The Effects of Negative Ion Indoor Air Filtration on Selected Biomarkers in Healthy Adults

A Randomized Double-Blind Crossover Trial

By

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Executive Summary

Air pollution is a significant challenge in environmental health. According to the World Health Organization (WHO), about 91% of world's population live in areas where WHO's air quality guidelines were not met. Ambient air pollution causes approximately 4.2 million premature deaths worldwide. While outdoor ambient air quality is associated with global mortality, indoor air pollution also possesses a significant challenge to people's health and well-being. In countries with less desirable air quality, the use of air filtration devices is common. Despite the rise of negative ion air filtration device usage, the purification efficiency and health effects of these devices remain unclear.

The goal of this project is to evaluate health impacts of indoor negative ion air filtration intervention in healthy young adults. The intervention is hypothesized to reduce indoor fine particulate matter exposure and reduce adverse health effects associated with indoor $PM_{2.5}$ pollution. We conducted a randomized, double-blind, cross-over study with two specific aims: first, to evaluate the effectiveness of $PM_{2.5}$ removal by negative ion air filtration device; second, to evaluate differences in health endpoints associated with $PM_{2.5}$ exposure between the two interventions.

Fifty-five healthy adults participated in this study. Each participant received a random sequence of true and sham filtration intervention, with two weeks of washout period in between. Before and after each intervention period, these participants provided biological samples so we could measure specific biomarkers of interest to assess health impacts of each intervention. My project only assessed urinary biomarkers.

Overall, only one out of the five biomarkers selected has statistically significant result. No significant difference between true and sham intervention is observed for urinary fMDA and (biomarkers of lipid peroxidation, reflecting cell membrane damage), 8-OHdG (a biomarker of oxidative damage to DNA), and 11-OHTXB1 (a biomarker of platelet activation, reflecting thrombosis risk). The findings indicate that the negative ion filtration did not lead to significant changes in biomarkers expected to be associated with fine particle exposure reduction. More research is needed to investigate other health endpoints and long-term changes associated with the use of negative ion air filtration device.

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1. Introduction

1.1 Air Pollution and Health

There is a clear, well-established association between fine particulate air pollution and mortality rates in the United States (Dockery *et al,* 1993). According to the World Health Organization (WHO) fact sheet released in 2018 on ambient air pollution, about 91% of world's population live in areas where WHO's air quality guidelines were not met, and it causes approximately 4.2 million premature deaths worldwide (WHO, 2018). Furthermore, this mortality associated with ambient air pollution is due to the exposure to small fine particulate matter with a diameter of 2.5 microns or less, also known as $PM_{2.5}$. WHO had estimated a majority of these premature deaths were attributable to ischemic heart disease, stroke, and chronic obstructive pulmonary disease (COPD). Long-term exposure to $PM_{2.5}$ contributes to higher risks of cardiovascular and respiratory diseases due to fine particular matter's ability to enter into human respiratory system, penetrate the lung-blood barrier, circulate in the vascular system, and affect cardiovascular and pulmonary function (Polichetti *et al.* 2009).

However, air pollution is not a problem limited to the outdoors. Indoor household air pollution also possesses a significant challenge to people's well-being. People are not only worried about the outdoor environment, but also are concerned about indoor exposure to ambient air pollutants. According to the newest Exposure Factors Handbook released by the U.S Environmental Protection Agency (U.S EPA), an average adult between 18 and 64 years of age would spend approximately sixty-six percent of their time indoors, and around eighty-two percent of the time indoors as toddlers and adults older than 64 years old (U.E EPA, 2011). It is evident that people spend the majority of their time indoor, and thus it is important to shift

research focus from outdoor ambient air quality to indoor air pollution as a result of poor ambient air quality and review potential health implications.

1.2 Air Filtration Technology

In countries with less desirable air quality, the use of air filtration devices is common. One of the most popular air purifier types is a HEPA air purifier. According to the U.S Department of Energy, HEPA air purifiers can efficiently remove more than 99.9% of particulate matter with a diameter greater than 0.3 microns, a size much smaller than the pollutant of concern established in this research (U.S. DOE, 2005). Correspondingly, research has demonstrated the effectiveness of HEPA air purification with positive health outcome associated HEPA intervention in healthy adults and asthmatic children (Allen *et al.* 2011; Lanphear *et al.* 2011)

Ito and Zhang estimated air purifier sale in China from January 2006 to December 2014, during which China had experienced its worst air pollution in major cities such as Beijing and Shanghai. They estimated that approximately 17% of households in Beijing and around 9% of households in Shanghai and Shenzhen had purchased some form of air purification device during the time period (Ito and Zhang, 2020). While most purchases involved HEPA filtration, some does not have HEPA filtration. Instead, negative ion, or anion, air filtration devices has become popular among households. Negative ion air filtration devices are marketed as a new generation of air filtration with health benefits associated with reduced $PM_{2.5}$ and exposure to anion. Comapred to HEPA filtration, the negative ion technology has lower initial and maintenance costs; it uses less electricity to achieve the same amount of PM removal; it also generates lower noise. However, the health effects of negative ion air filtration devices remains unknown despite its popularity.

1.3 Mechanistic Review of Fine Particulate Matter

In addition to technological, epidemiological, and public health perspectives of air pollution, it is critical to discuss air pollutants, particularly fine particulate matter, in the context of toxicology. Particulate matter (PM) is a complex mixture of chemicals with varying physical and chemical properties. Fine PM, or $PM_{2.5}$, are PM with a diameter of 2.5 microns or less. $PM_{2.5}$ is a ubiquitous air pollutant, often associated with anthropogenic activities such as fuel combustions and industrial emissions, can be inhaled through air exchange (U.S EPA, 2016).

