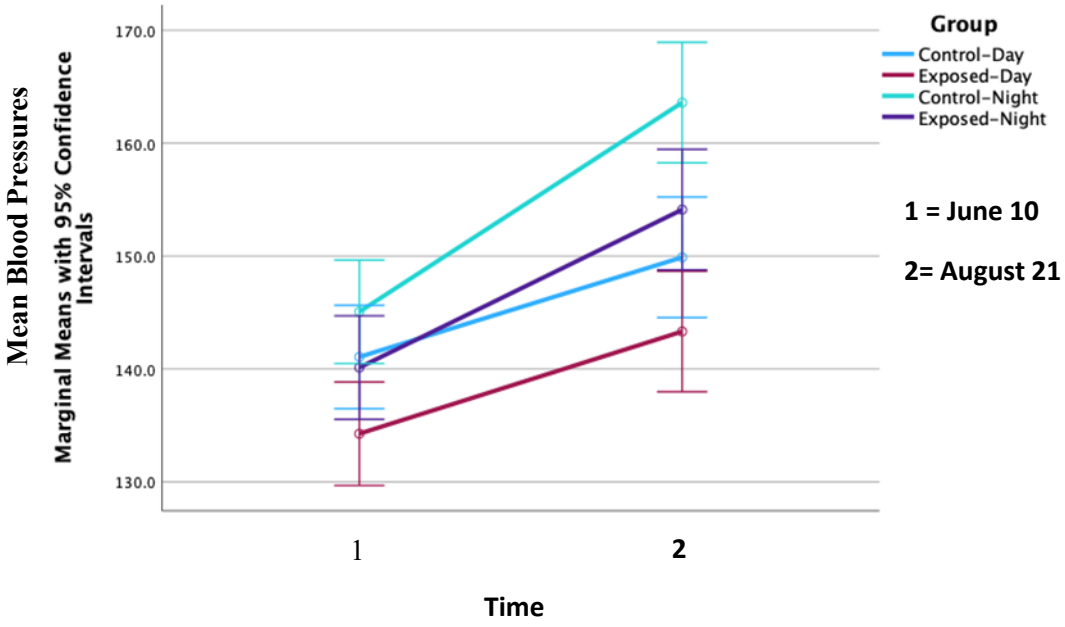


Figure 10. Beginning to End Mean Blood Pressures of Exposed and Control Rats Weekday.

There were statistically significant differences of mean blood pressure, weekday daytime vs nighttime of exposed rats (n =6, p = 0.007) and weekday daytime vs nighttime of control rats (n = 6, p = 0.001).

For the beginning to end of study analysis of weekend mean pressure, there was no statistically significant interaction effect, $F(3,20) = 0.98$, $p = 0.42$. There were no significant main effects detected at the beginning of the study, $F(3,20) = 0.38$, $p = 0.77$, or at the end of the study, $F(3,20) = 1.51$, $p = 0.24$. The marginal means with 95% confidence intervals for this analysis are presented in Table 8, and the interaction is depicted in Figure 11.

There were no statistically significant changes in mean blood pressures of exposed or control rats in the weekend, daytime vs nighttime.

Table 8. Beginning to End marginal mean blood pressures and 95% confidence intervals of Exposed and Control Rats Weekend- Interaction and Simple Effects Analysis.

Mean blood Pressure Weekend	Beginning to End	Group	Beginning			End	Main effect p value
		Control-Day	143.1 (138.4 – 147.7)			155.8 (150.5 – 161.2)	
		Exposed-Day	142.9 (138.3 – 147.6)			154.9 (149.5 – 160.2)	
		Control-Night	145.5 (140.8 – 150.1)			158.6 (153.2 – 164.0)	
		Exposed-Night	145.2 (140.5 – 150.0)			161.9 (156.6 – 167.3)	0.42
	Simple effects p-value		0.77			0.24	

