Reducing particulate matter-associated cardiovascular health effects for seniors. (RAPIDS2-Ypsi)

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SPECIFIC AIMS

Fine particulate matter (<2.5 μm, PM2.5) air pollution is the fifth leading risk factor for mortality worldwide[1-3]. Over 88 thousand deaths per year are attributable to PM2.5 in the US alone[1], with the largest portion from cardiovascular (CV) causes (myocardial infarctions, strokes, heart failure)[1,3]. While several mechanisms are likely responsible[1,4], we have identified PM2.5-induced elevations in blood pressure (BP) as a key pathway[4-8]. Beyond triggering acute CV events, the BP increase itself (2-10 mm Hg) is clinically relevant, promoting hospitalizations for high BP and chronic hypertension[4-9]. Unfortunately, more than 90% of the global population is exposed to PM2.5 levels above World Health Organization Air Quality Guidelines (<10 μg/m3)[2,3]. Despite improvements across the US, PM2.5 remains above 10 μg/m3 in numerous hot-spots (e.g., near roadways)[10-12]. Moreover, studies show that even PM2.5 levels <10 μg/m3 pose CV health risks[13-20]. Indeed, no safe lower threshold of exposure exists[13]. It is thus critical to develop *feasible* and *effective personal* strategies to protect "vulnerable" (urban/near-roadway) and "susceptible" (elderly) populations at risk to the harmful effects of PM2.5.

Emerging trials have shown that portable indoor air filtration units (AFUs) with high-efficiency particulate arrestance filters can reduce PM2.5 exposures by 30-50% and improve CV health endpoints (e.g., BP, vascular function)[21-29]. However, most positive studies focused on highly-polluted regions (Asia)22,23, representing only a proof-of-principle for Americans who typically face PM2.5 levels 5-10 times lower. Thus, we performed one of the few trials in a location typical of US environments, *Reducing Air Pollution in Detroit Study* (RAPIDS), among older adults living in low-income senior housing in Detroit[30]. Three-day use of two AFUs (bedroom and main living space) reduced personal-level PM2.5 exposures by 42% (15.7 to 9.1 μg/m3). This translated into a reduction in systolic BP (primary endpoint) by 3.2 mm Hg (95%CI -6.1, 0.2), a trend toward lower diastolic BP (-1.5 mm Hg; 95%CI 3.3, 0.2) and improved secondary outcomes (e.g., aortic hemodynamics). The exposure reduction was greatest during nighttime (10PM-8AM), accounting for 62% of the total decrease in 24-hr PM2.5 exposure[31]. This suggests that an AFU in the bedroom is a key determinant of the success of the intervention.

Our results confirm that even low PM2.5 levels pose significant risks to CV health and that AFUs represent a promising preventative strategy. However, several important questions remain. Almost all prior trials (including RAPIDS) have been of brief duration (2-3 days). No study has addressed whether exposure reductions and health improvements can be sustained over more clinically relevant periods of intervention (i.e., several weeks) which are required to plausibly yield decreases in actual CV events. In addition, the efficacy of a novel practical approach (i.e., less expensive and more feasible in real-world settings) of using a single AFU only in the bedroom to focus on reducing nocturnal PM2.5 exposure is unknown. Finally, the mechanisms by which PM2.5 increases BP remain incompletely understood[32]. As a logical extension of our prior grant (R01NR014484), we propose in this renewal project (**RAPIDS-2**) to build upon our findings by addressing two pragmatic issues fundamentally requiring resolution before this field can progress from a clinical standpoint **(Aims 1 & 2)** and by elucidating a novel mechanism potentially explaining PM2.5-induced cardiometabolic abnormalities **(Aim 3)**.

Aim 1: Determine if long-term AFU usage provides sustained reductions in PM2.5 exposure and persistent improvements in cardiometabolic outcomes. We propose a randomized double-blind 2-way crossover study (AFUs bedroom- vs. sham filtration) in 50 adults living in a low-income senior residence impacted by roadway pollutants. We posit that compared to sham, AFU use *long-term (4-week)* periods will **(1a)** lower resting morning BP (*primary outcome*), **(1b)** reduce indoor PM2.5 and 24-hr personal PM2.5 exposures, and **(1c)** improve secondary health outcomes: 24-hr and nocturnal BP, 24-hr and nocturnal aortic hemodynamics (BP, augmentation index), and insulin sensitivity.

Aim 2: Determine if nocturnal PM2.5 exposure reduction alone improves cardiometabolic outcomes. We posit that compared to sham, bedroom-only AFU use will **(2a)** lower resting morning BP. We further posit that **(2c)** 24-hr personal PM2.5 exposure and **(2d)** secondary health outcomes will improve – particularly nocturnal BP (a powerful

predictor of CV events)33-35.

Aim 3: Demonstrate the key role of adrenal activation as a novel mechanism explaining PM2.5-induced cardiometabolic effects. Recent data suggest PM2.5 provokes hypothalamic-pituitary-adrenal axis (HPAA) activation[32]. We posit **(3a)** AFU will reduce adrenal activation (comprehensive assessment by blood steroidomic profiling) after 4 weeks. Steroids with glucocorticoid and mineralocorticoid activity will be shown **(3b)** to be mediators of PM2.5-induced BP changes & insulin resistance.

PM2.5 is a leading risk factor for mortality. Our proposal, which will help validate the benefits of novel strategies to employ AFUs in an elderly vulnerable population living in a non-traditional setting, is directly responsive to the Program Announcement (PA-18-142), and is of global public health importance. Positive results would form a key part of the evidence base required to promote more wide-scale AFU use. In the long term, given the low cost and burden, AFU use could be up-scaled to help protect diverse populations across the world.

RESEARCH STRATEGY

SIGNIFICANCE

PM2.5 is a major contributor to the global epidemic of cardiometabolic diseases.

Fine particulate matter (< 2.5 μm, PM2.5) air pollution is largely derived from fossil fuel combustion processes[1-9]. More than 90% of the world's population faces levels above World Health Organization Air Quality Guidelines (AQG) (>10 μg/m3). PM2.5 is the fifth leading global risk factor for mortality, principally due to cardiovascular (CV) events1-4. While air quality in the US has improved, numerous hot spots remain (e.g., near roadways)10-12 such that 20% of Americans are exposed to PM2.5 levels above 10 μg/m3. Moreover, mounting studies show that PM2.5-induced CV events still occur even when levels are below AQG13-19. In two large studies of Medicare patients[18,19], exposures to low PM2.5 levels over both the short and long term increased mortality. Individuals at greatest risk were from "vulnerable" (i.e., more highly-exposed) populations, such as those living in urban or near-roadway settings, as well as "susceptible" (i.e., those biologically predisposed to the adverse effects of exposure) adults, such as the elderly and minorities. Thus, we designed our prior study, *Reducing Air Pollution in Detroit Study* (RAPIDS), and this renewal to focus on protecting a vulnerable *and* susceptible population.

A key mechanism underlying CV events (myocardial infarctions, strokes, heart failure) due to exposure is PM2.5-induced blood pressure (BP) elevations[4-9]. In over 20 publications by our group36-56 (and in studies across the world)[5-8] PM2.5 has proven to increase BP[4,5]. In a series of panel studies we have shown that exposures to ambient levels of PM2.5 over acute and subacute periods (1-7 days) both in lightly- (Michigan) and heavily-polluted (China) locations prompt clinically significant elevations in BP (2-10 mm Hg)35-41 and insulin resistance[39,40]. In epidemiological studies we and others have shown that beyond contributing to the triggering of acute CV events, the pro-hypertensive/diabetic responses translate into health threats of clinical importance in their own right. PM2.5 exposure promotes acute (hospitalizations for high BP)[48,49] and chronic (development of overt hypertension and diabetes)[50-54] adverse cardiometabolic health effects[4]. Several mechanisms have been elucidated including autonomic imbalance, reduced arterial compliance, inflammation, vasoconstriction, and vascular dysfunction[4]. Given the large body of evidence[4-8], both the American Heart Association (AHA)[1] and European Society of Cardiology (ESC)[9] have deemed PM2.5 a risk factor for CV disease.

Adverse cardiometabolic effects of PM2.5 may be reduced by portable indoor air filtration units (AFUs).

National regulations have lowered PM2.5 levels over the past few decades leading to improvements in life expectancy in the US21. However, millions of susceptible adults remain at risk and exposed to PM2.5 levels above AQG[2,3,12]. Moreover, ambient concentrations <10 μg/m3 still pose significant health risks[13-19], and additional efforts are urgently needed to help protect the American public to prevent the more than 88 thousand deaths per year in the US attributable to PM2.5[1-3]. In a recent review of strategies[21], we concluded that the most viable method is to specifically target "at-risk" adults for reductions in personal exposure. Americans spend 90% of their time indoors, with almost 70% in their own residence[57]. This makes relatively inexpensive (~$70) indoor AFUs a practical approach that fits the US population[21]. AFUs have proven to reduce PM2.5 exposures by 30-50% and mitigate adverse CV

responses (lowered BP, improved insulin sensitivity)[21-29]. However, notable limitations exist in prior trials. Many were conducted in heavily-polluted areas[22,23]. Almost all were brief (2-9 days) and enrolled healthy people. None evaluated personal-level PM2.5 exposure. This makes our recent AFU trial (RAPIDS) unique[30]. We evaluated: 1) an at-risk population (low-income elderly adults), 2) living in a typical US urban environment (Detroit), as well as 3) personal PM2.5 exposures. While our positive results showing a significant reduction in personal-level PM2.5 exposures and resting systolic BP levels by 3.2 mm Hg were encouraging, we recognize that an AFU intervention lasting 3 days still only represents a proof-of-principle.

Longer-term AFU studies over more clinically relevant periods of intervention are needed.

Several remaining scientific gaps must be addressed before authoritative medical bodies (e.g., AHA, ESC) have a compelling evidence base necessary to promulgate formal recommendations for use of AFUs20. Foremost is if AFUs can provide sustained reductions in PM2.5 exposures and persistent improvements in health outcomes over durations lasting longer than a few days as in most prior trials[21]. This question is critical because for AFU use to plausibly yield a reduction in actual CV events (e.g., myocardial infarctions), the health benefits will need to be maintained over clinically relevant periods (e.g., weeks). Possible changes in patient activities (e.g., compliance, time outdoors), filtration effectiveness (e.g., open windows, lower filtration efficiency) and biological factors (e.g., diminution of physiologic responsivity) could in theory attenuate the benefits over longer periods. The precise duration of intervention sufficient to provide convincing evidence that the benefits of AFUs persist over periods that can translate into clinical benefits is not known. We believe 4 weeks is a feasible duration that still allows for a crossover design yet also represents a major advancement compared to prior acute studies. If positive, we can next justify larger and longer-term follow-up trials.

A practical "bed-room only" AFU strategy more adoptable to real-life scenarios needs to be evaluated.

Most prior studies (including RAPIDS) evaluated the effect of AFUs in ≥2 rooms[21]. While it is logical to lower PM2.5 as much as possible throughout the indoor environment, many factors (e.g., multiple household rooms, variability in room sizes, indoor PM2.5 sources, outdoor PM2.5 penetration, and time spent in each room) may make this strategy untenable in most real-world settings. These factors could obviate the widespread applicability of this strategy if numerous AFUs are required, particularly for lower income populations. Importantly, we observed in RAPIDS that 62% of the total exposure reduction over the 24-hr period was achieved during nighttime alone[31]. This is most obviously explained by the fact that individuals remain indoors near a bedroom AFU while sleeping. As such, we posit that a novel, economical and more practical approach that employs a single AFU strategically located in the bedroom may lower overall 24-hr PM2.5 exposure by a sufficient degree to yield significant health benefits similar to those achieved using a multi-room intervention. Moreover, reducing nighttime exposure has a sound physiological basis for why it should prove to be highly protective and beneficial for CV health. It is logical to posit that this strategy should yield a robust decrease in nocturnal BP, which ranks among the most powerful determinants of CV outcomes and is a superior predictor of morbidity/mortality than clinical and daytime BP[33-35,58-62]. Thus, even if the bedroom-alone AFU strategy fails to lower daytime resting BP (primary outcome), a reduction in nocturnal BP alone would still be of major clinical relevance. One prior study showed that ambient PM exposure can raise nocturnal BP63; however, no study has evaluated the ability of AFUs to prevent nocturnal (or 24-hour long) elevations in BP (as measured by ambulatory BP monitoring). Other leading researchers have endorsed this hypothesis that placing an AFU in the bedroom is likely a key determinant of the success of the overall intervention58.

The mechanisms whereby PM2.5 causes cardiometabolic abnormalities require further elucidation.