The physical size of $PM_{2.5}$ undermines its exposure pathway in the human body and exacerbates adverse health outcomes associated with its exposure. $PM_{2.5}$ has the capacity to penetrate into the lung, irritate the alveoli, and induce an inflammatory response of the respiratory system in addition to its damages to the lung tissues. Currently in the research field, there are three proposed mechanisms of action of $PM_{2.5}$ in the respiratory system (Xing *et al.* 2016). The first potential mechanism is free radical peroxidation. Due to the complex mixture of $PM_{2.5,}$ it is possible that these components of $PM_{2.5}$ can induce free radicals combined with a decrease in antioxidant defense and result in oxidative stress (Kelly F, 2003). Further studies have demonstrated $PM_{2.5}$ ability to generate reactive oxygen species (ROS) such as the hydroxyl radical, that are known to cause oxidative damage to DNA (Dellinger *et al.* 2001; Vidrio *et al.* 2009). The second mechanism involves calcium-mediated inflammatory response and induced ROS and free radical generation (Brown *et al.*2004). The last mechanism is inflammatory damage. As the name suggest, it is proposed that $PM_{2.5}$ stimulates inflammatory cytokines expression and reduces inflammation-inhibiting cytokines expression, causing lung damage (Xing *et al.* 2016).

Based on previous research, key mechanisms of action of $PM_{2.5}$ includes ROS generation and induced oxidative stress, calcium-mediated inflammatory response and free radical generation, and inflammatory response. Understanding these mechanisms of action is critical in exploring and evaluating biomarkers associated with $PM_{2.5}$ exposure in this project, as the study is designed to examine the health effects of PM2.5 reduction brought by negative ion filtration.

1.4 Biomarkers of Interest

Given $PM_{2.5}$'s mechanisms of action in cardiovascular and respiratory system, it is important to assess health impacts of $PM_{2.5}$ and effectiveness of negative ion/anion air filtration device with given biomarkers, chemicals that are representative of the molecular processes or mechanisms of interest. As discussed in the previous section, lipid peroxidation by reactive oxygen species under oxidative stress is critical in evaluating the damages caused by $PM_{2.5}$ exposure. In addition, it is also essential to study the inflammatory response initiated under $PM_{2.5}$ exposure. Thus, the selection of urinary oxidative stress and inflammatory biomarkers, free malondialdehyde (fMDA), total malondialdehyde (TMDA), 8-isoprostane, 8-hydroxy-2'deoxyguanosine (8OHdG), and 11-dehyrothromboxane (11-OHTXB12) is appropriate to evaluate the effectiveness of negative ion air filtration device in reducing $PM_{2.5}$ pollutant exposure.

MDA, as a product of lipid peroxidation, is a recognized biomarker for oxidative stress. MDA occurs in biological systems as both free state and covalently bound form. MDA can be released from its bound forms by acid or alkali digestion through hydrolysis. The tribarituric acid (TBA) test has been widely used to measure lipid peroxidation since it was introduced, and measurement of MDA is further improved by eliminating interfering chromogens by High-Performance Liquid Chromatography (HPLC). Many studies have used MDA as a biomarker for

oxidative stress as a result of PM exposure. Positive association between personal PM2.5 exposure and blood plasma MDA were found in Sorensen et al. 2002, with no significant relationship found between background PM2.5 and biomarkers. Exposure to ultrafine particles results in an inhibition of anti-inflammatory capacity of lipoprotein and systemic oxidative stress characterized by significant increase in hepatic MDA levels, which again confirmed that adverse cardiovascular effects of ambient PM exposure (Araujo *et al.* 2008). In addition, MDA was measured in exhaled breath condensate to evaluate oxidative stress as a result of wood smoke Barregard *et al*. 2008; Gong *et al.* 2013). The use of fMDA and TMDA can facilitate a better understanding of MDA concentration in urine, thus both forms of MDA are measured. 8-Isoprostane is also a recognized biomarker of oxidative stress. 8-Isoprostane is formed through the peroxidation of arachidonic acid catalyzed by free radicals (Montuschi *et al.* 1999). 8-Isoprostane belongs to F_2-Isoprostanes which are produced via arachidonic acid peroxidation but can undergo beta peroxidation under normal conditions and found at elevated levels based on environmental exposure (Chen *et al.* 2007). 8OHdG is a critical biomarker of oxidative stress. 8OHdG is one of the critical forms of free-radical induced oxidative lesions. By measuring 8OHdG level in the body, it can be used as a measurement of endogenous oxidative DNA damage (Valavanidis *et al.* 2009). Urinary 11-OHTXB12 is a good indicator of platelet thromboxane generation in the blood. It is biologically inactive with a long circulating half-life. Studies have shown that an increase in particulate matter is associated with an increase in platelet activation that could lead to vascular dysfunction and inflammation, increasing the risk of thrombosis (Bacerra *et al*. 2017; Hoek *et al* 2013)

1.5 Study Aims

The goal of this project is to evaluate health impacts of indoor negative ion air filtration intervention in healthy young adults. The intervention is hypothesized to reduce indoor fine particulate matter exposure and reduce adverse health effects associated with indoor $PM_{2.5}$ pollution. There are two aims of this study: first, evaluate the effectiveness of $PM_{2.5}$ removal by negative ion air filtration device; second, evaluate differences in health endpoints associated with $PM_{2.5}$ exposure between the two interventions.

2. Methods

2.1 Study Design

This master project is a part of the research project on the health effects of negative ion air filtration in indoor environment conducted by Dr. Junfeng Zhang's team in 2017. The purpose of this project is to determine the effects of negative ion air filtration in indoor environment in healthy young adults. Specifically, there are three objectives. First, whether negative ion air filtration is an effective indoor particulate matter filtration method. Second, whether such intervention has an effect on indoor air pollutant associated adverse health impacts and last, the underlying biological mechanism of indoor air pollutants.

