RESEARCH PROTOCOL

HS-IRB # 2017-1090

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E-Cigarette Effects on Markers of Cardiovascular and Pulmonary Disease Risk (aka CLUES - Cardiac and LUng E-cig Smoking Study)

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Version 2.7, April 26th, 2021

Summary change in *Version 2.7, April 26th, 2021*

1. *Remove the dual users group. We have realized that they do not exist in significant numbers based on our inclusion criteria in the general population, making recruitment almost impossible for this type of user*
2. *Increase the smokers and e-cigarette users group size to 165 from 110 participants. This was adjustment was based on reassessed statistical power after removing the dual users group.*

Study Overview

E-cigarette use is increasing rapidly in the United States, especially amongst youth, underscoring the vital need to improve understanding of its health risks. Relevant data could inform policy, guide public health and clinical intervention efforts, and inform individuals who might use or who are using this product. This research will significantly enhance our understanding of the possible health effects of e-cigarette use by relating the acute and long-term use of e-cigarettes and conventional cigarettes ("*products*") to well-validated cardiovascular and pulmonary disease biomarkers. We will enroll 3 different "use-groups" of participants: exclusive e-cigarette users (n=165), exclusive cigarette smokers (n=165), and a "control" group of never-users (n=110). These groups reflect the primary decisions that people can make regarding their future tobacco use: to continue to smoke cigarettes, to switch to e-cigarettes, to use both cigarettes and e-cigarettes, or to avoid tobacco use entirely. It is essential that smokers and health care providers have accurate information on the health effect of these choices.

Product use will be related to well-validated biomarkers that sensitively and reproducibly reflect mechanisms, injury, and/or future risk related to cardiovascular or pulmonary disease. Biomarkers will be related to: 1) acute product use in the laboratory (exposure challenges), 2) lifetime history of product use, and/or 3) real-time measures of product use in participants' daily lives. The primary cardiovascular biomarkers are brachial artery flow-mediated dilation (a measure of endothelial function) and carotid intima-media thickness, a measure of subclinical arterial injury and atherosclerosis. The primary pulmonary disease biomarkers will be measures of lung volumes and flow rates (FEV1, FVC, FEV1/FVC, FEF25-75) obtained by spirometry. We also will perform treadmill exercise stress testing (to assess aerobic fitness), electrocardiography (to measure heart rate variability, HRV), and measure heart rate, blood pressure, lipids, HgbA1c, and inflammation/oxidation markers (leukocyte count, C-reactive protein, urinary F2 isoprostanes and exhaled nitric oxide). This research will show how product use-groups differ in response to acute product use and long-term use as they are related to key cardiovascular and pulmonary biomarkers. Objective measures of product use include exhaled CO and plasma nicotine/cotinine and urinary nicotine/cotinine concentrations. We also will determine how history of product use within use-groups is related to biomarker status.

The proposed research will yield vital and comprehensive data regarding product use, subclinical arterial injury, atherosclerosis burden, arterial and pulmonary function, cardiac and aerobic fitness, cardiac autonomic regulation, systemic and pulmonary inflammation, and oxidative stress, as well as other key outcomes. These data will serve as a foundation for future longitudinal investigations of e-cigarette health effects and will inform public policy decisions, clinical intervention, and patient guidance regarding e-cigarettes.

Study Aims

Aim 1. *To determine the acute effects of product use on primary cardiovascular (brachial artery flow-mediated dilation, FMD) and pulmonary (spirometry) disease risk biomarkers* after an acute product exposure challenge. We will compare acute responses to E-cigs in exclusive E-cig users with responses to cigarettes in exclusive smokers vs. no exposure (never-users). The primary acute exposure biomarkers will be brachial artery FMD for cardiovascular disease (CVD) and FEV1 for pulmonary disease. Secondary measures such as acute changes in HR, HRV, BP, FVC, FeNO, also will be assessed. Acute responses will be related to covariates

such as product use history, self-administration topography, nicotine levels, and other biological risk variables.