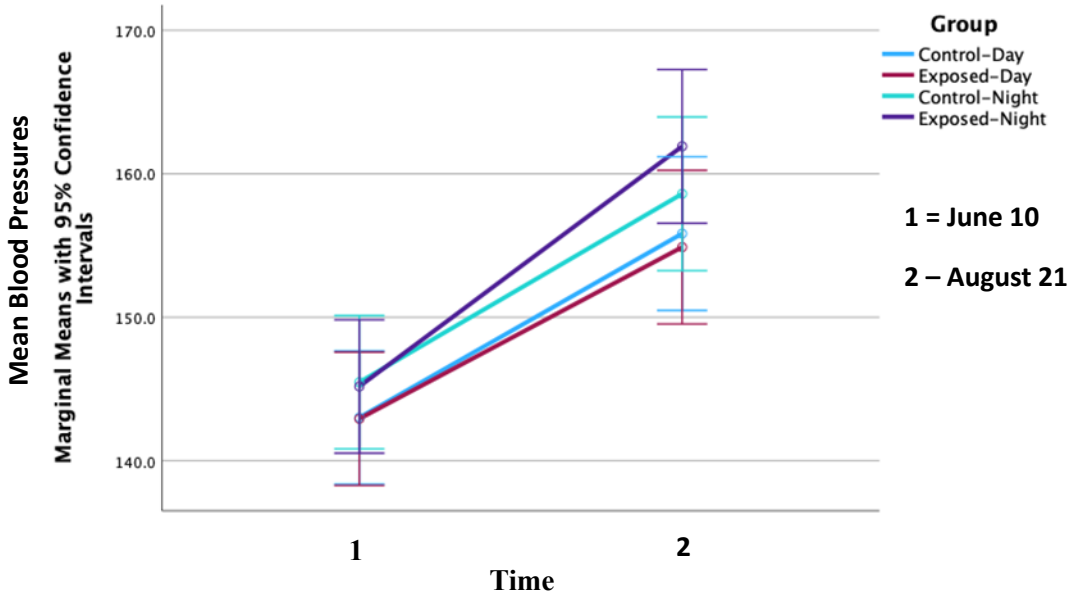


Figure 11. Beginning to End Mean Blood Pressures of Exposed and Control Rats Weekend.

7. DISCUSSION

Course of hypertension development in young spontaneously hypertensive rats (SHR)

The measurement of changes in systolic blood pressure (BP), body weight, and heart rate (HR) of SHRs and Wistar-Kyoto (WKY) rats at 2, 3, 4, and 6 weeks of age was performed by Dickhout and Lee[38]. Systolic BP was similar between SHR and WKY at 2 and 3 weeks of age. Differences were small in the 4th week, and by the 6th week, systolic BP in SHR had become significantly elevated compared with that in WKY. In the 2nd week, most of the variation in systolic BP was found between individuals. At 3rd and 4th week, differences between inbreeding lines accounted for most of the variation in systolic BP, with differences between strains smaller than those between lines. However, by the 6th week, differences between strains overshadowed the differences between inbreeding lines. The period of prehypertensive tachycardia for SHR was found between 2 and 4 weeks. The subsidence of tachycardia and an increase in systolic BP of SHR compared with that in WKY became apparent in rats older than 4 weeks.

The sympathetic component of the autonomic tone in both strains at 4 weeks of age increased the intrinsic HR an average of 16 beats/min in WKY and 31 beats/min in SHR or as a percentage of intrinsic rate (SHR = 23% and WKY = 20%). After the removal of all autonomic tones, SHR still showed a significantly higher intrinsic HR than that in WKY. The parasympathetic component of the autonomic tone of both strains at 4 weeks of age decreased the intrinsic rate by 16% in SHRs and 17% in WKY rats.