We have shown that a 2-hour exposure to high levels of concentrated PM2.5 (150 µg/m3) acutely raises BP by triggering autonomic imbalance[44]. However, the mechanisms by which far lower ambient levels of PM2.5 (5-25 µg/m3) cause BP elevations are less clear. In our and others' panel studies it has been consistently observed that subacute changes in ambient PM2.5 levels (1-5 days) cause somewhat delayed (e.g., 1-3 days later) pro-hypertensive responses that persist for several days following exposure4. These responses do not match what would be expected if they were prompted solely by hyper-acute (seconds-to-minutes) reactions mediated by autonomic pathways. Other potential causes for BP elevations include PM2.5-induced endothelial dysfunction and impaired arterial compliance[4,5]. However, our research suggests that changes in these outcomes represent parallel (not causal)

responses, as they sometimes (but not always) occur during overlapping (but not identical) periods and are thus not necessary responses for the genesis of BP elevations[4].

A recent study in Shanghai corroborated that AFU use for 9 days in healthy students reduces BP23. There were concomitant improvements in metabolomic profiles with lower circulating levels of cortisol, cortisone, epinephrine, adrenocorticotropic hormone (ACTH), insulin and glucose. These changes correlated with reductions in PM2.5 exposures. In an accompanying editorial, we interpreted these results to signify that PM2.5 triggers adverse cardiometabolic responses via hypothalamic-pituitary-adrenal-axis (HPAA) activation (Fig. 1)[32]. It is an established physiological principle that adrenal steroids with glucocorticoid and mineralocorticoid activities can increase BP and impair insulin resistance. However, they typically trigger these adverse responses in a slower (hours-to-days) but more persistent manner[32]. We outlined several pathways explaining how this may occur as supported by our prior experiments: a systemic "stress response," inhaled pollutants stimulating pulmonary afferents that trigger reflex hypothalamic activation, and inflammatory mediators (e.g., cytokines) released by pulmonary cells that spill over into the circulation and penetrate through CNS-permeable areas to instigate hypothalamic inflammation[31]. The slower onset and more persistent changes in BP and insulin resistance (as would be expected if mediated by HPAA activation) matches our prior panel study findings and the decreases in BP observed (peaking 2-3 days after PM2.5 reductions from AFU use) in RAPIDS. The totality of evidence thus supports that a slower but more persistent pathway (i.e., HPAA activation) likely underlies ambient PM2.5-induced cardiometabolic changes[32]. While intriguing, the recent Shanghai findings are limited by the fact that they were derived from a post hoc analysis in people exposed to very high PM2.5 levels. A pre-specified, hypothesis-driven trial at more globally relevant PM2.5 concentrations (as we herein propose) using comprehensive HPAA profiling would more conclusively help to establish the mechanistic role of subacute (by late-evening salivary cortisol and blood steroidomic profile) and chronic (by hair cortisol) HPAA activation as a causal mediator of PM2.5-induced cardiometabolic abnormalities. Beyond elucidating key physiological principles that further scientific knowledge, a better understanding of the mechanisms may help in the effort to devise further preventive strategies (beyond AFUs) and target specific pathways to reduce the harmful effects of PM2.5.

INNOVATION

We have a productive track record studying the cardiometabolic effects of air pollutants[36-56], and will leverage our unique collaborative team, experience, and resources established during the initial project (R01NR014484) and build upon our novel findings. This trial is innovative for several reasons. **(1)** This will be the first study of elderly adults living in a non-traditional setting (a low-income senior facility) in a typical US urban location (metropolitan Detroit) that will evaluate the efficacy and CV benefits of AFU use to reduce personal PM2.5 exposures over a more *clinically relevant, longer-term intervention period*. **(2)** We recognize the importance of demonstrating that AFUs can improve a primary outcome (resting BP) known to be adversely impacted by PM2.5 which is also a surrogate endpoint unquestionably linked to serious health ramifications[1,4,9]. However, we will also evaluate several novel secondary endpoints not previously studied in an AFU trial. This will be the first study to employ the gold-standard 24-hr ambulatory BP technique. This novel device allows for the measurement of 24-hr and nocturnal brachial BPs, as well as 24-hr aortic hemodynamics (BP levels, augmentation index [AIx]). Aortic BP often differs from brachial BP in the elderly[64]. Along with 24-hr and nocturnal brachial BP, aortic BP and AIx (arterial compliance) are independent predictors of CV risk[64-71]. **(3)** This will be the first study to test a novel intervention (bedroom-only AFU use) which may prove equally effective compared to a multi-room strategy. **(4)** Finally, we will comprehensively evaluate the mediating role of subacute and chronic HPAA activation – producing glucocorticoids, mineralocorticoids, and potentiating steroids – as a novel mechanism explaining the cardiometabolic effects of PM2.532. **We expect our findings will play a key role in validating the clinical benefits of practical inexpensive strategies using AFUs that at-risk individuals living in vulnerable communities across the US can readily adopt to further reduce the CV risks of PM2.5.**

APPROACH

PROGRESS REPORT (July 2014 – present)

We successfully completed the RAPIDS clinical trial protocol on schedule (R01 NR014484)21. As with all clinical trials, we had to await full completion prior to analysis, which has limited the number of papers for submission at this time. We have nonetheless been highly productive. Our main results (Specific Aim 1) are in press in a top-tier medical journal[30] ("Publication List" #1). We also published a review on strategies to reduce PM2.5 exposures[21] ("Publication List" #2). Our third manuscript, accepted for publication, characterizes numerous details of *personal* PM2.5 exposure reduction by AFUs[31] ("Publication List" #3). Two further manuscripts are currently in preparation. Major findings of these manuscripts are briefly discussed in below.

The specific aims for the original RAPIDS study were: (1) to determine whether filter-based interventions are capable of reducing PM2.5 exposures and thereby improve clinically-relevant intermediate CV health endpoints in a senior-citizen facility; (2) to identify which measurement of PM2.5 – outdoor, indoor or personal – is most closely associated with the CV health outcomes under three different filtration scenarios in a senior-citizen residential facility; and (3) To determine whether filter-based intervention reduce exposures to specific outdoor sources of PM2.5 and thereby improve the CV health endpoints in a senior-citizen residential facility.

Main results: We enrolled 40 nonsmoking elderly adults living a senior facility in midtown Detroit into a randomized double-blind crossover study with daily CV health outcomes and PM2.5 exposure measurements. There were no activity restrictions (e.g., window closure requirements) during the study. Participants were exposed to three blinded 3-day-long scenarios separated by one-week washout periods: unfiltered ambient air

Figure 1: Multiple pathways of HPAA activation due to $PM_{2.5}$ exposure

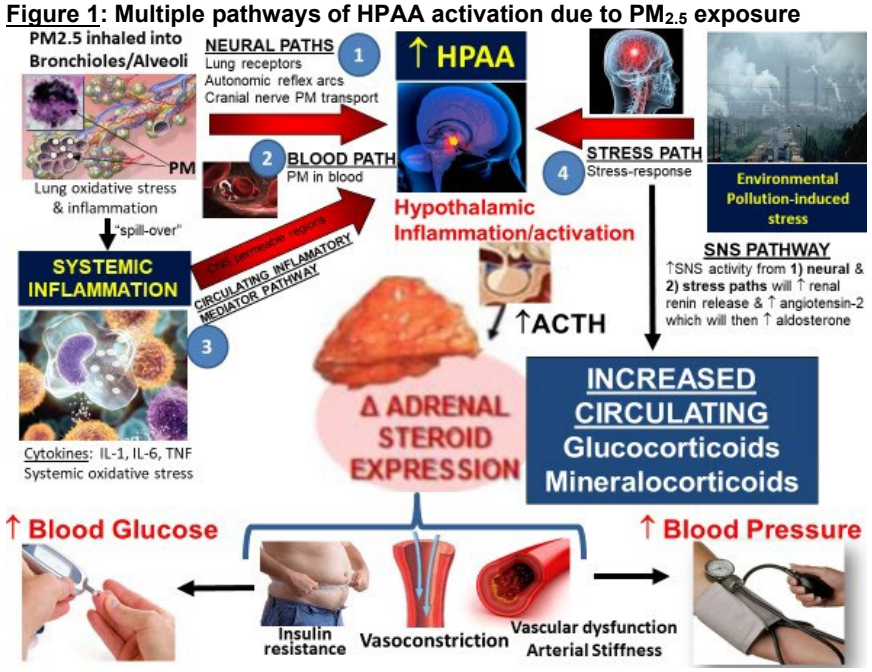


Figure 2: AFUs lowered evening-night personal $PM_{2.5}$ exposures by the greatest extent. During night alone (10pm-8am), 62% of the total 24-hr exposure reduction occurred[31].

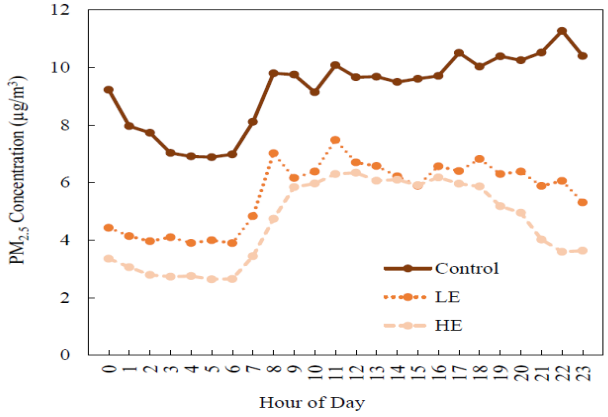


Figure 3: Any AFU usage lowered 3-day average BP levels[30].

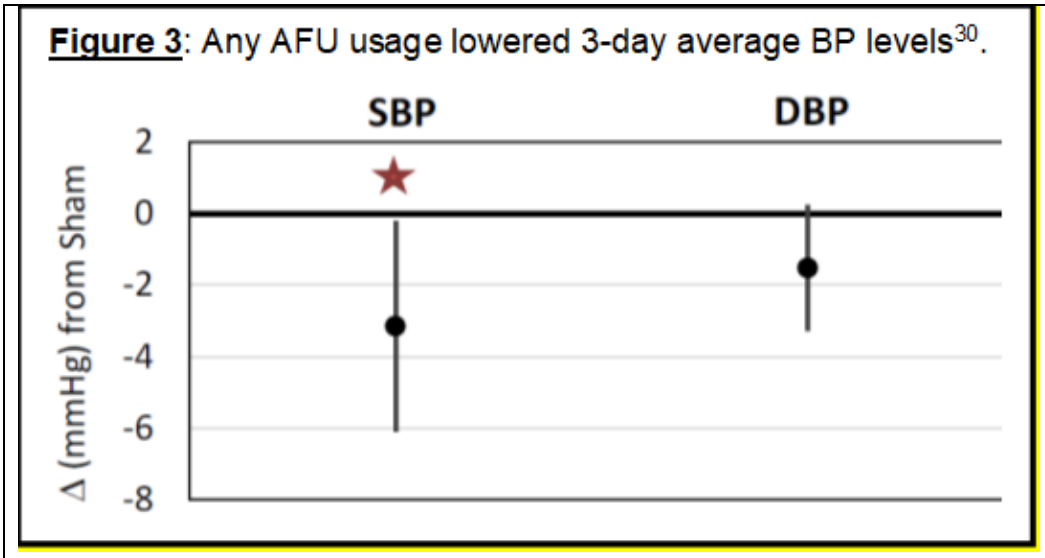
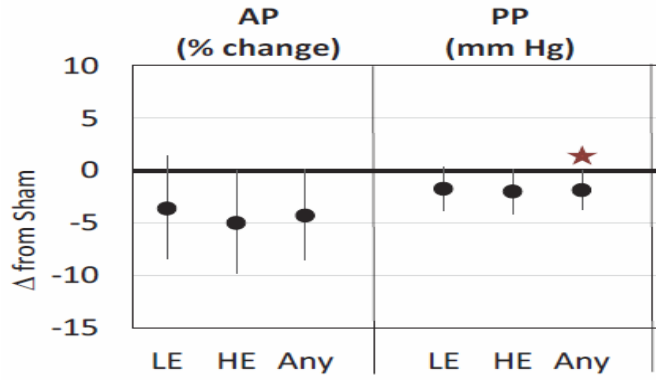


Figure 4: AFU use and reductions in central aortic AP (augmentation pressure) and PP (pulse pressure)[30].