A total of fifty-six study participants were recruited under the following criteria: healthy adults over age 18 currently enrolled in a university with no history of cardiovascular, respiratory, and other chronic disease with a body mass index less than 30 kg/m2. A person will be excluded from the study if the person meets one of the following conditions: history of smoking, acute infection, chronic respiratory or cardiovascular disease, pharmaceutical drug use within the past month, body mass index greater than 30 kg/m2, or leave school on weekends. The purpose of these conditions is to minimize confounding variables of this study. Medical conditions such as acute infection and chronic cardiovascular disease could affect biomarkers of interest such as lung function and inflammatory response. In addition, students who would leave the campus on weekends could be expose to elevated or decreased concentrations of air pollutants and particulate matter that are not representative of indoor exposure.

After recruitment, the participants are divided into two groups to receive the intervention. The study is a randomized, double-blind, crossover trial. Negative ion air filtration system and "sham" air filtration system were installed at students' dorm room for a crossover intervention in

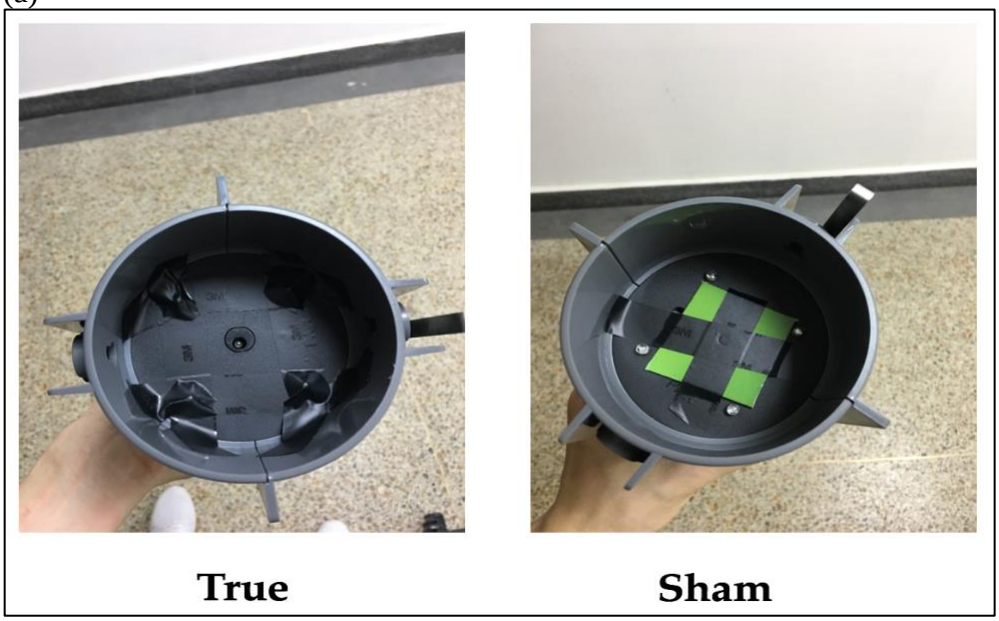
addition to four visits over the course of the intervention. In this study, Bentax Corporation Type A6E negative ion air filtration unit is used as the true intervention (Figure 1).

Figure 1. Outer packaging of Bentax Corporation Type A6E negative ion air filtration unit.

All other ventilation system in each door room remains the same. True negative ion air filtration is a normal, functional, negative ion filtration unit, whereas sham filtration unit cannot filter any indoor air pollutants. There is no visual, sound, size, and other noticeable differences between “true” and “sham” negative ion air filtration units (Figure 2a). Prior to the start of the seven-day intervention period, each door room was cleaned to confirm that there was no distinct source of indoor air particulate matter. Each intervention lasts seven days, and for the duration of each intervention period, the students were asked to stay in their door rooms as long as possible with closed windows and continuously running the negative ion air filtration units. An hour before and an hour after each intervention period, the students were asked to go to the designated clinics to collect biological samples and test for lung function, FeNO, and airway obstruction assessment.

All study participants had the same time with the true filtration unit, the washout period, and the sham filtration unit. Biological samples were collected before and after each intervention period. During the first seven-day intervention period, half of the participants are randomly assigned and received the true filtration unit while the other half of the participants are randomly assigned the sham unit. Both the participants and researchers did not know the order of interventions. After the first intervention period, filtration units were removed, and the second visit was conducted. Following the intervention period was a two-week long washout period, in which the participants did not receive any form of intervention. The second intervention period began with the filtration units switching among the participants. The participants that previously received true filtration units now received the sham units, and the participants the previously received sham units now received the true filtration units. This crossover design allows each participant to act as his or her own control, and the double-blind nature of this study minimizes biases within the researchers and the participants. Below is a figure of this study design (Figure 2b).

(a)



(b)

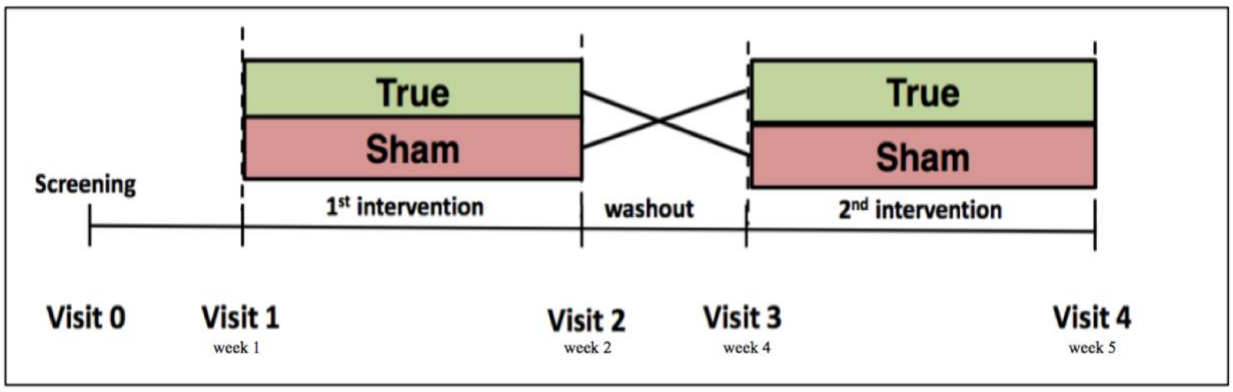


Figure 2 (a) true and sham setting of negative ion air filter. (b) Study design. (credit: Dr. Xiaoxing Cui)

Indoor $PM_{2.5}$ and PM_{10} concentrations were measured with DustTrak TMII 8530 Aerosol Monitor placed inside and outside of each dorm room. Continuous indoor and outdoor $PM_{2.5}$ and PM_{10} monitoring data and activity pattern of the study participants were used to quantify personal exposure to $PM_{2.5}$ and PM_{10}. Anion concentrations were quantified using AirCheck XR 5000 air sampling pump at 4L/min followed by GC-MS to separate anion and cation concentrations over each intervention period.