In our study, there were two important findings:

1. Statistically Significant Reduction of Heart Rates of Exposed Rats after Repeated $PM_{2.5}$ Exposure. The average age of our exposed and control rats is 8 weeks old. If we extrapolate from the graph of Heart Rate in Figure 12, the expected heart rate at 8-week-old is about 350 beats per minute (bpm). Figure 13 shows that the intrinsic heart rate of spontaneously hypertensive rats at 4 weeks old has a mean of 410 bpm and a range from 350 bpm (with parasympathetic stimulation) to 510 bpm (with sympathetic stimulation). As the spontaneously hypertensive rat becomes older, the intrinsic heart rate should also decline, with a mean of nearly 350 bpm. In our experiment, there is a statistically significant reduction of the heart rates of exposed rats to 275 bpm (daytime weekday), 305 bpm (nighttime weekday), and 290 bpm (daytime weekend),

There are two possible explanations for the reduction of average heart rate following $PM_{2.5}$ exposure:

A. Exposed rats initially had a highly responsive and competent parasympathetic nervous system, which reduces the heart rate and counters the elevation of blood pressure caused by $PM_{2.5}$-induced sympathetic nervous system activation.

B. SHRs develop an adaptive response to repeated $PM_{2.5}$ exposure by strengthening the parasympathetic nervous system, leading to a continuous reduction of average heart rates later.

Previous research studies show an increase in heart rate and mean blood pressure in SHRs. Chang et al. (2004)[39] reported that increased exposure to concentrated $PM_{2.5}$ may be responsible for the increase in heart rate and mean blood pressure in spontaneously hypertensive rats (SHRs) in the spring and summer months. In this study, SHRs were exposed to concentrated

ambient particles for 2 days in February, 2 days in March, 5 days in June, and 1 day in July. Also, SHRs served as exposure and control groups alternatively. Wagner et al. (2014)[40] showed that mean heart rate and blood pressure were increased, while the heart rate variability was decreased over 4 days of exposure to $PM_{2.5}$. Our experiment was conducted over a period of 11 weeks, thus the data reflecting the change in the average heart rates of the exposed rats on a sub chronic basis.

For our experiment, the temperature was kept constant for both the exposed rats and the control rats. Wang and Ogawa (2015)[41] reported that precipitation had a negative correlation with $PM_{2.5}$, while temperature was positively correlated with $PM_{2.5}$. In other words, a rise in temperature would correlate with an increase in the number of $PM_{2.5}$ in the air.

2. Statistically Significant Increase of Mean Blood Pressures of Exposed and Control Rats after Repeated $PM_{2.5}$ Exposure Weekday, Daytime vs Nighttime. And No Statistically Significant Differences in Mean Blood Pressure on the Weekend.

Spontaneously hypertensive rats are prone to develop higher blood pressure with age. With repeated $PM_{2.5}$ exposure, the elevation of mean blood pressures of exposed rats is not as vigorous as the control rats during the weekday, daytime (Figure 8). The finding implies that the exposed rats may have developed an adaptation to the elevated blood pressure, thereby suppressing the effects of the sympathetic nervous system. This adaptation appears to be transient as the blood pressures of the exposed rats rise above those of the control rats in the weekend nighttime (Figures 9, 11). Our findings are consistent with prior studies (Zhang et al.

2022)[42], Ying et al. (2014)[43], and Liang, et al. (2014)[44], which reported a positive association between acute and chronic $PM_{2.5}$ exposure and blood pressure.

Average Heart Rate and Mean Blood Pressure Relationship

In our study, the reduction of the average heart rate (HR) of exposed rats appears to compensate for the increase in the mean BP, thus allowing them to maintain a state of homeostasis. With prolonged $PM_{2.5}$ exposure, there is a general trend of increase in heart rate x blood pressure product. The finding implies that most of the increase is caused by an elevation of blood pressure**.**

Possible Mechanisms Proposed for Elevation of Blood Pressures after $PM_{2.5}$ Exposure.

$PM_{2.5}$ was found to increase the blood pressure of rodents by activating toll-like receptor 3 (TLR3)[45]. Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) that recognize molecular patterns correlated to microbial pathogens and defense against pathogens[46]. TLRs activation can induce low-grade vascular inflammation, modulate vascular function, and thus lead to hypertension.