(sham), low-efficiency (LE) "HEPA-type", and high-efficiency (HE) "true-HEPA" filtered air using AFUs (one in the bedroom and one in the main living space). The primary outcome was average resting brachial systolic BP (BpTRU: unattended automated mean of 5 repeats) measured the morning of all 3 days of each intervention. Secondary outcomes included aortic hemodynamics and pulse wave velocity (SphygomoCor) and heart rate variability. PM2.5 exposures were measured by gravimetric analyses of filters collected over each of the 3-day interventions in each participant's living room and by personal-level monitoring (pDR-1500).

Participants were 67 ± 8 years old (38% female). Three-day personal PM2.5 exposures were reduced by AFUs from 15.7 µg/m3 (sham) to 10.9 (LE) and 7.4 (HE) µg/m3 ($p<0.05$). Indoor levels in the living room were also reduced from 17.5 (sham) to 8.4 (LE) and 7.0 (HE) µg/m3; however, they differed from personal levels, confirming the importance of 24-hour personal exposure monitoring to accurately characterize the success of "true" exposure reduction by in-home AFUs, as we herein propose (Fig. 2; "Publication List" #2). Personal exposure reduction was **most effective at night** (10PM-8AM). Compared to sham, any air filtration (both LE and HE) for 3 days decreased 3-day average brachial systolic BP (SBP) and diastolic BP (DBP) by 3.2 mmHg (95% CI: -6.1, -0.2) and 1.5 mmHg (CI: -3.3, 0.2), respectively (Fig. 3; "Publication List" #1). Though quantitatively similar, BP reductions measured on each of the 3 individual lag days during active filtration were of borderline significance. This demonstrates the key importance of repeating BP measurements over ≥3 days to accurately assess the benefits of AFUs (as we herein propose). It also suggests that longer periods of AFU use will likely provide persistent and more stable improvements in BP. The findings also support the use of the gold-standard 24-hr BP[64-69] (as we herein propose) to provide a more sensitive

evaluation of BP responses. Several secondary outcomes (aortic BP) also showed trends towards improvements during AFU use (Fig. 4). This supports that our intended use of the more sensitive technique evaluating aortic BP over a 24-hr period is likely to be successful and warranted.

For Aim 3, we have completed detailed chemical analyses of outdoor PM2.5 samples, and we are now finalizing receptor modeling results (via Positive Matrix Factorization). Our preliminary data show contributions from outdoor sources including local traffic, iron-steel industry, refinery, incinerators and regional transport.

No changes in heart rate variability or pulse wave velocity were seen in RAPIDS, suggesting that the BP reduction was not due to improved autonomic activity or arterial stiffness and further supporting our aim to assess HPAA activation as the mechanism. Finally, in post hoc analyses the 19 obese (body mass index, BMI ≥30 kg/m3) participants were found to have a significantly greater reduction in systolic BP from any AFU use (- 6.9 mm Hg). Given the high prevalence of obesity in the US (>40% in RAPIDS), these suggestive findings support our pre-specified plan to evaluate BMI as an effect modifier of the BP reductions in this follow-up study. Altogether, our results from RAPIDS support the rationale and hypotheses of this renewal proposal.

3.3.2. STUDY DESIGN AND EXECUTION

We will use well-validated methods with which we have extensive experience through our initial RAPIDS

project30 (R01 NR014484) and in prior studies[36-56] to characterize PM2.5 exposures and health responses. The rationale and design/methods of our proposed renewal trial (RAPIDS-2), primary outcome (systolic BP), study power and AFU intervention are well-justified by our initial project findings.[30] We have also intentionally designed the trial to minimize risk and burden to the elderly participants. The protocol will be conducted entirely within a senior living facility and will evaluate clinically informative yet minimally-invasive endpoints (Fig. 5). Our design using a randomized double-blind cross-over trial and our method for obtaining our primary outcome (the average systolic BP measured 4 days in a row by unattended automated 5 repeated measurements) are intended to address NIH's Rigor and Reproducibility guidelines. The trial is principally designed and powered to validate the real-world clinical cardiometabolic benefits of AFUs (Aims 1 and 2). While we will explore a novel mechanistic underpinning (Aim 3), we recognize that invasive procedures (that might provide more detailed biological understandings) are not feasible in this setting and could jeopardize the success of Aims 1 and 2. Thus, we designed Aim 3 to be informative but also as unobtrusive as possible. While our secondary outcomes have never been evaluated in an AFU trial (including steroidomic profiling), we have extensive experience with each endpoint and thus will have no difficulties in adapting them into the study protocol.

Trial Overview

We propose a randomized double-blind placebo-controlled (sham filtration) crossover trial comparing the effectiveness of bedroom-only AFU use to reduce personal PM2.5 exposures and improve cardiometabolic health (Fig. 5). The health benefits (primary outcome: resting BP) of both active AFU strategies will be evaluated over acute (4-day) and long-term (4-week) periods in 50 nonsmoking elderly adults living in a senior facility impacted by near-roadway pollutants. The rationale for assessing the 4-day period is to corroborate the findings from RAPIDS but in a different, yet highly relevant (near-roadway), environment common in the US. The rationale for the 4-week intervention is to demonstrate for the first time that AFUs can lower exposures and improve CV endpoints over a longer-term, more clinically relevant period. The rationale for the bedroom-only AFU intervention is to show that this novel (more practical, less expensive) approach is also an effective strategy., a participant may undergo the following ordering: Block 1, bedroom AFU; Block 2, bedroom- sham filtration .

Study Participants: We will enroll n=50 participants.

Inclusion criteria: 1) Nonsmoker, 2) ≥60 years old, 3) residing in Carpenter Place Apartments. 4) SBP>115 mm Hg

These criteria were selected as they focus on the adverse health effects of PM2.5 on an urban/near-roadway ("vulnerable") and elderly ("susceptible") population. They are also highly responsive to the Program Announcement: *Environmental Exposures and Health: Exploration of Non-traditional Setting (PA-18-142).*

Exclusion criteria: 1) Active cigarette smoker 2) daily secondhand smoke exposure (self-report), 3) any CV event (myocardial infarction, stroke, heart failure, revascularization) in the past 3 months, 4) unstable CV condition or risk factor (uncontrolled diabetes, class 3-4 angina or heart failure) or any medical condition that would place the participant at risk from participation or jeopardize study integrity (per investigators), 5) expected overnight travel outside their apartment during the 14-week study period, 6) unable to provide informed consent, 7) lung disease requiring oxygen, 8) renal dialysis, 9) cancer receiving active treatment or chemotherapy, 10) severe uncontrolled high BP ≥160/100 mm Hg or SBP<115 mm Hg.11) CV medication change in the prior month. If participants are on medications for high BP, diabetes, or a CV condition, they will need to have stable therapy during the prior month with no planned changes during the study period.

Hypertension or other stable cardiometabolic risk factors or diseases are not exclusions to this trial given their prevalence in the elderly. They also enhance the sensitivity to the health impacts of air pollution and are thus important to include in the trial to assure the real-world applicability of the findings. Finally, these factors (e.g., hypertension status, basal BP, diabetes) did not modify (mitigate or augment) the BP reduction provided by AFUs in RAPIDS. We will collect complete health and medication information during screening visits and will plan subgroup and effect modification analyses by these and other key clinical characteristics.

Study location: The study will be conducted within Carpenter Place Apartments, a nonsmoking facility for low-income seniors ≥60 years of age. The complex houses over 150 residents and is located at 3400 Carpenter Road, Ypsilanti, MI 48197. We selected this site because it is important to corroborate the effectiveness of an AFU intervention at a different location from that of our initial RAPIDS trial30. The facility is ideal for this renewal project (RAPIDS-2) trial because it houses low-income elderly adults (a population known to be susceptible to the health effects of PM2.5)[1,18,19] and it is located within 150 meters of a 4-lane US highway (US-23; ~78,000 vehicles per day72). Each residence consists of a one-bedroom apartment (576 sqft). Given this relatively small living space, the apartments are ideal to test the effectiveness of AFUs (positioned in the main room and/or bedroom). We have obtained approval from the management of Carpenter Place to conduct RAPIDS-2 in this facility and have observed enthusiasm from the residents regarding participation.

Pollutant levels: Preliminary data during Winter/Spring of 2018 demonstrate that this complex is impacted by near-roadway air pollutants. Average outdoor PM2.5 levels were 16.6 ± 10.7 μg/m3. This is above AQGs and levels seen in RAPIDS in Detroit (15.7 μg/m3) where we have already shown the benefits of lowering exposure to PM2.5 using AFUs[30]. Traffic pollutant levels, including black carbon (1.3 ± 0.5 μg/m3) and ultrafine particles (10,545 ± 2,317 particles/cc), were also relatively high.

Recruitment: Potential participants will be recruited directly from Carpenter Place Apartments. Investigators will post fliers in public areas and investigators will hold "town-hall" style meetings to help disseminate study information. Investigators will note each resident's status as interested, not interested, or undecided in an attempt to recruit all necessary subjects from this limited pool, and to assure residents are disturbed as little as possible. Fliers may be slid under each apartment door to reach subjects and flier information may be included in the resident newsletter.

Screening Visit: Interested individuals will contact investigators and undergo screening (fasting >8 hours) in a private room at Carpenter Place. Information will be collected on all key demographic factors (age, race, sex), cardiometabolic risk factors (diagnosed hypertension, diabetes, hyperlipidemia), prior smoking history (pack years, when quit), and CV disease history (e.g., prior MI, stroke) as well as all medications and doses. BP and finger blood glucose (glucometer) will be measured. Urine dipstick will be performed to corroborate non-smoker status (i.e., no tobacco during past 10 days by NicAlert <100 ng/mL). Inclusion and exclusion criteria will be reviewed and the study will be discussed in detail with each individual. Individuals who wish to participate and who satisfy all study entry criteria will sign a written informed consent document and be enrolled into the trial on a rolling basis. Height and weight will then be measured for calculation of BMI.

Study Execution: A private room in Carpenter Place will be allocated for all visits. Only one participant will be permitted in this room at any given time to protect privacy. The temperature will be maintained between 70-75 ◦F for all visits to limit any immediate impact on BP. ***The entire trial will be conducted within the apartment complex.***

This assures minimal risks and burden to the participants and reduces the potential impact of outdoor exposures (noise, temperature) immediately prior to study outcomes. Once enrolled, participants will be randomized into the first study block. The study consists of a 10-week, crossover trial (Fig. 5).

Activity restrictions: Participants will be provided with an activity log-sheet to record periods of: exercise, over-night stays outside their residence (if any), time periods of open outdoor apartment windows, or other concerns. We will ask participants to sleep overnight in their bedroom at Carpenter Place during all nights of the study and to notify investigators (and note in their activity log) if they must spend a night offsite. If unexpected overnight travel occurs, this will not prohibit continued participation unless they spend a total of more than 4 days during any single study block offsite. In this case the participant will discontinue the study, and their data will be excluded from analysis due to this pre-defined protocol deviation. There are no limitations on air conditioning, heating, or indoor sources of pollution (e.g., cooking). All activities will be allowed except no exercise during periods of 24-hour BP and personal PM2.5 monitoring. We will place no restrictions on opening outside apartment windows to keep the trial as real-world as possible. In RAPIDS we did not require any restriction in this regard and nevertheless we achieved the reported significant reduction in indoor/personal PM2.5 exposures and improved BP levels. Participants will be instructed to record in their logs any periods that windows were opened to facilitate determining its impact on AFU efficacy for reducing PM2.5 exposure and BP reductions in sensitivity analyses (although there were no such adverse impacts found in RAPIDS).

Visit logistics: All study visits will commence at the same time for each participant between 8AM-10AM, after fasting for >8 hours (prior to breakfast, any caffeine) and before any medications**. All BP measurements will be obtained prior to antihypertensive medications to obtain a "trough" level, per standard clinical trial practices70.** After visits, participants will continue daily routines (and take any medications) without restrictions.

Block 1:

Week 1/day 1: An investigator will place one AFU in the bedroom of each participant's apartment. Each AFU will be set to either "active mode" (i.e., a HEPA filter inside) or "sham mode" (i.e., no HEPA filter inside) in ***randomized double-blind fashion.*** We have documented that both modes exhibit similar noise and identical appearance. This will be accomplished by having a different (unblinded) investigator set the AFU modes offsite beforehand – so that neither the on-site investigator nor the participants will be aware of the settings during all study blocks. This offsite unblinded investigator will be the only team member aware of the randomization order. For the ***placebo intervention***: both AFUs will be set to "sham mode". The AFUs will remain running in the same mode for the entire 4-week study block.