2.2 Biomarker Measurements

Urine samples were taken one hour before and after each intervention period. This master project focus on five biomarkers of interest, they are: free MDA, total MDA, 8-OHdG, 8-isoprostane, and 11-OHTXB12. Each biological sample contains 10 mL of urine and they are stored in -80°C refrigerator until testing. HPLC and LC-MS were used to measure biomarkers of interest.

2.3 Statistical Analysis Methods

The change in each participant's biomarker concentration before and after each intervention is calculated. This value indicates within-participant change during the intervention.

These biomarker concentration differences, including creatine-adjusted and specific gravity-adjusted values, are tested for normality with a Shapiro-Wilk normality test. Basic participant characteristics is also analyzed to provide an overview of the given study cohort. In addition, descriptive statistics for participants' baseline information, exposure data during each intervention, and biomarker values are also calculated. Paired t-test of internal biomarker concentration difference is used to examine the effects of negative ion air filtration intervention.

3. Results

3.1 Participant Characteristics

A total of 56 healthy young adults between the age of 18 and 31 were recruited to participate in the study. A basic description of the study cohort is provided in Table 1.

Table 1. Basic description and distribution of study participants.

Description	Median	Minimum	Maximum
Age (year)	22	18	31
Height (cm)	169.5	153	188
Weight (kg)	60.5	43.5	92
BMI (kg/cm2)	22	16.8	27.50

Gender	Number of Participants	Percentage
Male	33	58%
Female	23	42%

Of the 56 healthy young adults, there are 33 male and 23 female participants. The median age is 22 years old, with a minimum age of 18 and maximum age of 31 years old. The median height of the cohort is 169.5cm, and the minimum height is 153cm and the maximum is 188cm. The median weight is 60.5kg, the minimum weight is 43.5 kg and the maximum weight is 92 kg. The median BMI is 22 kg/cm2, the minimum BMI is 16.8 kg/cm2 and the maximum is 27.5 kg/cm2. All of the participants meet the criteria given during the recruitment process.

3.2 Tests of Normality

Shapiro Wilks Normality tests are used to test the normality of dependent variables in this analysis. The tests indicate the majority of biomarker concentrations are normally distribution ($p>0.05$), with the exceptions of fMDA concentration differences from both true and sham interventions.

3.3 Air Quality Measurements

Indoor personal $PM_{2.5}$ and anion exposure measurements were recorded during each intervention period. Personal exposure levels are calculated based on $PM_{2.5}$ active sampling data from both indoor and outdoor environment. These exposure levels are measured in average daily exposure concentration in $\mu g/m^3$ provided in Table 2.

Table 2. Personal $PM_{2.5}$ Daily Exposure Concentration During Each Intervention.

Intervention Period	**Mean $PM_{2.5}$ ($\mu g/m^3$)**	**Median $PM_{2.5}$ ($\mu g/m^3$)**	**Range of $PM_{2.5}$ ($\mu g/m^3$)**
True Filtration	18.73	14.60	5.10 – 71.10
Sham Filtration	31.49	26.60	11.30 – 67.60

Indoor anion exposure is also recorded during each intervention period. These exposure levels are provided in Table 3.

Table 3. Anion Exposure During Each Intervention.

Intervention Period	**Mean Anion**	**Median Anion**	**Range of Anion**
True Filtration	39696	38079	10662 - 74366
Sham Filtration	63.05	64.50	40 - 96

Overall, there is a lower level of $PM_{2.5}$ exposure during true filtration period compare to PM2.5 exposure during sham filtration period. There is also a higher level of anion exposure during true intervention compare to its exposure during sham intervention. The differences of anion and $PM_{2.5}$ between the interventions was found to be significant, both with a $p < 0.001$, rejecting the hypothesis of no difference in air quality between these interventions.

3.4 Statistical Analysis

A paired t-test was conducted for biomarkers of interest to assess the difference of these biomarkers between the interventions. Urinary samples were taken before and after each intervention period, and correspondingly, biomarker concentration data were available for each visit. This analysis obtained the difference in biomarker measurement for each intervention

period by subtracting individual participant's pre-intervention urinary biomarker concentration from post-intervention urinary biomarker concentration for true and sham interventions. The differences obtained from this subtraction indicate the change in individual's biomarker concentration as a response to study intervention. A paired t-test is then used to evaluate the mean difference between sham and true interventions' change in selected biomarker concentrations. Non-adjusted fMDA and TMDA data cannot fully address potential dilution in participants' urine samples, while specific gravity adjusted data could underestimate the real concentration; thus creatinine-adjusted concentrations are selected in this study.

Change in creatinine-adjusted fMDA (Δ fMDA**)** concentration is compared between true and sham interventions. The mean Δ fMDA for true intervention is 7.489 mg/L with a range of -606.1 to 710.4 mg/L. The mean Δ fMDA for sham intervention is 97.56 mg/L with a range of -971.6 to 1687 mg/L (Figure 3, Table 4). A paired t-test yields an insignificant p value (0.12), failing to reject the null hypothesis that the true mean difference of Δ fMDA between the interventions is equal to zero. This can be interpreted as there is no statistical difference between Δ fMDA concentration between the true and sham interventions.