Cuimei et al. (2021)[47] suggest that paternal $PM_{2.5}$ exposure causes hypertension in offspring. The mechanism that could be involved in paternal $PM_{2.5}$ exposure-associated oxidative stress induces the elevated renal G-protein–coupled receptor kinase type 4 (GRK4) level, leading to the enhanced angiotensin II type 1 receptor (AT_1R) expression and its-mediated sodium retention, consequently, causes hypertension in male offspring. See Figure 12.

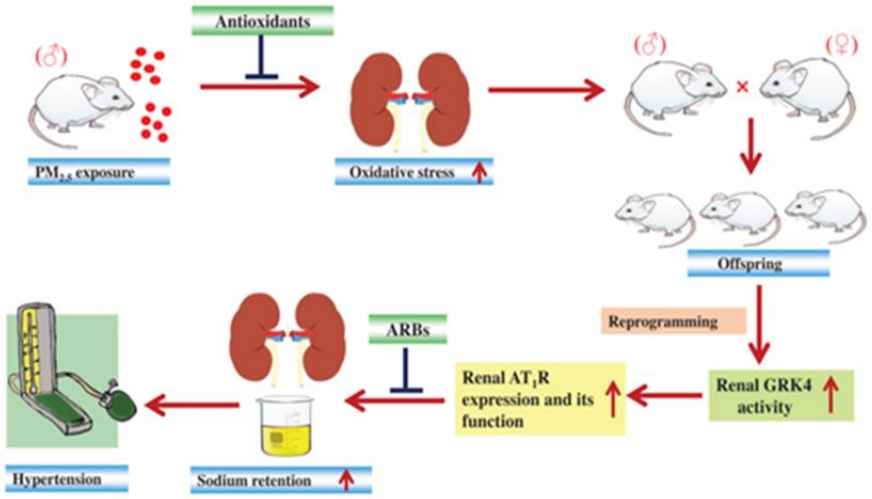


Figure 12. Paternal long-term $PM_{2.5}$ exposure causes hypertension via increased renal AT1R expression and function in male offspring. (Adapted from Cuimei et al, 2021)[47].

Aztatzi-Aguilar et al. (2015)[48] found that acute and subchronic exposure to air particulate matter induces the expression of angiotensin and bradykinin-related genes in the lungs and heart. Angiotensin-II type-I receptor serves as a molecular target of particulate matter exposure.

Finally, Ying et al, (2014)[49], proposed that long-term exposure to concentrated ambient $PM_{2.5}$ increased basal blood pressure (BP) by inducing an inflammatory response in the arcuate nucleus of the hypothalamus.

Mechanism of $PM_{2.5}$ Effects on the Cardiovascular System

There is a proposed model that explains the pathophysiological and molecular mechanisms of atmospheric $PM_{2.5}$ affecting cardiovascular health[50]. See Figure 13.

As $PM_{2.5}$ penetrates deeply into the respiratory tract, it may disrupt multiple physiological barriers to integrity and translocate from the lung into the systemic circulation, gaining access to a range of secondary target organs, including the heart, kidney, liver, spleen, lymph nodes, and brain in humans and animal models[51,52].