Aim 2. *To determine relations of chronic product exposure with primary cardiovascular (carotid intima-media thickness, IMT) and pulmonary (spirometry) biomarkers of injury, disease risk, and their mechanisms.* We will contrast product use-groups to determine their status on each disease biomarker and within each group, we will relate measures of chronic product exposure to disease biomarkers. The primary exposure biomarker will be carotid IMT for CVD and the primary pulmonary biomarker will be FEV1. Primary analyses will determine the relations of E-cig and cigarette product use groups with each biomarker. In addition, within-group analyses will relate history of prior product use (e.g., duration, patterning, and maximal intensity) with each biomarker. Biomarkers used in secondary analyses will include baseline measures from Aim 1 (such as FMD, HR, HRV, BP, FeNO) as well as carotid echogenicity, PWA, treadmill stress testing parameters, and markers of inflammation and oxidation. Historical and biological covariates (i.e., age, sex, and CVD risk factors) will be used in exploratory moderation and sensitivity analyses to determine the robustness of identified relations. This research will relate acute use of E-cigs and cigarettes with putative cardiovascular and pulmonary disease mechanisms and relate longer term patterns of E-cig and cigarette use with measures of cardiovascular and pulmonary dysfunction and injury.
Aim 3. (Exploratory aim) To examine the relationship between monocyte genomic features and e-cig use status
We will perform linear regression analyses with genomic features as response and status of e-cig use as predictors. Covariates, such as technical factors, will be included in the model. We will use the FDR q-value method to correct for multiple testing. We will compare e-cig users, smokers and control groups. This will only be done prospectively in 50 e-cig users, 25 matched controls and 25 smokers.
Aim 4 (exploratory hypothesis): Plasma from E-cig and Cigarette users will have higher levels of inflammatory cytokines compared to non-users, and these inflammatory cytokines will activate arterial endothelial cells in an in vitro model.

Recruitment & Study Entry

Participants from the greater Madison, WI area will be recruited via the media recruitment methods (i.e., newspaper, and earned media) that have recruited over 2000 smokers over 2 years. In addition, we will use Internet/Facebook advertisements that have been successful in our recent dual use study (HS-IRB #2012-0844). Interested individuals will be asked to call the study telephone number and leave their name and telephone number for a return and phone screen. Potential participants who pass the phone screen will be invited to attend a study session (Visit 1 Orientation) at the UW Madison University Hospital-Atherosclerosis Imaging Research Program, where they will learn more about the study, have eligibility confirmed, and provide written informed consent. During the phone screen we will collect demographic and contact information (address for mailing reminder appointments and phone number) and document the responses to these screening questions.

If we are not successful in meeting recruiting goals, we will recruit from Milwaukee, WI as we have in numerous previous studies. All research activities will still be performed at UW-CTRI and UW Hospital (CSC).

Inclusion/Exclusion

The eligibility (inclusion) criteria are: ≥18 years old, able to read and write English, *no* plans to quit smoking and/or e-cig use in the next month, not using cigars/smokeless/snus tobacco ≥1 time/week, having a stable pattern of current product use, and able to walk at least 2 blocks without assistance or stopping. For the different user-groups, participants must meet the criteria listed in Table 1. The exclusion criteria are: current use of a smoking cessation medication, women who are pregnant or plan to get pregnant in the next month, women who might be pregnant, incarcerated individuals.

Table 1. Use Groups/Eligibility				
Group	**Cigarette Use**	**E-cig Use£**	**CO†**	**Cotinine‡**
Exclusive smokers	Smokes daily; ≥5 cigs/day‡‡ for last 6 mos	<3 uses lifetime	≥5	>100
Exclusive E-cig users	≤2 days/month for last 6 mos	≥5 days/week for last 3 mos	≤4	>100
Dual users	≥5 cigs/day‡‡, ≥5 days/wk for last 6 mos	≥5 days/week for last 3 mos	≥5	>100
Never users	<100 cigs/lifetime, none for >5 years	<3 uses lifetime	≤4	<100
£Predominant use of a tank system; †≥5 ppm indicates smoking; ‡>100 ng/ml indicates nicotine intake; ‡‡average consumption on smoking days.				

Study Design

We will enroll 165 exclusive E-cig users, 165 exclusive cigarette smokers, and 110 former (never-) smokers. We will examine tobacco product use via time-line follow-back use-history measures, real-time measures, and lab measures. Potential participants will be assessed for eligibility via a phone screen. Because biomarker status may be affected by age and sex, we will stratify groups equally for gender (48/52 male/female) and age (≤35/>35 years).