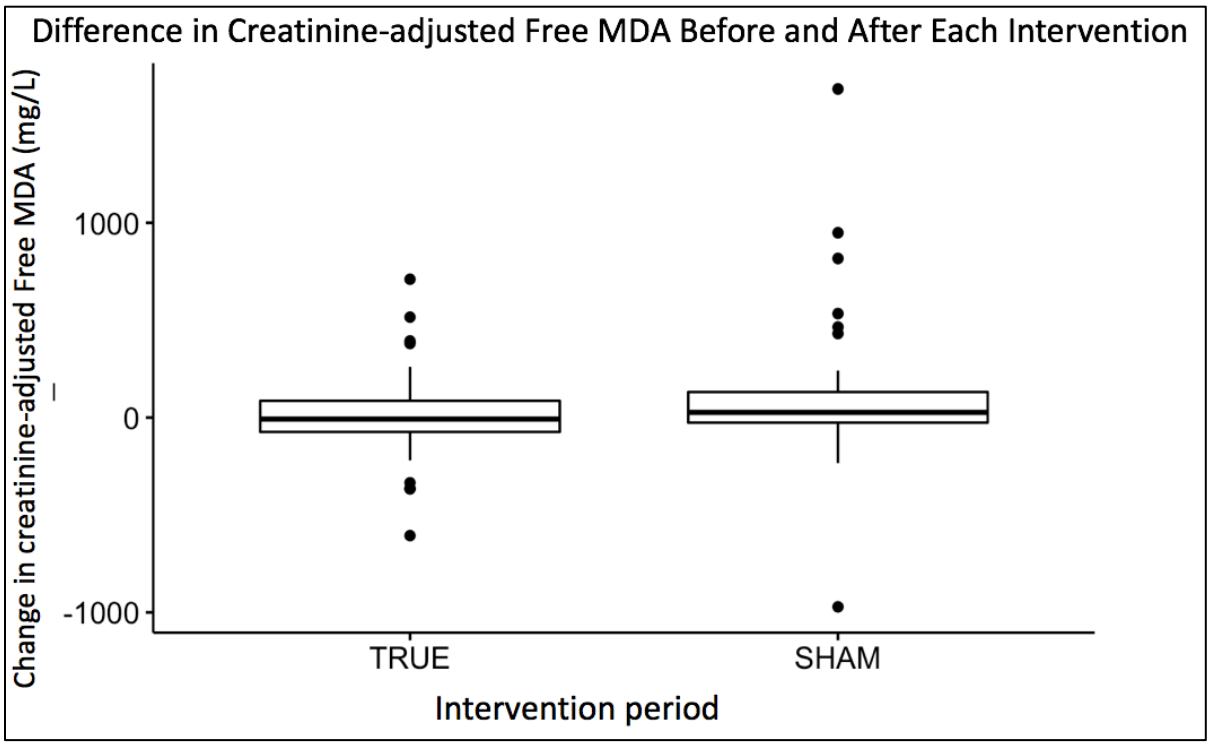


Figure 3. Visualization of change in creatinine-adjusted fMDA concentration for true and in sham intervention.

Table 4. Δ fMDA Concentration Between Interventions.

Intervention Period	**True Filtration**	**Sham Filtration**
Mean Δ fMDA (mg/L)	7.489	97.56
Median Δ fMDA (mg/L)	-7.800	26.37
range Δ fMDA (mg/L)	-606.1– 710.4	-971.6 – 1687

Change in creatinine-adjusted TMDA (Δ TMDA**)** concentration is compared between true and sham interventions. The mean Δ TMDA for true intervention is 2.132 mg/L with a range of -2883 to 2954 mg/L. The mean Δ TMDA for sham intervention is 594.4 mg/L with a range of -1536 to 4111 mg/L (Figure 4, Table 5). A paired t-test yields a significant p value (0.03), rejecting the null hypothesis that the true mean difference of Δ TMDA between the interventions is equal to zero. This can be interpreted as there is a statistical difference between Δ TMDA concentration between the true and sham interventions. The overall value of Δ TMDA

concentration over true intervention is lower than the value of Δ TMDA concentration over sham intervention. Because Δ TMDA concentration is calculated by subtracting initial biomarker concentration from final, post intervention biomarker concentration, a negative value of Δ TMDA concentration indicates a decrease in TMDA concentration after the intervention. Thus, based on this interpretation, true filtration intervention demonstrates a greater reduction in TMDA concentration compared to sham filtration.