Week 1/days 2-4: Participants will be asked to sleep every night in their own apartment and measure their blood pressure using a home BP device we provide just prior to going to bed and upon waking up. At the end of the visit on day 4, participants will have heart rate variability determined with a holter monitor for 6 minutes and blood pressure measured using the BPTru device. Subjects will be fitted with 2 portable devices: an ambulatory BP monitor and a personal PM2.5 monitor. At all times during the 24-hour period, they will wear the PM monitor or have it near them (e.g., on bedside stand while sleeping). We have demonstrated that these devices can be worn together for 24 hours without excessive burden[38,40]. During this period, participants will be instructed not to exercise.

Week 1/day 5: Upon arrival the personal monitoring devices will be collected.

Weeks 2-3: Participants will undertake their usual activities (except for overnight travel restrictions).

Week 4: Study visit days 2-5 will be repeated as during week 1.

Visit day 5 is completed and prior to eating or taking medications, participants will have blood drawn in the study room by an investigator. An investigator will then remove the AFUs from participants' apartments.

Washout period: Participants will undergo a 1 or 2-week washout period without restrictions on activities (including overnight travel). No AFUs will be in participant's apartments during this time. We have shown in RAPIDS that a 1-week washout period is adequate, with no evidence for a carryover effect from the previous intervention on endpoints (BP, PM2.5 exposures). There was no statistically significant effect (interaction, confounding) from the order of the intervention on the results[30]. PM2.5 exposures and BP returned to baseline after one week.

Block 2 (crossover phase): Participants will undergo the same protocol as during Block 1, the only difference being that on *week 1/visit day 1* of both blocks, the AFUs will be set to different modes (active vs. sham) from prior blocks(in randomized order).

Retention plan: Based on our experience with RAPIDS, we do not anticipate retention problems. During weeks 2 and 3, investigators will contact each participant at least twice per week (phone, email, or in-person) to assure continued participation and compliant usage of AFUs, and to address any study problems.

Timeline: In RAPIDS, only 1/41 participants dropped out after enrollment. However, given the longer duration of this protocol, we intend to be realistically conservative in our estimates for RAPIDS-2. We will allow for a 10% dropout rate and thus plan to enroll 55 people to meet our goal of n=50 participants completing the entire study.

Aim 1: Determine if long-term AFU usage provides sustained reductions in PM2.5 exposure and persistent improvements in cardiometabolic outcomes.

Aim 2: Determine if nocturnal PM2.5 exposure reduction improves cardiometabolic outcomes.

Aim 3: Demonstrate the key role of adrenal activation as a novel mechanistic pathway explaining PM2.5-induced cardiometabolic effects.

Experimental Procedures and Protocols

AFU intervention: We have carefully designed this study to accommodate the needs and limitations of the volunteer subjects and minimize changes and burdens on their lifestyle by incorporating noninvasive daily home BP testing while also assuring detection of outcome-exposure associations based on our previous studies. As described above in the Progress Report section, despite the fact that the RAPIDS protocol did not restrict subjects' activities (e.g., window opening), we were able to reduce personal PM2.5 exposure and observe improved CV health outcomes. Thus, we will maintain the same protocol and study real-world exposure to the extent possible. Apartment central air conditioning and heating can be used without limitations. Participants will record in activity logs any period in which windows are opened. We will employ the same commercially available AFU brand (Holmes) proven in RAPIDS to effectively reduce indoor and personal PM2.5 exposures and lower BP among participants[30]. For the "gold standard" comparison (2-room AFU use), we will follow the same deployment protocol successfully used in RAPIDS30 and supported by EPA guidance and expert scientific opinions[73-76]. At 8AM Monday (week 1, visit day 1) of each study block, a blinded onsite investigator will place AFUs in each participant's residence. One AFU each will be placed in both the bedroom and main living room. Each AFU will be set to the randomized mode ("active" or "sham") per the description in prior sections in a *randomized double-blind fashion* by a different offsite investigator beforehand so that neither the onsite investigator nor the participant will be aware of the settings (the HEPA filter is not visible from the outside). In both rooms the AFU will be a Holmes HAP8650B-NU-1, designed for spaces up to 310 square feet. Under the "active" mode, the AFU will be fitted with a new "true HEPA" filter (Holmes HAPF600TCS), validated to remove 99.97% of particles at 0.3 µm in size[73]. Ultrafine (10-100 nm) particles (e.g., from traffic) and larger particles (e.g., coarse PM) are typically filtered even more effectively by HEPA filters[73-76]. Under the "sham" mode, a HEPA filter will not be installed in the AFU and there will be no significant removal of PM2.5. Each AFU will be fitted with a cumulative power consumption meter (Kill-A-Watt EZ) to verify continuous operation at the correct fan speed setting for the entire intervention period. The low noise level of the AFUs will be indistinguishable between active and sham

Table 1: List of Study Endpoints

Health Endpoints	Endpoint designation
Unattended automated systolic BP 4-day average (Tues-Fri) in weeks 1 and 4	***Primary study outcome (Aims 1a, 2a/2b) Study is a priori powered for this endpoint***
24-hour BP levels & aortic hemodynamics	Secondary endpoints (Aims 1c & 2d)
Insulin sensitivity (HOMA-IR)	Secondary endpoint (Aims 1c & 2d)
Adrenal steroid panel (blood)	Secondary mechanistic endpoints (Aims 3a/3b)
Exposure Endpoints	
24-hr personal $PM_{2.5}$ (filter-gravimetric mass)	Primary exposure outcome (Aims 1b, 2c)
Daily indoor $PM_{2.5}$	Primary exposure outcome (Aims 1b)
Black carbon, hourly personal $PM_{2.5}$ levels	Secondary exposure endpoints

modes. In RAPIDS, no participant complained or required the AFU to be stopped due to noise30. The AFUs will run in the same blinded mode continuously for the entire 4-week block. During the washout periods, the offsite investigator will remove or install the HEPA filters in the AFUs prior to starting the next block. The HEPA filters are rated to be effective for the entire 8 weeks of active usage and will not need to be replaced.

Protocols for cardiometabolic health endpoints

Home BP: This BP measurement will be the primary outcome (Table 1). The ***rationale*** is that high BP is a well-established predictor of CV risk[68-71], the leading risk factor for global mortality[3], increases due to short-term PM2.5 exposure[5-8,36-56], and was shown by our RAPIDS study30 to be reduced by AFUs. Unattended automated BP more accurately reflects 24-hour BP, is less impacted by variability and the white coat effect and is superior to the standard technique as a predictor of hypertensive complications[71]. The repeat-measured morning BP average using the BpTRU device was the primary outcome in RAPIDS30, thus it is important to use this same method in this renewal project. **Methods**: Guidelines for BP measurement will be meticulously followed (e.g., no caffeine, exercise >1 hr prior)[70]. The BpTRU device will be located in a quiet room where participants will have seated BP measured71. The proper cuff size will be fitted on their upper right arm and participants will rest seated with their feet uncrossed on floor and their arm supported at midsternal level for 5 minutes. A first BP will be determined to assure proper monitor function. Afterward, personnel will leave the room and the BpTRU will measure 5 automated unattended BP readings (1-min intervals) with the participant alone. The average of these 5 unattended BP levels and heart rates will be recorded as the endpoint for each visit. The **primary outcome** is average systolic BP (determined as above) obtained over 4 consecutive (Tue-Fri) mornings (Fig. 5). The 4-day average (not 3-day as in RAPIDS) will be used because the results showed that the BP reduction tended to become more consistent with increasing time of filtration. This will improve the stability of BP levels and increase the power for detecting changes in the primary outcome[30]. **The 4-day average of unattended fully-automated 5-repeated BPs is a rigorous and repeatable outcome. BP will be determined fasting in the morning before participants leave the residence and prior to other factors potentially altering BP (e.g., noise, traffic, activity, outdoor temperature). This is a uniquely powerful method to capture an effect of any intervention (i.e., AFU) on lowering BP compared to using conventional clinic-based methods as done in other trials[21-29]. This explains our unparalleled capability to detect a BP-lowering effect of AFU use (i.e., even a small - yet clinically significant - decrease in systolic BP by ~3 mm Hg as in RAPIDS)30.**

24-hour BP and aortic hemodynamics: We have extensive experience with and will use the SphygmoCor XCEL PWA system which non-invasively measures upper arm BP and central aortic hemodynamics over a 24- hour period. These endpoints will all be considered secondary study outcomes. The ***rationale*** is that aortic BP levels differ from arm levels depending on a variety of factors (e.g., height, age, heart rate, arterial stiffness). They are validated superior predictors of CV target organ damage and future CV events[64-67]. Aortic BP levels also change in response to interventions (e.g., medications) and possibly to PM2.5 exposures discordant from those detected by traditional arm BP measurement. Hence, measuring aortic hemodynamic responses to AFU intervention will provide novel insight into the totality of hemodynamic responses induced by PM2.5 exposures. The device calculates aortic BPs (systolic, pulse pressure) and hemodynamics: augmentation pressure and index (AIx@75), a metric of arterial pressure wave reflection from the periphery back to the aorta and left ventricular load adjusted for a heart rate at 75 beats/min), and coronary perfusion index (CPI)[64-67]. These parameters are calculated by pulse wave analyses (PWA) of arterial waveforms recorded from a specialized upper arm cuff of an Oscar-2 (SunTech Medical) 24-hour ambulatory BP monitor by a mathematical generalized transfer function. PWA has been used in hundreds of studies[64-67]. The device will be used to determine brachial BP and aortic hemodynamics every 30 minutes over a full 24-hour period. The

rationale is that 24-hour BP levels are the "gold standard" endpoint most predictive of CV risk[60,68,69]. They can change discordantly (e.g., white coat or masked high BP) from resting seated values60. Finally, **nocturnal BP** is important to characterize. In RAPIDS, AFU use reduced nighttime PM2.5 exposures by the greatest extent because an AFU was situated in each participant's bedroom (Fig. 2). Given the robust prognostic value of nocturnal BP[63], we will also explicitly determine the impact of AFU use on reducing PM2.5-mediated nocturnal BP elevations. **Methods**: We will follow guidelines for 24-hour BP monitoring[69] and all manuals for the SphygmoCor XCEL (http://atcormedical.com/healthcare-professionals/). The appropriate size cuff will be fitted on the right upper arm of participants. This cuff is connected to the BP monitor (Oscar-2) worn on a strap. The monitor will record BP every 30 minutes for a 24-hr period. Subjects will be instructed to have their right arm resting by the side of their body during each BP measurement. Instructions for troubleshooting (e.g., loose cuff) will be provided[69]. Participants will record the times they went to bed and awoke to identify nocturnal periods. The data from the Oscar-2 monitor will be analyzed by the SphygmoCor system. **Outcomes**: 24-hr, daytime and nighttime averages of brachial systolic/diastolic BP, pulse pressure, and heart rates; and aortic hemodynamics (systolic BP, pulse pressure, augmentation pressure, AIx@75, and CPI).

Protocols for exposure monitoring

Indoor exposure monitoring: An indoor PM2.5 sampler will be positioned in the main room at the furthest point away from the AFU with samples being collected onto Teflon filter media using Teflon-coated aluminum cyclone sample inlets at a nominal flow rate of 16.7 L/min. This sampler will use pump systems of a type

designed and used for previous studies30. These pump systems use acoustically-insulated wood cases designed for operation in home environments, minimizing pump noise. Filter samples will be collected from exposure periods starting at 8 AM on visit day 1 until 8 AM on visit day 5 during weeks 1 and 4 of each study block. For the entire duration of each block (including weeks 2-3), an indoor PM2.5 air pollution sensor (PurpleAir sensor; PA-II-SD) will be used to measure continuous PM2.5 concentrations. The PurpleAir is a low-cost, light-scattering-based PM sensor and is currently in widespread use[77]. In order to ensure that the sensor performance is equivalent to the gravimetric federal reference method, a correction factor will be calculated by comparing sensor data against gravimetric filter measurements from the indoor sampler described above. The filter samples will also allow for particle characterization/source apportionment. In addition, an aethalometer (MicroAeth AE 51, Aethlabs) – used to continuously measure black carbon concentrations, provide data logging and a continuous readout – will be located near the PurpleAir sensor. ***Rationale:*** To determine indoor integrated 4-day, as well as daily, indoor PM2.5 and black carbon (traffic particles) during the period of health outcomes.