Screening Call ("Call" - Table 2)

Interested individuals after verbal consent using an approved script, will complete an initial phone screen. Eligible, interested participants will attend an Orientation Visit (visit 1 Baseline)

Visit 1 ("Baseline" - Table 2)

Participants will attend a single or group information session where the study will be explained in detail and they will be given time to read the consent. The participants that still express interest, will be lead to a private room where they can ask any remaining questions, and provide written informed consent. Then they will provide biological samples indexing their tobacco product use (*i.e.,* exhaled CO and urine cotinine) to establish their final eligibility and complete questionnaires that assess cigarette and/or E-cig dependence:

The CLUES Baseline Questionnaire (appendix) includes segments of several validated surveys to assess cigarette and e-cigarette/Juul dependence motives, tobacco withdrawal, smoking history using time-line follow-back to quantify history of tobacco product use including heaviness of use across time, quit attempts, age of use onset, and exposure to environmental smoke/vapor and symptoms and health effects associated with use. Additionally, they will complete a Medical History Questionnaire (appendix). Participants will bring a list of their medications and cigarettes, E-cigs, and e-juice so we can obtain specific information on product features, including E-cig battery voltage, resistance, flavoring .and nicotine concentration.

Participants will be provided with and trained to use a research smartphone with our previously developed app for EMA tracking of E-cig and cigarette use for 1 week before Visit 2. They will press a button marked "C" every time they start a new cigarette and to press one marked "E" every time they vape (a vaping episode is ≥2 puffs on an E-cig that are close together in time). The phone is programmed to prompt them to fill a report every night to gather additional data on recent product use/exposure (*e.g.,* context of use, exposure to others vaping or smoking), symptom reports of common nicotine/smoking/vaping adverse events, and withdrawal symptoms and craving (to determine heavy, dependent use: report duration). The questions from the report widget are in the appendix. Based on previous studies experience, participants will spend <5 minutes/day recording cigarette/E-cig use and answering questions. EMA eliminates the problems of recall bias and prefilling/back-filling of paper-pencil diaries. Smartphones will track data wirelessly in real-time so we can rapidly address assessment non-adherence. Note: For the few participants that do not feel comfortable using a phone we will provide a booklet with the nightly survey to be completed every day for 1 week between V1 and V2. This will be assess and decided during V1 phone ap training.

To enhance compliance with EMA recording, participants will be paid $2 for each programmed report (≈$14/ for a 1-week assessment window), and $36 for returning the smartphone, for a maximum total EMA payment of ≥$50. Over the last 3 days of the EMA reporting period, participants will be sent text messages and an email indicating the time of Visit 2 at CTRI, that they need to bring in relevant smoking or vaping materials to the visit, and that they abstain from food, caffeine and tobacco/nicotine use for 8 hours on the day of the visit. Visit 2 will occur ideally within 2 weeks of Visit 1. In cases where participant become febrile and need to recover from an infection, we will allow up to 1 month between visits to allow them to come back to normal values (CRP levels, glucose levels, urinary Isoprostanes, white cell count and brachial artery reactivity responses). Additionally as a precaution from the current COVID-19 outbreak.

Table 2. Schedule of Events

Visit	Call	V1 BASELINE	V2 CHALLENGE (5-15 days after V1)		
Location	**Screening**	**AIRP**	**CTRI-pre hut**	**CTRI-post hut**	**AIRP**
Pre-visit instructions	**X**	**NO need to fast and FREE to smoke**	**Fasting and NO smoking or vaping for 8 hs**		**Refrain from smoking after Hut**
Partial eligibility questions	**X**				
Informed consent		**X**			
Provide CDC and QUITLINE information		**X**			
Baseline use Questionnaire (QUALTRICS)		**X**			
Collect medical history (QUALTRICS) and medication list		**X**			
Obtain height, weight and waist circumference		**X**			
Collect product use data, photos		**X**	**X**		
Urine cotinine (NiCheck1)		**X**	**X**		
CO (x1)		**X**	**X**[c]	**X**	
Confirm eligibility, assign arm, confirm V2 date/time/instructions		**X**			
Provide smart phone + training		**X**[b]			
Collect phones			**X**[b]		
Fasting labs (sent to UWHC core lab)			**X**		
Urinary Isoprostane/creatinine (CHL)			**X**		
Pre and post Challenge QUALTRICS survey			**X**	**X**	
Vital signs			**X**[a]		
IMT (M9)			**X**		
Radial Tonometry (PWA)			**X**		
Spirometry (x3)			**X**	**X**	
FeNO (x1)			**X**	**X**	
Plasma Nicotine/cotinine (CHL)			**X**	**X**	
BART (M9)			**X**	**X**	
HRV and BP			**X**	**X**	
Light snack				**X**	
Resting 12 lead ECG				**X**[e##]	**X**
ETT					**X**