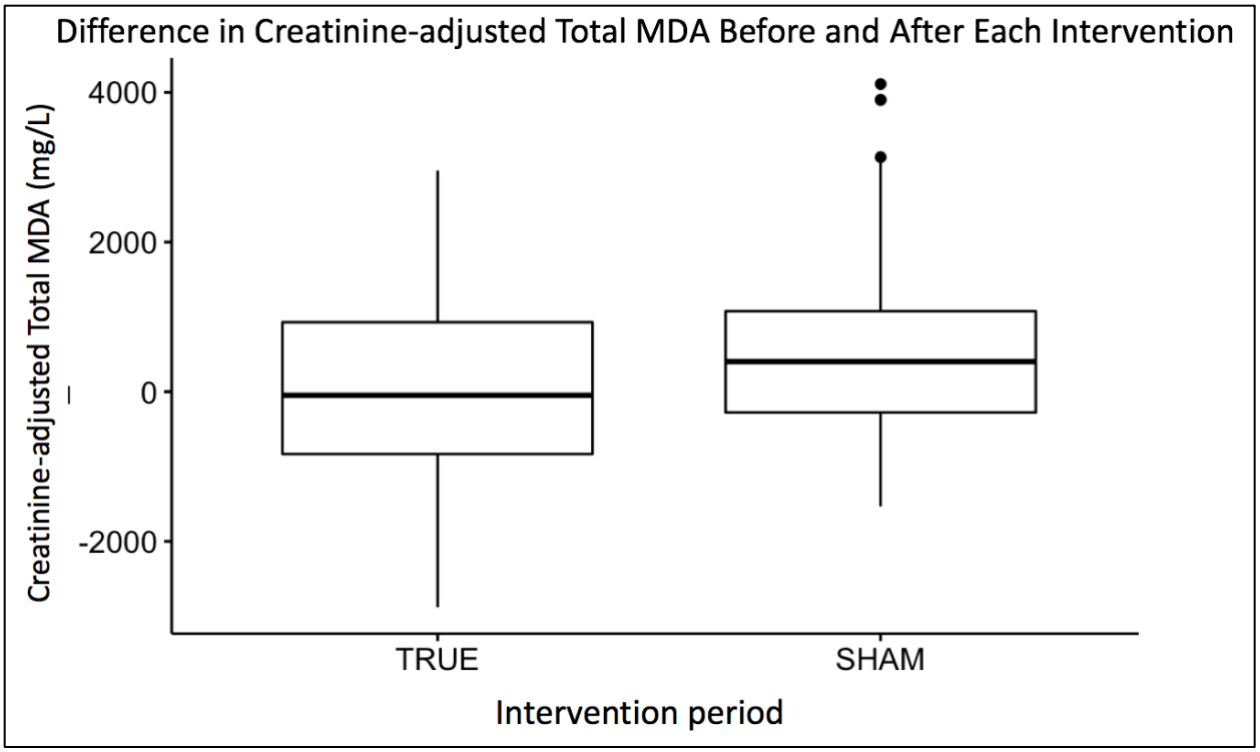


Figure 4. Visualization of change in creatinine-adjusted TMDA concentration for true and in sham intervention.

Table 5. Δ TMDA Concentration Between Interventions.

Intervention Period	**True Filtration**	**Sham Filtration**
Mean Δ TMDA (mg/L)	2.132	594.4
Median Δ TMDA (mg/L)	-47.00	402.5
range Δ TMDA (mg/L)	-2883 – 2954	-1536 – 4111

Change in urinary 8OHdG (Δ 8OHdG**)** concentration is compared between true and sham interventions. The mean Δ 8OHdG for true intervention is -0.64 ng/L with a range of -56.3 to 76.1 ng/L. The mean Δ 8OHdG for sham intervention is -1.34 ng/L with a range of -85.6 to 98.2 ng/L (Figure 5, Table 6). A paired t-test yields an insignificant p value (0.56), failing to reject the null hypothesis that the true mean difference of Δ 8OHdG between the interventions is equal to zero. This can be interpreted as there is no statistical difference between Δ 8OHdG concentration between the true and sham interventions.

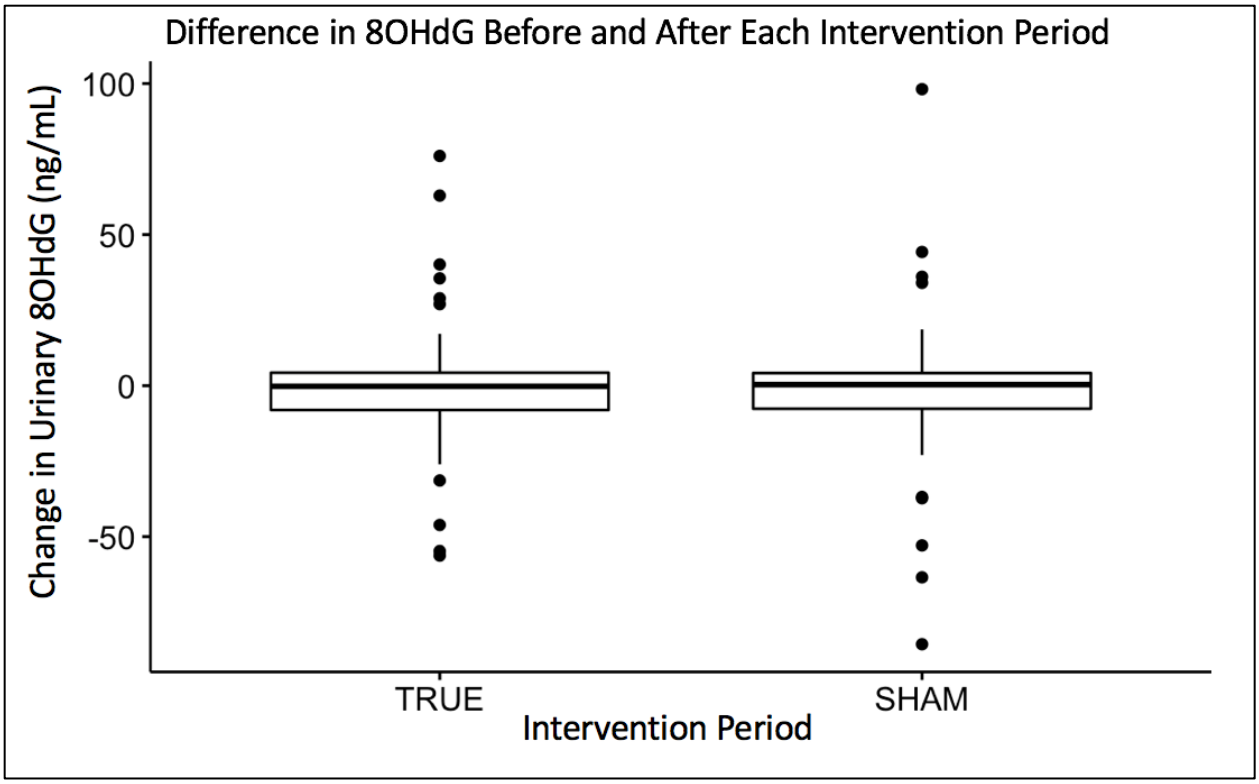


Figure 5. Visualization of change in 8OHdG concentration for true and in sham intervention.