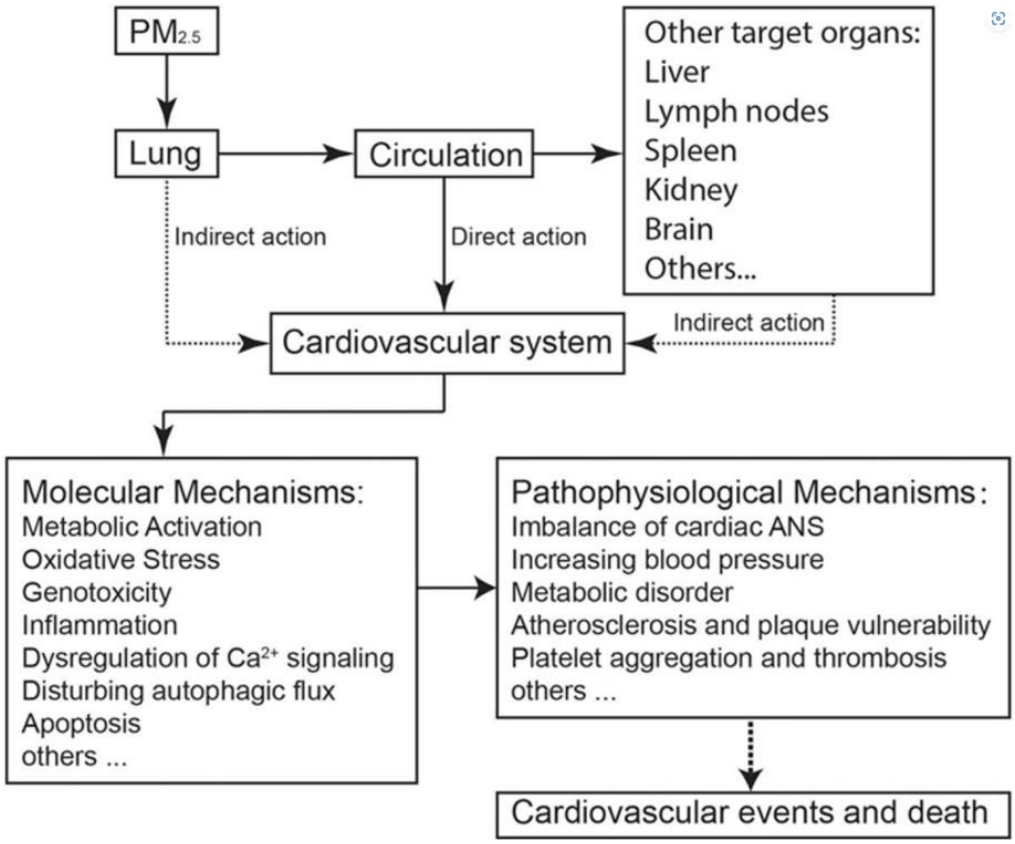


Figure 13. Pathophysiological and molecular mechanisms of $PM_{2.5}$ affecting cardiovascular health (adapted from Shaolong et al, 2023)[50].

$PM_{2.5}$ can affect the cardiovascular systems through molecular mechanisms such as metabolic activation, oxidative stress, inflammation, dysregulation of Ca^{2+} signaling, and apoptosis. PM inhalation activates inflammatory responses in the lung, leading to systemic

inflammation, which promotes thrombosis, endothelial dysfunction, and atherosclerosis. Inhaled PM can also activate sensory receptors in the lung, leading to imbalance of the autonomic nervous system (ANS), favoring sympathetic pathways, and leading to alterations in heart rate, vasoconstriction, endothelial dysfunction, and hypertension.

A number of studies have shown that $PM_{2.5}$ can impair the function of the cardiac autonomic nervous system (ANS) and lead to a decline in heart rate variability (HRV), which is considered an independent risk factor for cardiovascular morbidity and mortality[53,54]. Induced oxidative stress and inflammatory impairments in the central nervous system, especially in the hypothalamus, were suggested to be the important mechanisms underlying this abnormal activation of the ANS[49, 54]. The $PM_{2.5}$-induced oxidative stress and inflammation in the hypothalamus may lead to dysfunctions of its neuroendocrine, such as an increase of norepinephrine and 5-hydroxy-indole acetic acid in its paraventricular nucleus and corticotrophin-releasing hormone levels in the median eminence. As a result, $PM_{2.5}$ exposure would elicit the dysfunctions of the ANS, leading to a decline in HRV and ultimately increasing cardiovascular diseases.