Personal exposure monitoring: During each 24-hr period of personal monitoring, participants will wear (on a belt or strap) a small (2 lbs) battery-powered personal particulate monitor (Thermo Scientific pDR-1500). This scientifically-validated device provides time-stamped continuous data storage of both ambient temperature and PM2.5 exposures. While time frames as brief as 5 minutes can be analyzed by the continuous data collection, we plan to evaluate 60-min exposure widows as in our prior study (Fig. 2). ***Rationale:*** We employed this device in RAPIDS and demonstrated the importance of characterizing time-resolved reductions in personal PM2.5 exposures, which differed from indoor PM2.5 levels due to variations in daily activity. The personal particulate monitors will be provided to the participants after visit 4 is completed and will be worn as much as possible for the ensuing 24 hours both indoors and outdoors. While sleeping, monitors will be placed on a nearby nightstand. Participants may also remove the monitor during the daytime for certain activities (e.g., showering); however, they will be instructed to keep it within close proximity. The monitor has adequate battery power to provide 24 hours of continuous monitoring. The pDR-1500 also collects sampled particles onto 37-mm Teflon filters for gravimetric and chemical analyses of PM2.5. Continuous PM2.5 data will be validated by comparing results to 24-hr integrated mass values obtained by "gold-standard" gravimetric measurements of filters. Using continuous exposure windows in addition to 24-hr filter-based measurements will allow for a shorter time frame resolution of intra-day exposures and for characterizing hourly exposure reductions achieved by the AFU (Fig. 2).

Activity log: During each 24-hour period of personal monitoring, participants will be asked to keep a log of specific activities: sleep/awake time, outdoor windows open, time outdoors, and time in traffic. They will also keep a log during the entire study to record: open window times, exercise, nights spent outside their residence.

Filter analysis: Sample handling, processing, and analysis will take place in a Class 100 ultraclean room at the Michigan State University Exposure Science Laboratory. Gravimetric determinations of PM2.5 mass will be made using a microbalance (XP6UD5, Mettler Toledo) in a temp/humidity-controlled room as described elsewhere[30,31].

Protocols for blood outcomes

Blood draws collection: On week 4/visit day 5 (Blocks 1-3) after BP measurement, an experienced investigator will draw fasting blood from the participant's antecubital fossa using clean universal precautions. We will draw 10 ml of blood each time, which will also allow for stored aliquots for post hoc studies. We will obtain blood for steroidomics profiling at the designated time points from as many participants as possible. Missing any blood collection may weaken our final power to meet aim 3; however, it will not impact eligibility for participants to remain in the study as it will not adversely affect the main study objectives (Aims 1 and 2).

Comprehensive steroidomic profiling: The HPAA is highly responsive to stressors such as air pollution, as we herein hypothesize. The ***rationale*** for focusing on HPAA activation as a central mechanism of PM2.5-induced cardiometabolic effects has been addressed (Fig. 1)[32]. We will measure **ACTH levels** (a metric of central nervous system hypothalamic/pituitary activation) by the MDRC clinical laboratory. Dr. Richard Auchus has performed extensive work to develop comprehensive steroid profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS)[78-87]. By simultaneously measuring multiple steroids across several adrenal pathways, we can identify patterns and biomarkers for stress-responses, including aldosterone[87], steroid sulfates and 11-oxyandrogens as biomarkers of acute and chronic HPAA activation. Among the advantages of LC-MS/MS is the capacity to interrogate multiple adrenal steroids, including mineralocorticoids, cortisol, and 11-oxygenated steroids (glycerrhetinic acid-like factors)[86] that inhibit 11β-hydroxysteroid dehydrogenases and thus potentiate the hypertensive effect of cortisol. Our unique LC-MS/MS method allows us to uncover the full extent of adrenal pathway activation contributing to hypertension via multiple mechanisms with unprecedented biochemical sensitivity and specificity from a small blood sample (<0.25 mL of serum) and measures up to 35 steroids (Table 2). In this panel are multiple parent steroids, hybrid steroids and steroid precursors that possess glucocorticoid and mineralocorticoid activity. Synthesis and release of all or some of these steroids can increase due to stress responses (multiple potential pathways elicited by PM2.5 exposure)[32] and play a key mechanistic role in promoting both pro-hypertensive responses and metabolic insulin resistance[32]. **Methods:** Aliquoted serum samples will be stored in a -80°C freezer for annual batched analyses. For steroid analysis, serum samples (0.1 mL) are mixed with internal standards and collected via supported-liquid extraction, resolved by 2-dimensional chromatography (Agilent 1260 and 1290 binary pump systems), and measured using multiple reaction monitoring with an Agilent 6495 triple quadrupole LC-MS/MS operating in positive electrospray ionization mode as described[79-84]. The Delta-5/5α-Panel is analyzed as oxime derivatives, and steroid sulfates are analyzed in negative ion mode[79,83]. Cortisol in saliva and hair is extracted with methanol and measured as above85. **Thus, we will perform a comprehensive assessment of HPAA activity, and the benefits of AFU usage, across several biologically important timescales. This includes a disruption in diurnal rhythm which alone can cause adverse effects (11PM saliva cortisol), as well as subacute HPAA (serum ACTH and steroidomics panel) and chronic HPAA activation (hair cortisol) induced by PM2.5.**

Table 2. Steroid panels using LC-MS/MS

Delta-4/Estrogens Panel (0.1 mL)

Cortisol Precursors

- Progesterone
- 11OH-Progesterone
- 16OH-Progesterone
- 17OH-Progesterone
- 11-Deoxycortisol
- 21-Deoxycortisol

Delta-4/Estrogens Panel (0.1 mL)

Cortisol, Cortisone, & Hybrid Steroids

- Cortisol*
- Cortisone*
- **18OH-Cortisol***
- 18-oxo-Cortisol*

Androgens & Precursors

- Androstendione

Delta-5/5□- Panel (0.1 mL)

- Pregnenolone
- 17OH-Pregnenolone
- Dehydroepiandrosterone
- Allopregnanolone
- 5□-Pregnane-3□,17□-diol-20-one
- Androsterone
- Dihydrotestosterone

Steroid Sulfate Panel (0.01 mL)

- Pregnenolone Sulfate
- 17OH-Pregnenolone Sulfate
- Dehydroepiandrosterone Sulfate
- 5-Androstenediol Sulfate
- Androsterone Sulfate

Mineralocorticoids/ Precursors

- 11-Deoxycorticosterone*
- Corticosterone*
- **18OH-Corticosterone***
- Aldosterone*
- *Estrogens*
- Estrone
- Estradiol
- Testosterone
- 11OH-Androstenedione
- 11Keto-Androstenedione
- 11OH-Testosterone
- 11Keto-Testosterone
- Etiocholanolone Sulfate

Bio-banked blood: We will store 0.5-ml aliquots of serum and plasma at the end of each study block. We have access to a -80°C freezer with alarms and backup power in our research room at the University of Michigan Cardiovascular Medicine outpatient clinical facility at Domino's Farms Lobby A. This will allow for post hoc analyses of many possible biomarkers after study completion depending upon the nature of our results.

Study Pitfalls and Remediation Plans

We do not anticipate difficulties successfully completing this trial or utilizing the methods proposed as they have all been performed in our laboratories and many have been conducted in our previous clinical trials (e.g., RAPIDS). It will be critical to maintain robust recruitment and retention of subjects. We plan to further reinforce mutually beneficial relationships that have already been established with residents and facility staff and address concerns about the study on an ongoing basis (e.g., townhall meetings). We will adopt and modify the study (as long as it does not compromise the science) if deemed required by community feedback during the start-up period in year 1 and during the study if such a need arises. Other pitfalls are described in individual subsections.

Outreach Activities and Knowledge Dissemination

A social ecological approach[88] will be used to translate the findings of the study into health-promoting actions that can be taken by older adults, family caregivers, housing managers, health care providers, and policy makers to reduce the health risks of exposure to air pollution in congregate housing for the elderly. Common values of community-based health promotion include:

- Stakeholder engagement in the design and evaluation of translation strategies[89,90]
- A comprehensive evaluation of process, implementation, and sustainability[91]
- A dissemination plan[90]

Evaluation strategies applied to the health education materials and methods will include process evaluation, assessment of short term learning outcomes, and a six-month follow-up on the extent of adoption and maintenance of behavioral or environmental changes among selected housing communities. Evaluation criteria for selected target audiences (housing managers; community-based network) will address standards of utility, feasibility, propriety, and accuracy (fidelity)[90]. The study team will:

1. Consult with study participants and key stakeholders throughout the study to help develop the strategies and media for educating older adults and providers of housing for the elderly.
2. Conduct tailored, one-to-one educational sessions with study participants to share results and implications, and to develop educational materials and methods.
3. Design and conduct communications to address potential mobility, cognitive, and sensory limitations of older adults (e.g., access to group meetings; impaired vision and hearing), and health literacy.
4. Develop and evaluate fact sheets in cooperation with the Residents' Council and Activities coordinators for distribution through existing channels (e.g., meetings, flyers).

5. Schedule meetings for presentations by study team members[89] to the residents, staff and key associates.
6. Utilize the network of community-based organizations and advocacy groups[92] to reach the larger population of managers of senior housing communities, health care providers and policy makers via presentations at professional meetings. Key dissemination networks include marketing and networking trade meetings of senior housing providers; the Area Agencies on Aging, and the Michigan Association of Homes and Services to the Aging.
7. Disseminate findings for broader application by health professionals through channels of peer-reviewed publications and presentations at conferences on aging.

Statistical Analyses and Data Management

Sample size, power considerations, and statistical analysis plans are provided in full in the Study Record: the PHS Human Subjects and Clinical Trials Information document.

Project Management and Schedule

As described in the multiple PI leadership plan, Drs. Brook and Morishita together will provide oversight of the entire project. Since the proposed research is interdisciplinary and involves multiple schools/departments, a governance structure will be implemented in order to ensure effective management and coordination. The proposed research will follow a work plan that will meet the milestones shown in the "Study Timeline" attached to the Study Record: the PHS Human Subjects and Clinical Trials Information document. We will hold bi-monthly general project meetings involving all staff and investigators to present the latest data and to coordinate study logistics and analysis. The team of investigators on this proposal is well-poised to integrate their various disciplines and ensure a high probability of success for accomplishing the proposed specific aims.

DATA MANAGEMENT AND STATISTICAL CONSIDERATIONS

Study Power and Analysis Plans:

With 50 subjects randomized to blocks 2 ("bedroom alone AFU"), and ("control") in a random sequence, we anticipate adequate power using our proposed cross-over study design for observing significant reductions in ***average systolic BP (primary endpoint)***. With the repeated BP measurements collected in this cross-over design, we will do paired analysis for power calculations, since each subject can serve as their own control given sufficient washout periods and <u>because we have a prori pre-specified the group comparison hypothesis for each aim clearly beforehand</u>. We will be able to achieve 90.3% power in **Aim 1a** for detecting a 3 mm Hg in mean systolic BP difference comparing the "bedroom AFU" to the "control" group at both the acute (4-day) and chronic (4-week) time periods, assuming that the standard deviation (SD) of systolic BP is 8.2 mm Hg. The detectable mean difference of systolic BP between "bedroom FU" vs "control" with the corresponding statistical powers that we can achieve is as follows:

Detectable mean BP difference between "active intervention" vs. "control" (Hg)	2.4 mm	2.6 mm	2.8 mm	3 mm Hg	3.2 mm	3.4 mm
Statistical Power	75.4%	81.9%	86.1%	90.3%	93.6%	95.4%

Within the range of the SD of systolic BP measurements from 6.2 to 9.8, the statistical power for aim 1a we can achieve ranges between 72.1% and 97.5% to detect a 3 mm Hg change in mean systolic BP. Similar powers will be achieved for **Aim 2a** as well, comparing "bedroom alone AFU" (block 2) versus "control" (block 3) group. In our previous AFU study (RAPIDS, see: Progress Report) we observed a 3.2 mm Hg reduction in systolic BP by active versus control intervention. Therefore, the pre-specified effect size (3 mm Hg systolic BP reduction) for which we have designed this renewal proposal to have adequate power to observe is well justified. <u>The study is a priori designed and</u>

powered to determine the pre-specified outcomes in aims 1a, 2a, and 2b. Other comparisons evaluated in aims 1b (24-hr personal-$PM_{2.5}$ exposures); 1c (secondary health endpoints); 2c (24-hr personal-$PM_{2.5}$ exposures); 2d (secondary health endpoints); as well as in aims 3a (differences in steroid levels between blocks) and 3b (mediation analyses) are all deemed secondary study outcomes. We will collect the data as proposed and analyze the results using the same statistical methods as outlined for the primary study outcomes. However, since these are secondary endpoints only (i.e., hypothesis generating results), we will analyze the data for trends and for "nominal" statistical significance. We will also not perform a priori power calculations for all secondary endpoints.