Table 6. Δ 8OHdG Concentration Between Interventions.

Intervention Period	**True Filtration**	**Sham Filtration**
Mean Δ 8OHdG (ng/L)	-0.64	-1.34
Median Δ 8OHdG (ng/L)	-0.22	0.34
range Δ 8OHdG (ng/L)	-56.3 – 76.1	-85.6– 98.2

Change in urinary 8-Isoprostane (Δ 8-Isoprostane**)** concentration is compared between true and sham interventions. The mean Δ 8-Isoprostane for true intervention is 7.77 ng/L with a range of -117 to 152 ng/L. The mean Δ 8-Isoprostane for sham intervention is 4.23 ng/L with a range of -125 to 161 ng/L (Figure 6, Table 7). A paired t-test yields an insignificant p value (0.62), failing to reject the null hypothesis that the true mean difference of Δ 8-Isoprostane between the interventions is equal to zero. This can be interpreted as there is no statistical difference between Δ 8-Isoprostane concentration between the true and sham interventions.

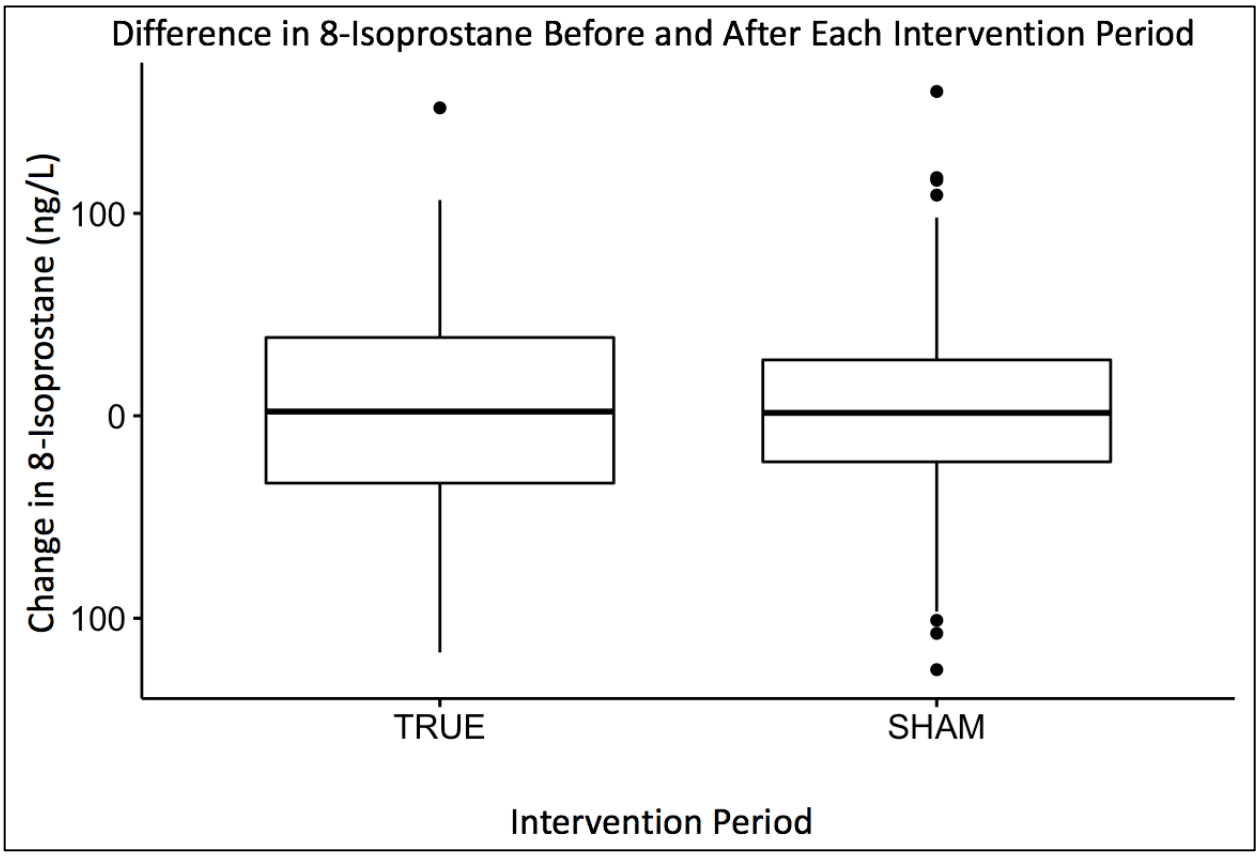


Figure 6. Visualization of change in 8-Isoprostane concentration for true and in sham intervention.

Table 7. Δ 8-Isoprostane Concentration Between Interventions.

Intervention Period	**True Filtration**	**Sham Filtration**

Mean Δ 8-Isoprostane (ng/L)	7.77	4.23
Median Δ 8-Isoprostane (ng/L)	2.12	1.45
range Δ 8-Isoprostane (ng/L)	-117 – 152	-125.41 – 161

Last but not least, change in urinary 11-OHTXB12 (Δ 11-OHTXB12**)** concentration is compared between true and sham interventions. The mean Δ 11-OHTXB12 for true intervention is -1.86ng/L with a range of -592 to 725 ng/L. The mean Δ 11-OHTXB12 for sham intervention is 75.8 ng/L with a range of -784 to 852 ng/L (Figure 7, Table 8). A paired t-test yields an insignificant p value (0.28), failing to reject the null hypothesis that the true mean difference of Δ 11 OHTXB12 between the interventions is equal to zero. This can be interpreted as there is no statistical difference between Δ 11 OHTXB12 concentration between the true and sham interventions.