$PM_{2.5}$ can also trigger a battery of pathophysiological responses that increase blood pressure and result in the development of hypertension[49,54]. The specific biological mechanisms have been suggested to include an increase in sympathetic tone and/or the modulation of basal systemic vascular tone[49] and endothelial and vascular dysfunctions[55]. The endothelium acts to maintain vascular homeostasis. The systemic inflammation and oxidative stress following $PM_{2.5}$ exposure will trigger endothelial dysfunction and lead to vasoconstriction[56], resulting in higher vascular resistance and, ultimately, hypertension.

Moreover, $PM_{2.5}$ exposure has been shown to link to cardiovascular diseases via accelerated atherosclerosis (AS), which is a chronic disease of the arterial wall[57]. Both inflammation and endothelial dysfunction are considered to be the important mechanisms that trigger AS[58].

Also, $PM_{2.5}$ may directly act on the heart and induce cardiac tissue remodeling and function altering, leading to the occurrence and development of cardiac diseases. Cardiac histopathology results have revealed $PM_{2.5}$ deposition and myocardial inflammation in the tested rats[54]. Long-term $PM_{2.5}$ exposure could induce obvious myocardial ultra-structural changes (with increased hypertrophic markers), and lead to adverse ventricular remodeling[54,59].

In summary, following repeated $PM_{2.5}$ exposure, there was a statistically significant reduction in average heart rates and a statistically significant increase in mean blood pressures of exposed rats. The reduction in heart rate is mainly due to overcompensation of the parasympathetic system to counter the elevation of blood pressure and maintain a state of homeostasis. The implications are as the blood pressure continues to rise, the heart must work harder, and the blood vessel walls stiffen. The cardiovascular end results are uncontrolled hypertension, arrhythmia, myocardial infarction, and heart failure. In particular, for individuals with a preexisting elevation of blood pressure, repeated exposure to $PM_{2.5}$ appears to accelerate the progression of hypertension and its effects on the end organs such as the heart, brain, and kidneys.

Strengths of the study

The main strength of this study is the continuous monitoring of the average heart rates and mean blood pressures of exposed and control rats over the course of 11 weeks of $PM_{2.5}$ exposure. Also, the control conditions of exposure and the use of the SHR model.

Limitations of the study

There are limitations to this study. First, the study has a small sample size of 6 exposed SHRs and 6 control SHRs, which limits its power. Secondly, all the study rats are male. In this regard, it is uncertain where the heart rates and blood pressures of female SHRs would respond to repeated $PM_{2.5}$ exposure in the same way as their counterparts. Finally, the study rats were only exposed to concentrated ambient particles (CAPs). The advantage of using CAPs is that the $PM_{2.5}$ exposures closely match the human exposures in the regional population. The main disadvantage is that these exposures are difficult to compare when obtained from different regions because $PM_{2.5}$ composition will vary widely both regionally and temporally.

8. CONCLUSIONS

Following repeated $PM_{2.5}$ exposure over the course of 11 weeks, there was a statistically significant reduction in average heart rates and a statistically significant increase in mean blood pressures of spontaneously hypertensive rats**. These findings support the hypothesis that repeated exposure to $PM_{2.5}$ decreases the average heart rate and increases the mean blood pressure of spontaneously hypertensive rats.**

9. FUTURE STUDIES

Our study has shown a statistically significant reduction in average heart rates and a statistically significant increase in mean blood pressures of male spontaneously hypertensive rats after repeated exposure to $PM_{2.5}$. However, the current study has a small group size and comprises only male subjects. Future endeavors should include a larger study to determine which systemic biochemical markers (c-reactive protein (CRP), sedimentation rate, fibrinogen, or lactate) would assist in identifying the pathological processes that precede the onset of hypertension or in tracking its progression, as well as for predicting greater risk of developing hypertension in exposed individuals. In addition, future studies could focus on the effects of $PM_{2.5}$ on the heart rate variability and blood pressure of normotensive young male and female rats. Recognition and treatment of hypertension in young individuals would likely prevent the dreaded complications of uncontrolled hypertension in the future.

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