Both the primary endpoints and the secondary measures such as diastolic BP, 24-hour BP levels and HOMA-IR will be reported as mean (median) ± SD (IQR). One-sample paired t-test will be performed to compare each outcome variable between active intervention group and the control group. In case there are extreme values or that the variables are not normally distributed, Wilcoxon signed-rank test, a non-parametric test for comparing repeated measurements' mean ranks, will be performed for robustness[1]. When the distribution of an outcome variable is not anticipated to follow a normal distribution, we will transform it to meet normality assumptions as best as possible before we fit regression models to adjust for confounders (age, sex, BMI, dose of BP tablets, type of BP control medication). Non-parametric correlation coefficients, such as Kendall's tau[7] or spearman's rho[8], between the principal outcomes and exposure measures will be calculated at each time point. Such measures provide robust inferences. Multiple hypothesis testing will be adjusted through the Holm's procedure[9].

More specifically, to adjust for other confounders, we will use linear mixed models[2-4] to evaluate the effect of interventions on the outcomes (e.g. BP levels) adjusting for confounders. The mixed models will fully represent the structure of the data by taking into account the within-subject correlations of the outcome variables measured repeatedly at different time points over the study design. We will also evaluate the impact of other variables that may impact the results. To be more specific, a mixed effect model with a random intercept and a random slope[2-4] will be fit for the longitudinal measurement of the outcomes: $\mathrm{Y_{ij}} = \mathrm{a_0} + \mathrm{a_1 t_{ij}} + \mathrm{a_2} I(\mathrm{B_{ij}} = 1) + \mathrm{a_3} I(\mathrm{B_{ij}} = 2) + \mathrm{a_4 X_{ij}} + \mathrm{b_{i0}} + \mathrm{b_{i1} t_{ij}} + \mathrm{e_{ij}}$, where $\mathrm{Y_{ij}}$ denotes the repeated average BP outcomes for subject i at block j, $\mathrm{B_{ij}}$ denotes what intervention is subject i randomized to at occasion j, and $\mathrm{X_{ij}}$ are the other covariates that would be controlled in the model (age, sex, BMI, dose of BP tablets, type of BP control medication). So in **Aim 1a**, we hypothesize $\mathrm{a_2} \neq 0$ and test for $\mathrm{H_0} : \mathrm{a_2} = 0$, and in **Aim 2a**, we hypothesize $\mathrm{a_3} \neq 0$ and test for $\mathrm{H_0} : \mathrm{a_3} = 0$, and in **Aim 2b**, we are testing $\mathrm{a_2} = \mathrm{a_3}$. Similar models will be fit for other secondary outcome variables, such as ABPM, HOMA-IR, etc. When it happens some outcome variables have inflated zero measures, we will adopt zero-inflated regression models or a two-part mixture regression (tobit) model[5,6] on the outcome changes between "active intervention" vs. "control".

Other statistical considerations:

Missing data: We will try to avoid missing data when collecting data through the study design. When missing data happens, we will test if the data are missing completely at random (MCAR). If it is MCAR or the amount of missing data is less than 10%, analysis will be performed based on the complete data. If the amount of missing data is more than 10%, multiple imputations (5-10 times) will be utilized through mi package in R[10]. The analysis will be performed on each imputed data set, and later merged through mitools package in R[11]. Multiple imputations are based upon the missing-at-random (MAR) assumption[12]. Sensitivity analysis[13] will be examined when inferences depart from the MAR assumption.

Data Monitoring and Coordination: The Data Coordinating Center (DCC) at U-M will ensure the proposed research collect and manage data in a confidential and secure manner. U-M DCC will review data to control its data quality and integrity, organize data using uniform formats and vocabulary to facilitate analysis and document protocols for collecting, filtering and formatting the data. All files stored at the U-M database will be managed by Dr. Wang as was done previously for other successful grants. The database provides a central location for disseminating and retrieving study information, including codebooks, common data element definitions, synopses of studies, data collection forms (if necessary), and analyses in progress. We will create and maintain a webpage for any distributed software packages, with detailed documentation as well as contact information. We will also archive all relevant publications from the proposed research by following the law of copyrights.

Quality Management Plan: All study outcomes have been performed by our research team in prior studies and are well-established research endpoints using in hundreds of prior experiments. We will follow the developed protocols used in our prior experiments and established manual of operations (Dr. Brook, available on request). All proposed blood outcomes will be measured in accredited clinical laboratories. We will plan regular (quarterly) group meetings during the trial to identify and mitigate any unanticipated study pitfalls that develop during the trial.

Bibliography & References Cited

1. Brook RD, Rajagopalan S, Pope CA III, Brook JR, Bhatnagar A, Diez-Roux A, Holguin F, Hong Y, Luepker, RV, Mittleman M, Peters A, Siscovich D, Smith SC Jr, Whitsel L, Kaufman JD. Particulate matter air pollution and cardiovascular disease. An update to the scientific statement from the American Heart Association. *Circulation* 2010; 121: 2331-78.

2. Lelieveld J, Evans JS, Fnais M, Giannadaki D and Pozzer A. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature*. 2015;525:367-71.

3. Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, Balakrishnan K, Brunekreef B, Dandona L, Dandona R, Feigin V, Freedman G, Hubbell B, Jobling A, Kan H, Knibbs L, Liu Y, Martin R, Morawska L, Pope CA 3rd, Shin H, Straif K, Shaddick G, Thomas M, van Dingenen R, van Donkelaar A, Vos T, Murray CJL, Forouzanfar MH. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet* 2017; 389: 1907-1918.

4. Brook RD, Newby DE. Rajagopalan S. Air pollution and cardiometabolic disease: An update and call for clinical trials. *Am J Hypertens* 2017; 31: 1-10.

5. Giorgini P, Di Giosia P, Grassi D, Rubenfire M, Brook RD, Ferri C. Air pollution exposure and blood pressure: An updated review of the literature. *Curr Pharm Design* 2015: 22; 28-51.

6. Liang R, Zhang B, Zhao X, Ruan Y, Lian H, Fan Z. Effect of exposure to PM2.5 on blood pressure: a systematic review and meta-analysis. J Hypertens 2014; 32: 2130-40

7. Cai Y, Zhang B, Ke W, Feng B, Lin H, Xiao J, Zeng W, Li X, Tao J, Yang Z, Ma W, Liu T. Associations of Short-Term and Long-Term Exposure to Ambient Air Pollutants with Hypertension: A Systematic Review and Meta-Analysis. *Hypertension.* 2016; 68: 62-70.

8. Rao X, Montresor-Lopez, Puett R, Rajagopalan S, Brook RD. Ambient air pollution: An emerging risk factor for diabetes mellitus. *Curr Diabetes Rep* 2015; 15: 33.

9. Newby DE, Mannucci PM, Tell GS, Baccarelli AA, Brook RD, Donaldson K, Forastiere F, Franchini M, Franco OH, Graham I, Hoek G, Hoffmann B, Hoylaerts MF, Künzli N, Mills N, Pekkanen J, Peters A, Piepoli MF, Rajagopalan S, Storey RF. Expert position paper on air pollution and cardiovascular disease. *Eur Heart J* 2015; 36: 83-93b.

10. Ghosh R, Lurmann F, Perez L, Penfold B, Brandt S, Wilson J, Milet M, Künzli N, McConnell R. Near-Roadway Air Pollution and Coronary Heart Disease: Burden of Disease and Potential Impact of a Greenhouse Gas Reduction Strategy in Southern California. *Environ Health Perspect* 2016; 124: 193-200.

11. Kheirbek I, Haney J, Douglas S, Ito K, Matte T. The contribution of motor vehicle emissions to ambient fine particulate matter public health impacts in New York City: a health burden assessment. *Environ Health* 2016;15: 89.

12. https://www.epa.gov/air-trends/particulate-matter-pm25-trends

13. Burnett RT, Pope CA 3rd, Ezzati M, Olives C, Lim SS, Mehta S, Shin HH, Singh G, Hubbell B, Brauer M, Anderson HR, Smith KR, Balmes JR, Bruce NG, Kan H, Laden F, Prüss-Ustün A, Turner MC, Gapstur SM, Diver WR, Cohen A. An integrated risk function for estimating the global burden of disease attributable to ambient fine particulate matter exposure. *Environ Health Perspect* 2014; 122: 397-403.

14. Crouse DL, Peters PA, van Donkelaar A, Goldberg MS, Villeneuve PJ, Brion O, Kahn S, Atari DO, Jerret M, Pope CA III, Brauer M, Brook JR, Martine RV, Steib D, Burnett RT. Risk of nonaccidental and cardiovascular mortality in relation to long-term exposure to low concentrations of fine particulate matter: A Canadian national-level cohort study. *Enivon Health Perspect* 2012; 120: 708-14.

15. Pinault L, Tjepkema M, Crouse DL, Weichenthal S, van Donkelaar A, Martin RV, Brauer M, Chen H, Burnett RT. Risk estimates of mortality attributed to low concentrations of ambient fine particulate matter in the Canadian community health survey cohort. *Environ Health* 2016; 15: 18.

16. Weichenthal S, Villeneuve PJ, Burnett RT, van Donkelaar A, Martin RV, Jones RR, DellaValle CT, Sandler DP, Ward MH, Hoppin JA. Long-term exposure to fine particulate matter: association with nonaccidental and cardiovascular mortality in the agricultural health study cohort. *Environ Health Perspect.* 2014; 122: 609-15.

17. Thurston GD, Ahn J, Cromar KR, Shao Y, Reynolds HR, Jerrett M, Lim CC, Shanley R, Park Y, Hayes RB. Ambient Particulate Matter Air Pollution Exposure and Mortality in the NIH-AARP Diet and Health Cohort. *Environ Health Perspect* 2016; 124: 484-90.

18. Di Q, Wang Y, Zanobetti A, Wang Y, Koutrakis P, Choirat C, Dominici F, Schwartz J. Air Pollution and Mortality in the Medicare Population. *New Engl J Med* 2017; 376: 2513.

19. Di Q, Dai L, Wang Y, Zanobetti A, Choirat C, Schwartz JD, Dominici F. Association of short-term exposure to air pollution with mortality in older adults. *JAMA* 2017; 318: 2446-2456.

20. Brook RD, Newby DE, Rajagopalan S. The Global Threat of Outdoor Ambient Air Pollution to Cardiovascular Health Time for Intervention. *JAMA Cardiol.* 2017;2(4):353-354.

21. Morishita M, Thompson KC, Brook RD. Understanding air pollution and cardiovascular diseases: is it preventable? Review, *Curr Cardiovasc Risk Rep* 2015; 9:1–9.

22. Chen R, Zhao A, Chen H, Zhao Z, Cai J, Wang C, et al. Cardiopulmonary Benefits of Reducing Indoor Particles of Outdoor Origin: A Randomized, Double-Blind Crossover Trial of Air Purifiers. *J Am Coll Cardiol* 2015; 65: 2279-2287.

23. Li H, Cai J, Chen R, Zhao Z, Ying Z, Wang L, Chen J, Hao K, Kinney PL, Chen H, Kan H. Particulate matter exposure and stress hormone levels: A randomized, double-blind, crossover trial of air purification. *Circulation* 2017; 136: 618-27.

24. Bräuner EV, Forchhammer L, Møller P, Barregard L, Gunnarsen L, Afshari A, et al. Indoor particles affect vascular function in the aged: an air filtration-based intervention study. *Am J Respir Crit Care Med* 2008; 177: 419-25.

25. Karottki DG, Spilak M, Frederiksen M, Gunnarsen L, Bräuner EV, Kolarik B, et al. An indoor air filtration study in homes of elderly: cardiovascular and respiratory effects of exposure to particulate matter. *Environmental Health* 2013; 12: 116.

26. Allen RW, Carlsten C, Karlen B, Leckie S, van Eeden S, Vedal S, Wong I, Brauer M. An air filter intervention study of endothelial function among healthy adults in a woodsmoke-impacted community. *Am J Respir Crit Care Me*. 2011; 183: 1222-30.

27. Kajbafzadeh M, Brauer M, Karlen B, Carlsten C, van Eeden S, Allen RW. The impacts of traffic-related and woodsmoke particulate matter on measures of cardiovascular health: a HEPA filter intervention study. *Occup Environ Med* 2015; 72: 394-400.

28. Padró-Martínez LT, Owusu E, Reisner E, Zamore W, Simon MC, Mwamburi M, Brown CA, Chung M, Brugge D, Durant JL. A Randomized Cross-over Air Filtration Intervention Trial for Reducing Cardiovascular Health Risks in Residents of Public Housing near a Highway. *Int J Environ Res Public Health* 2015; 12: 7814-38.