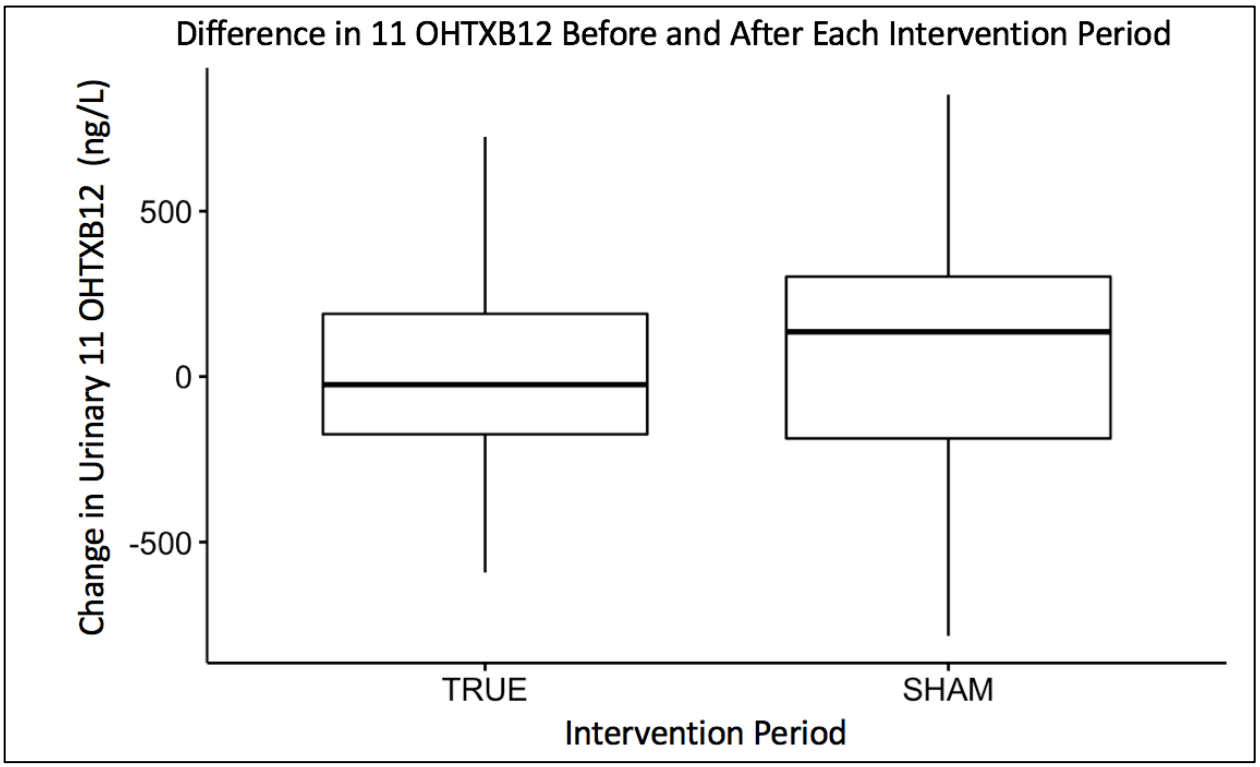


Figure 7. Visualization of change in 11-OHTXB12 concentration for true and in sham intervention.

Table 8. Δ 11 OHTXB12 Concentration Between Interventions.

Intervention Period	**True Filtration**	**Sham Filtration**
Mean Δ 11 OHTXB12 (ng/L)	-1.86	75.8
Median Δ 11 OHTXB12 (ng/L)	-24.5	135
range Δ 11 OHTXB12 (ng/L)	-592-735	-784-852

Overall, most of the biomarkers measured did not show any significance difference between the two interventions ($p>0.05$). The only exception is TMDA, which exhibits some significance with a greater reduction of TMDA in true filtration intervention, consistent with my initial expectation. However, with only one biomarker exhibiting significance, I cannot make any conclusive statement regarding my results.

4. Discussion and Conclusions

This study examined the health effects of negative ion air filtration in an indoor environment with a double-blind, randomized, crossover design. In this study, we evaluated the effectiveness of a negative ion air filtration unit, health impacts associated with each intervention, and interaction between negative ion and $PM_{2.5}$ in biomarker endpoints.

This study first found a significant evidence of the effectiveness of negative ion air filtration unit in reducing indoor $PM_{2.5}$ concentration and increasing negative ion concentration. Despite a couple of outliers of estimated personal daily $PM_{2.5}$ concentration exposure, the overall estimated daily exposure concentration was much lower during true intervention period than sham intervention. This suggests the negative ion air filtration unit was successful in reducing $PM_{2.5}$ concentration in the indoor environment. The overall estimated negative ion released during each intervention period also varied significantly. There was a more than three-fold increase in the indoor anion value during the true intervention. This confirms that the negative ion air filtration units were working properly during the true intervention periods, and indoor anion level was greater during the true intervention than sham intervention. The personal $PM_{2.5}$ exposure outliers found during true intervention period could be a result of study participant's activity level, time spent outdoors, and weather variations. Overall, our findings confirm our first hypothesis.

Use of paired t-test showed that the negative ion air filtration intervention does not have a significant effect on multiple oxidative stress biomarkers within the study cohort.

However, based on literature review, some research has suggested that there is a likelihood of anion-induced oxidative stress. This could potentially offer an explanation to our result. While the reduction of indoor particulate matter could reduce oxidative stress; the presence of elevated anion concentration could induce oxidative stress, causing more harm or

cancelling out the health benefits associated with reduced $PM_{2.5}$. These actions and counteractions could result in a net-zero situation, in which health benefits and harms are almost equivalent, and there is no observable improvement in health as a result.

There is a limited understanding of the negative health impacts associated with negative ion exposure. More research is needed to gain a better insight to the results of this intervention among participants.

The limitations of this study are data availability, exposure measurements, and uncertainties associated with human subjects. There is limited information regarding the biological mechanisms of negative ion and associated health effects. In addition, exposure measurements are limited to an estimate of personal exposure, rather than a complete measurement over time. This is associated with the uncertainties associated with human subjects since their movements are not limited to their dorm rooms; and participants' exposure occurring outdoors and in other indoor environments were not controlled.

5. References

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