[a]*Vital signs will include: heart rate and blood pressure.*
[b]*Participants will receive the smartphone at visit 1 and return it at Visit 2.*
[c]*CO levels will determine if study should be rescheduled because of recent smoking.*
[d]*CO after challenge is measured after FeNO.*
[e##]*single resting ECG done at CTRI instead of AIRP prior to stress testing only in cases that are disqualified for ETT.*
When necessary, V2 visit window could be extended up to one month post V1, in cases of fever or infection where 14 days of recovery might be needed (in addition to safety concerns due to COVID-19 outbreak).

Visit 2: Acute Product Use Challenge Visit (Table 2)

Research smartphones will be collected. Participants will be asked about their food and tobacco product use over the last 8 hours and rescheduled if they are non-abstinent. Fasting urine (for cotinine and F_2 isoprostane/creatinine ratio) and pre- and post-acute challenge plasma nicotine/cotinine concentrations will be collected to be sent to ClevelandHeart labs for analysis and pre-challenge blood samples for fasting complete blood count [CBC], lipid panel, HgbA1c, and CRP are sent to the UW-Core lab for processing.

We will then perform the following tests in this order: spirometry(x3), FeNO(x1), CO (x1), carotid ultrasound, 5-minute rest, radial tonometry for augmentation index, and ultrasound brachial artery reactivity study (simultaneous with HR, HRV, BP), pre-challenge, and collected again post-challenge (except carotid ultrasound) within ~30 minutes. Phlebotomy will be performed from the left arm (contralateral arm used in brachial ultrasound testing) for plasma nicotine and cotinine just prior to the product *challenge* test. Only 100 participants are invited to be part of an epigenetics sub-study (50 E-cig users, 25 smokers and 25 controls), prior to smoking, an additional 16 ml of blood will be collected in Vacutainer CPT™ cell separation tubes for PBMC Isolation containing sodium citrate (Becton Dickinson, Rutherford, NJ). These samples will be pre-processed for monocyte isolation at UW (DrJarjour's lab) and frozen and after all 100 participant's samples are collected they will be shipped them in bulk in dry ice for final processing and storage in a liquid nitrogen tank at Dr. Liu's lab in the Duke Molecular Physiology Institute. These samples will be coded (with the link kept at the UW) and leftover samples will be disposed at Duke. They will not run full genome sequencing, neither will they post this information in a public data repository

The challenge test is designed to quantify the acute effects of vaping or cigarette smoking on selected pulmonary and cardiovascular measures intended to index disease mechanisms or dysfunction that are likely to respond rapidly (~5-40 minutes) to acute inhalation of E-cig vapor or tobacco smoke. Participants will report their withdrawal symptoms and then will be allowed to smoke (*e.g.,* exclusive smokers) or vape (*e.g.,* exclusive E-cig users) *ad libitum*, using their own cigarette brand/E-cig type for 20 minutes or less. Product use challenge activities will occur in a dedicated smoking laboratory (Butt Hut room 10'L x 8'W x 10' high, equipped with special ventilation, a door and 2 windows) so study staff are not exposed to smoking or vaping side-products. Participants will be told to use their product in the challenge as they would outside the laboratory. Product self-administration will be videotaped to permit determination of number of puffs duration of puffs, and inter-puff interval. The recorded video session of participants vaping or smoking, will be stored on a secure digital memory card. After a session has been recorded, the card will be uploaded to the CTRI secure server and the memory card will be erased. At the end of the night the camera and memory card will be stored in a locked cabinet. The recordings will be stored on the server until all recordings have been transcribed, at which point they will be deleted. Two trained, independent observers, will assess topography measures and inter-observer agreement will be determined. Then, participants will rate their withdrawal symptoms, satisfaction from smoking/vaping, and other physical sensations associated with smoking, vaping, or acute nicotine effects (*e.g.,* throat "hit," throat irritation, nausea, racing heart, chest congestion/breathing difficulty, and so on) using the post-challenge QUALTRICS survey. They will provide such ratings over 5´ while being prepared for the post-challenge assessments which will be performed in the following order: spirometry (6´), FeNO (2´), CO (2´), repeat blood draw for nicotine/cotinine (3'), rest (5´), ultrasound brachial artery reactivity study (10´), and simultaneous acquisition of HR, HRV, and BP.