29. Chuang HC, Ho KF, Lin LY, Chang TY, Hong GB, Ma CM, Liu IJ, Chuang KJ. Long-term indoor air conditioner filtration and cardiovascular health: A randomized crossover intervention study. *Environ Int* 2017; 106: 91-96.

30. Morishita M, Adar SD, D'Souza J, Ziemba RA, Bard RL, Spino CA, Brook RD. Portable Air Filtration Systems, Personal Exposure to Fine Particulate Matter, and Blood Pressure among Residents in a Low-Income Senior Facility in Detroit: A Randomized Trial. *JAMA Internal Medicine*, 2018 (in press).

31. Maestas MM, Brook RD, Ziemba RA, Li F, Crane RC, Klaver ZM, Bard RL, Adar SD, Spino CA, Morishita M. Reduction of Personal $PM_{2.5}$ Exposure via Indoor Air Filtration Systems in Detroit Intervention Study. *Journal Exposure Science and Environmental Epidemiology* 2018 (in press).

32. Brook RD, Rajagopalan S. “Stressed” about air pollution: Time for personal action. *Circulation* 2017; 136: 628-631.

33. Salles, GF, Reboldi G, Fagard RH, Cardoso CRL, Pierdomenico SD, Verdecchia P, Equchi K, Kario K, Hoshide S, Polonia J, de la Sierra A, Hermida RC, Dolan E, O’Brien E, Roush GC, ABC-H Investigators. Prognostic Effect of the Nocturnal Blood Pressure Fall in Hypertensive Patients. The Ambulatory Blood Pressure Collaboration in Patients With Hypertension (ABC-H) Meta-Analysis. *Hypertension* 2016; 67: 693-700.

34. Yano Y, Kario K. Nocturnal blood pressure and cardiovascular disease: a review of recent advances. *Hypertens Res* 2012; 35: 695-701.

35. Fan HQ, Li Y, Thijs L, Hansen TW, Boggia J, Kikuya M, Björklund-Bodegård K, Richart T, Ohkubo T, Jeppesen J, Torp-Pedersen C, Dolan E, Kuznetsova T, Stolarz-Skrzypek K, Tikhonoff V, Malyutina S, Casiglia E, Nikitin Y, Lind L, Sandoya E, Kawecka-Jaszcz K, Imai Y, Ibsen H, O'Brien E, Wang J, Staessen JA; International Database on Ambulatory Blood Pressure In Relation to Cardiovascular Outcomes Investigators. Prognostic value of isolated nocturnal hypertension on ambulatory measurement in 8711 individuals from 10 populations. *J Hypertens* 2010; 28: 2036-45.

36. Dvonch JT, Kannan S, Schulz AJ, Keeler GJ, Mentz G, House J, Benjamin A, Max P, Bard RL, Brook RD. Acute effects of ambient particulate matter on blood pressure: differential effects across urban communities. *Hypertension* 2009; 53: 853-859.

37. Brook RD, Bard RL, Burnett RT, Shin HH, Vette A, Croghan C, Phillips M, Rodes C, Thornburg J, Williams R. Differences in blood pressure and vascular responses associated with ambient fine particulate matter exposures measured at the personal versus community level. *Occup Environ Med* 2011; 68: 224-30.

38. Giorgini P, Rubenfire M, Das R, Gracik T, Wang L, Morishita M, Bard RL, Jackson EA, Fitzner CA, Ferri C, Brook RD. Particulate matter air pollution and ambient temperature: opposing effects on blood pressure in high-risk cardiac patients. *J of Hypertens* 2015; 33: 2032-8.

39. Zhao X, Sun Z, Ruan Y, Yan J, Mukherjee B, Yang F, Duan F, Sun L, Liang R, Lian H, Zhang S, Fang Q, Gu D, Brook JR, Sun Q, Brook RD, Rajagopalan S and Fan Z. Personal black carbon exposure influences ambulatory blood pressure: air pollution and cardiometabolic disease (AIRCMD-China) study. *Hypertension* 2014; 63: 871-7.

40. Brook RD, Xu X, Bard RL, Dvonch JT, Morishita M, Kaciroti N, Sun Q, Harkema J and Rajagopalan S. Reduced metabolic insulin sensitivity following sub-acute exposures to low levels of ambient fine particulate matter air pollution. *Sci of Total Environ* 2013; 448: 66-71.

41. Brook RD, Sun Z, Brook JR, Zhao X, Ruan Y, Yan J, Mukherjee, M, Rao X, Duan F, Sun L, Liang R, Lian H, Zhang S, Fang Q, Gu D, Sun Q, Fan Z, Rajagopalan S. Extreme Air Pollution Conditions Adversely Affect Blood Pressure and Insulin Resistance. The Air Pollution and Cardiometabolic Disease Study. *Hypertension* 2016; 67: 77-85.

42. Liu S, Brook RD, Huang W, Fan Z, Xu H, Wu R, Sun X, Zhao X, Ruan Y, Yan J, Sun L, Liang R, Lian H, Gu D, Rajagopalan S. Extreme levels of ambient air pollution adversely impact cardiac and central aortic hemodynamics: The AIRCMD-China study. *J Am Soc Hypertens* 2017; 11: 754-61.

43. Brook RD, Brook JR, Urch B, Vincent R, Rajagopalan S, Silverman F: Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* 2002; 105: 1534-1536.

44. Urch B, Silverman F, Cory P, Brook JR, Lukic KZ, Rajagopalan S, Brook RD. Acute blood pressure responses in healthy adults during controlled air pollution exposures. *Environ Health Perspect* 2005; 113: 1052-55.

45. Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, Morishita M, Kaciroti N, Harkema J, Corey P, Silverman F, Wellenius G, Mittleman MA, Rajagopalan S, Brook JR. Insights into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans. *Hypertension* 2009; 54: 659-667.

46. Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang H-Y, Spino C, Mukherjee B, Kaplan MJ, Yalavarthi S, Oral EA, Ajluni N, Sun Q, Brook JR, Harkema J, Rajagopalan S. The hemodynamic,

autonomic, and vascular effects of exposure to coarse particulate matter in a rural location. *Environ Health Perspect* 2014; 122: 624-30

47. Byrd JB, Morishita M, Bard RL, Das R, Wang L, Sun Z, Spino C, Harkema J, Dvonch, JT, Rajagopalan S, Brook RD. Acute Increase in Blood Pressure during Inhalation of Coarse Particulate Matter Air Pollution from an Urban Location. *J Am Soc Hypertens* 2016; 10: 133-139.

48. Huang W, Wang L, Li J, Liu M, Xu H, Liu S, Chen J, Zhang Y, Morishita M, Bard RL, Harkema JR, Rajagopalan S, Brook RD. Short-term Blood Pressure Responses to Ambient Fine Particulate Matter Exposures at the Extremes of Global Air Pollution Concentrations. *Am J Hypertension* 2018 (in press).

49. Szyszkowicz M, Rowe BH, Brook RD. Even low levels of ambient air pollutants are associated with increased emergency department visits for hypertension. *Can J Cardiol* 2012; 28; 360-6.

50. Brook RD, Kousha T. Air Pollution and Emergency Department Visits for Hypertension in Edmonton and Calgary, Canada: A Case-Crossover Study. *Am J Hypertens* 2015;28:1121-6.

51. Coogan PF, White LF, Jerrett M, Brook RD, Su GC, Seto E, Burnett RT, Palmar JR, Rosenberg L. Air pollution and incidence of hypertension and diabetes in African American women living in Los Angeles. *Circulation* 2012; 125: 767-772.

52. Coogan PF, White LF, Yu J, Burnett RT, Seto E, Brook RD, Palmer JR, Rosenberg L, Jerrett M. $PM_{2.5}$ and Diabetes and Hypertension Incidence in the Black Women's Health Study. *Epidemiology* 2016; 27: 202-10.

53. Coogan PF, White LF, Yu J, Burnett RT, Marshall JD, Seto E, Brook RD, Palmer JR, Rosenberg L, Jerrett M. Long term exposure to NO_2 and diabetes incidence in the Black Women's Health Study. *Environ Res* 2016; 148: 360-366.

54. Coogan PF, White LF, Yu J, Brook RD, Burnett RT, Marshall JD, Bethea TN, Rosenberg L, Jerrett M. Long-Term Exposure to NO2 and Ozone and Hypertension Incidence in the Black Women's Health Study. *Am J Hypertens* 2017; 30: 367-372.

55. Pope CA, Turner MC, Burnett R, Jerrett M, Gapstur SM, Diver WR, Krewski D, Brook RD. Relationships between fine particulate matter air pollution, cardiometabolic disorders, and cardiovascular mortality. *Circ Res* 2015: 116: 108-115.

56. Chen H, Burnett RT, Copes R, Kwong, JC, Villeneuve PJ, Goldberg MS, Brook RD, van Donkelaar A, Jerrett M, Martin RV, Brook JR, Kopp A, Tu JV. Ambient fine particulate matter and mortality among survivors of myocardial infarction: Population-based cohort study. *Environ Health Perspect* 2016; 124: 1421-8.

57. Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, Behar JV, Hern SC, Engelmann WH. The National Human Activity Pattern Survey (NHAPS): A resource for assessing exposure to environmental pollutants. *J Expo Anal Environ Epidemiol* 2001; 11: 231-52.

58. Brugge D, Simon MC, Hudda N, Zellmer M, Corlin L, Cleland S, Lu EY, Rivera S, Byrne M, Chung M, Durant JL. Lessons from in-home air filtration intervention trials to reduce urban ultrafine particle number concentrations. *Build Environ* 2017; 126: 266-275.

59. Hermida RC, Ayala DE, Mojón A, Fernández JR. Decreasing sleep-time blood pressure determined by ambulatory monitoring reduces cardiovascular risk. *J Am Coll Cardiol* 2011; 58: 1165-73.

60. Banegas JR, Ruilope LM, de la Sierra A, Vinyoles E, Gorostidi M, de la Cruz JJ, Ruiz-Hurtado G, Segura J, Rodríguez-Artalejo F, Williams B. Relationship between Clinic and Ambulatory Blood-Pressure Measurements and Mortality. *N Engl J Med* 2018; 378: 1509-1520.

61. Hermida RC, Ayala DE, Smolensky MH, Fernández JR, Mojón A, Portaluppi F. Sleep-time blood pressure: Unique sensitive prognostic marker of vascular risk and therapeutic target for prevention. *Sleep Med Rev* 2017; 33: 17-27

62. Hermida RC, Ayala DE, Smolensky MH, Fernández JR, Mojón A, Portaluppi F. Chronotherapy with conventional blood pressure medications improves management of hypertension and reduces cardiovascular and stroke risks. *Hypertens Res* 2016; 39: 277-92.

63. Tsai DH, Riediker M, Wuerzner G, Maillard M, Marques-Vidal P, Paccaud F, Vollenweider P, Burnier M, Bochud M. Short-term increase in particulate matter blunts nocturnal blood pressure dipping and daytime urinary sodium excretion. *Hypertension* 2012 Oct; 60: 1061-9.

64. Hashimoto J. Central hemodynamics for management of atherosclerotic diseases. *J Atheroscler Thromb* 2017;24(8):765-778.

65. Kollias A, Lagou S, Zeniodi ME, Boubouchairopoulou N, Stergiou G. Association of central versus brachial blood pressure with target-organ damage. *Hypertension* 2016; 67: 183-190.

66. Vlachopoulos C, Aznaourids K, O'Rourke ME, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systemic review and meta-analysis. *Eur Heart J* 2010; 31: 1865-71.

67. Vlachopoulos C, Aznaourids K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systemic review and meta-analysis. J Am Coll Cardiol 2010; 55: 1318-27.

68. Siu AL; U.S. Preventive Services Task Force. Screening for high blood pressure in adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2015; 163: 778-86.

69. Parati G, Stergiou G, O'Brien E, Asmar R, Beilin L, Bilo G, Clement D, de la Sierra A, de Leeuw P, Dolan E, Fagard R, Graves J, Head GA, Imai Y, Kario K, Lurbe E, Mallion JM, Mancia G, Mengden T, Myers M, Ogedegbe G, Ohkubo T, Omboni S, Palatini P, Redon J, Ruilope LM, Shennan A, Staessen JA, vanMontfrans G, Verdecchia P, Waeber B, Wang J, Zanchetti A, Zhang Y; European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability. European Society of Hypertension practice guidelines for ambulatory blood pressure monitoring. *J Hypertens* 2014 Jul; 32: 1359-66.

70. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2017; Nov 13.

71. Myers MG, Kaczorowski J, Dawes M, Godwin M. Automated office blood pressure measurement in primary care. *Can Fam Physician* 2014; 60: 127-32.

72. Michigan Department of Transportation. (2016) 2016 Average Daily Traffic (ADT) Maps. http://www.michigan.gov/mdot/

73. https://www.epa.gov/indoor-air-quality-iaq/guide-air-cleaners-home

74. Batterman S, Du L, Mentz G, Mukherjee B, Parker E, Godwin C, Chin JY, O'Toole A, Robins T, Rowe Z, Lewis T. Particulate matter concentrations in residences: an intervention study evaluating stand-alone filters and air conditioners. *Indoor Air* 2012; 22: 235-52.

75. Peck RL, Grinshpun SA, Yermakov M, Roa MB, Kim J, Reponen T. Efficacy of portable HEPA air purifiers against traffic related combustion particles. *Building Environ* 2016; 98: 21-29.

76. Brugge D, Simon MC, Hudda N, Zellmer M, Corlin L, Cleland S, Lu EY, Rivera S, Byrne M, Chung M, Durant DL. Lessons from in-home air filtration intervention trials to reduce urban ultrafine particle number concentrations. *Building Environ* 2017; 126: 266-75.

77. Kelly KE, Whitaker J, Petty A, Widmer C, Dybwad A, Sleeth D, Martin R, Butterfield A. Ambient and laboratory evaluation of a low-cost particulate matter sensor. *Environ Pollut*. 2017. 221:491-500.

78. Nakamura Y, Rege J, Satoh F, Morimoto R, Kennedy MR, Ahlem CN, et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein corticosteroids before and after adrenocorticotropic hormone stimulation. *Clin Endocrinol (Oxf).* 2012;76:778-784.

79. Rege J, Nanba AT, Auchus RJ, Ren J, Peng HM, Rainey WE, Turcu AF. Adrenocorticotropin Acutely Regulates Pregnenolone Sulfate Production by the Human Adrenal In Vivo and In Vitro. *J Clin Endocrinol Metab.* 2018;103:320-327.

80. Rege J, Karashima S, Lerario AM, Smith JM, Auchus RJ, Kasa-Vubu JZ, et al. Age-dependent increases in adrenal cytochrome b_5 and serum 5-androstenediol-3-sulfate. *J Clin Endocrinol Metab*. 2016;101:4585-4593.

81. Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC, et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *J Clin Endocrinol Metab*. 2013;98:1182-1188.

82. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano TJ, Shields JJ, et al. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. *Eur J Endocrinol.* 2016;174:601-609.

83. Turcu AF, Rege J, Chomic R, Liu J, Nishimoto HK, Else T, et al. Profiles of 21-carbon steroids in 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2015;100:2283-2290.

84. Turcu AF, Mallappa A, Elman MS, Avila NA, Marko J, Rao H, Tsodikov A, Auchus RJ, Merke DP. 11-Oxygenated Androgens Are Biomarkers of Adrenal Volume and Testicular Adrenal Rest Tumors in 21-Hydroxylase Deficiency*. J Clin Endocrinol Metab*. 2017;102:2701-2710.

85. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. *Clin Endocrinol (Oxf).* 2015;83:162-166.

86. Morris DJ, Latif SA, Lo YH, Abrampah K, Brem AS, Lichtfield WR, Williams GH. Correlation of glycyrrhetinic acid-like factors (kidney 11β-HSD2-GALFs) with urinary free cortisol and plasma renin activity in essential hypertension*. J Am Soc Hypertens*. 2008;2:286-293.

87. Markou A, Sertedaki A, Kaltsas G, Androulakis II, Marakaki C, Pappa T, Gouli A, Papanastasiou L, Fountoulakis S, Zacharoulis A, Karavidas A, Ragkou D, Charmandari E, Chrousos GP, Piaditis GP.

Stress-induced Aldosterone Hyper-Secretion in a Substantial Subset of Patients With Essential Hypertension. *J Clin Endocrinol Metab.* 2015;100:2857-2864.

88. Richard L, Gauvin L, Ducharme F, Leblanc M, Trudel M. Integrating the ecological approach in disease prevention and health promotion programs for older adults: An exercise in navigating the headwinds. *Journal of Applied Gerontology*, 2012; 31 (1), 101-125.

89. Israel BA, Eng E, Schulz AJ, & Parker EA. 2005. Methods in community-based participatory research for health. San Francisco, CA: Jossey-Bass.

90. CDC. 1999. Centers for Disease Control and Prevention. Framework for program evaluation in public health. Morbidity and Mortality Weekly Report, 48(RR-11). Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4811a1.htm

91. Bryant LL, Altpeter M, Whitelaw NA. Evaluation of health promotion programs for older adults: An introduction. *Journal of Applied Gerontology*, 2006; 25 (3), 197-213.

92. Beattie BL, Whitelaw NA, Mettler M, Turner D. A Vision for older adults and health promotion. *American Journal of Health Promotion*, 2003;*18* (2), 200-205.

Protocol Modifications to the RAPIDS-YPSI Clinical Trial

In light of COVID-19 and the high-risk nature of the study population (elderly adults >60-65 years of age living in a low-income senior home and many with pre-existing high-risk conditions), our objective is to modify the protocol to eliminate all direct in-person contact between study research personnel and participants.

All interactions will be via email, mail, and telephone and can be accomplished without detrimental impact to the study primary objective. Several secondary aims that involve more complicated endpoints, including 24-hour ambulatory blood pressure (BP) and blood draws, that necessitate direct subject contact will be removed from the protocol.

Summary of changes:

Visits/participant interactions: All communication with subjects, including study enrollment and informed consent and all study visits, will be conducted through remote visits via the telephone.

Endpoints: Remove blood draws, heart rate variability, BpTRU BP measurements, WatchPAT, and 24-hour ambulatory BP monitoring endpoints. Continue home BP monitoring using the same protocol as original trial. However, we will change BP measurements to be performed twice per day (AM+PM) of days 2-5 (Tues – Fri) of week 1 and week 4 of both study blocks.

PM2.5 monitoring: Continue with in-room (PurpleAir) monitoring per original study design for full 4 weeks of both study blocks. We will use personal-PM2.5 monitoring (pDR-1500); however, it will not occur during week-1 but only at end of week-4 for both study blocks (once per study block).

Air filter intervention: No changes. We will continue the protocol using portable air cleaners (PACs) in the bedroom without change for 4 weeks in active and sham conditions.

Study flow: All study devices (home BP monitors, pDR-1500 personal PM2.5 monitors, PurpleAir in-room PM monitors, PACs) will be cleaned and sterilized (per COVID-19 protocols) before they are provided to each subject. The devices will be provided to the subjects without any contact with the research team members. At the appropriate time in the protocol, the subjects will be notified by phone and the equipment and instructions for usage and set up will dropped off for the participants at their doorway at a pre-arranged time.

Advanced Covid Restrictions:

Study staff will not enter residences or have any personal contact with any subject or potential subject. In the event of elevated Covid concerns the study staff will not enter hallways and will deliver equipment to the front doorway to Carpenter Place. In cases where the subject agrees, the study staff may enter apartments to set up the equipment. Our 2021 experience has proven that some subjects have physical limitations to moving the equipment (frailty, walkers and small apartments, etc)

Study Protocol:

Recruitment: Advertisements and recruitment will be done without personal contact. We will provide fliers for recruitment with a contact number to the research coordinator for interested residents. The staff members who work and are present at Carpenter Place will post the fliers in Carpenter place bulletin boards and notify residents that the study is active and has been modified to be simpler and to eliminate any contact with the research team. Advertisements will continue in the residential newsletter.

Enrollment: Interested residents will call the study research coordinator. Study inclusion/exclusion criteria will be reviewed (no changes). All subjects must attest to be non-smokers living in non-smoking household. Consents will be left at the office or subjects will be emailed or mailed the consent form and subjects will be given ample time to review the consent and consider their participation independently. Study team will review the consent document over the telephone just as they would during a face to face meeting. Signature will be obtained on the consent form and provided to the study staff via mail or left for pickup by study staff in the same manner as equipment is dropped off with no contact. This cohort is unlikely to benefit from electronic consenting procedures based on the lack of internet access, the poor internet provided by Carpenter Place, the willingness of the participants, and the added burden it would place on this older population of subjects participating in a low risk study.

Study Flow: The modified trial will be conducted per the original design except for no face-to-face interactions between research staff and subjects and the protocol has been simplified. Subjects will be assigned a research ID number. They will be randomized in a blinded fashion to either active vs sham PAC use for intervention block 1. After completing block 1, they will washout for 1 week and then crossover to block 2 and undergo the same trial randomized to the alternative PAC intervention (sham vs active).

BLOCK 1 (2)												
	Week 1 (6)					**Wk 2-3 (7-8)**	**Week 4 (9)**					**Washout**
	Day						**Day**					
	1 M	**2 T**	**3 W**	**4 Th**	**5 F**		**1 M**	**2 T**	**3 W**	**4 Th**	**5 F**	
PAC INTERVENTION (2 limb x-over)												
Randomized PAC	↓	x	x	x	x	---------->	x	x	x	x	↑	
TESTING												
Home BP: AM		✓	✓	✓	✓			✓	✓	✓	✓	
PM		✓	✓	✓	✓			✓	✓	✓	✓	
PM MONITORING												
In-room PM (Purple Air)	↓	x	x	x	x	---------->	x	x	x	x	↑	
Personal PM (PDR1500)										↓	↑	

↓ = start; x = continue; ↑ = stop; ✓ = check BP; ----------> = continues throughout period

Week-1/day-1: PAC Intervention/bedroom PM monitoring: Start in subject's bedroom at 9 am

Home BP: am= when first wake up in bedroom; pm=just before going to sleep in bedroom

Personal PM monitoring: Wear/have on person during daytime. Put by bedside at night-time.

Week-1/day-1: Staff will drop off the study equipment between 8-9 AM. The equipment will all have been cleaned and sterilized beforehand. It includes a home BP monitor (and instructions), the blinded-randomized (active or sham) indoor PAC (and instructions for its use), and a PurpleAir monitor (and instructions for positioning and use).

At 9 AM, the subject will position the PAC in their bedroom per instructions, plug it in and turn it on. They will keep the PAC running at the highest tolerated setting (per protocol and instructions) and keep it running throughout weeks 1-4 in their bedroom. The subject will also plug in the PurpleAir and position it at least 6 feet away from the PAC in their bedroom per instructions (off the floor on a table). They will make sure it is running for continued air pollution monitoring throughout weeks 1-4 in their bedroom per provided instructions.

Home BP monitoring: The subject will measure home BP upon awakening in the morning and just before bedtime on week-1 (days 2-5 = Tuesday through Friday) and on week-4 (days 2-5 = Tuesday through Friday). They will follow instructions for home BP monitoring without change from original protocol. They will sit 5 minutes prior to measurement with right arm resting at heart level on a table. They will measure BP in triplicate on right arm. The Omron-10 series will be set to measure BP in triplicate at 1-minute intervals. The device stores all time-stamped BP readings and subjects will record on a log for safekeeping of data.

Subject reminders: The study coordinator will contact the subject week-4/day-1 (mon) to remind the subject to begin home BP monitoring on week-4/day-2 for the next 4 days.

Personal PM-monitoring (week 4): The study coordinator will call the subjects and Carpenter Place staff to remind them about personal PM monitoring which will occur on week-4/day-4 starting at 9-AM and continue for 24-hours through day-5 at 9-AM. The coordinator will drop off the pDR-1500 (with instructions for use) before 9-AM on day-4 at the front lobby of Carpenter Place. The subject will pick-up the monitor, turn it on, and wear it (on belt or strap) for the next 24-hours as per instructions. During sleep or showering the monitor can be kept close to their person (bedside).

Week-4/Day-5: On the final day of each study block, the coordinator will contact the subject. They subject will be instructed to place the equipment (randomized PAC, PurpleAir, pDR-1500, and home BP device) in the bin with wheels and bring it to the front lobby of Carpenter Place. The coordinator will then pick-up the equipment.

Data collection: The coordinator will collect/data-base the time-stamped home BP readings from week-1 and week-4. They will download all the time/date-stamped PM2.5 data from the PurpleAir and data-base the results. They will also download and data-base the 24-hour personal PM2.5 exposure results from the pDR-1500.

As-needed visits: During the study period, the subject can call and schedule an as-needed telephone or video visit with the research coordinator at any time to discuss any study-related issues or problems.

Washout-period. During the washout period the coordinator will clean and sterilize all the equipment. They will change the PAC to the alternative randomized blinded mode (active vs sham). They will call the subject and schedule the timeline for Block-2 (week-1/visit-1).

Block-2: This block will proceed exactly as block-1, except the subject will be provided the alternative randomized (blinded) PAC intervention.