The post-challenge tests will start ~*5 minutes after the product use challenge* has been completed, a time period during which these cardiovascular and pulmonary biomarkers have been demonstrated to change in response to cigarettes and/or E-cigs use, in prior studies. Several studies of the acute effects of cigarettes and limited data on acute effects of E-cigs suggest that HR, HRV, BP, and FMD change within ~5-10 minutes of exposure with maximum effects by ~30 minutes, so each test *will be obtained within 30 minutes of challenge*

completion, but after PFTs and FeNO, which change and resolve more quickly. This time period may also yield nicotine values that reflect prior product use and correlate well with nicotine levels occurring over ~60 minutes.
The CO and nicotine/cotinine tests will provide information on product use efficiency; the other tests will reflect the acute effects of smoking or vaping on disease biomarkers. These data complement both time-line follow-back and EMA assessment of product use data; the latter reflects duration and frequency of use in real world conditions whereas the challenge test reflects intensity and efficiency of use in a use episode. The EMA/text prompts prior to Visit 2 will remind participants to bring their products with them, and, depending on group, ensure that their E-cig device has a charged battery and enough fluid. Never-smokers will *not* use any nicotine product during the challenge period nor will they complete product use rating measures. They will, however, provide data 30 minutes apart for pre- and post-challenge biomarkers to obtain values for no-product-use, and to control for the effects of time and repeated measures. We then will encourage participants who smoke to quit and offer connection to the Wisconsin Tobacco Quitline.

After completion of the post challenge assessment, water and a small snack are provided before they drive to the AIRP lab (UW Hospital) for the final assessments, which include: a resting 12 lead ECG, and exercise treadmill test (ETT). The participants are instructed to refrain from using any tobacco products while driving to the hospital. At least one hour from their last e-cig or cigarette should pass before the stress test can start. Note: In cases of obvious atrial fibrillation or other exclusionary reason for performing an ETT detected at V1 or V2, those participants will receive a 12-lead ECG at CTRI to avoid the extra trip and the UW AIRP stress test will be canceled.

Summary of Experimental Procedures

Vital signs

Height, weight, heart rate and blood pressure. These results will be provided to the participant.

Blood Pressure Measurement

After resting supine for 5 minutes in a temperature-controlled quiet room, baseline blood pressure will be obtained by oscillometric sphygmomanometry (Dinamap Pro400, GE Healthcare). First, we will record 2 consecutive right brachial blood pressures and determine that the participant has reached a steady state of relaxation (*i.e.,* when 2 consecutive blood pressure and heart rate measurements are within 8 mmHg and 5 beats/minute of each other). We will use the average of those last 2 measurements as the right-side value. Then the cuff will be positioned on the left brachial artery and measurements obtained.

A consistent BP difference between both arms of greater than or equal to 20 mmHg also will be a sign of possible significant peripheral arterial disease (see CLUES Incidental Findings and Alert MOP). Subsequent BPs will be recorded from the left arm, to monitor changes during brachial artery reactivity test and during the supine-to-standing HRV protocol before and after the acute challenge.

Blood Draws

During Visit 2, at CTRI, a phlebotomist will obtain pre- and post-smoking challenge samples for nicotine/cotinine in plasma to be analyzed at ClevelandHeart labs.

In collaboration with investigators from the University of Wisconsin Morgridge Institute, we will explore the effects of inflammatory cytokines present in plasma from smokers, e-cig users and non-users on endothelial cell cultures, and study the inflammatory pathways that are activated after that exposure. The plasma has already been collected for nicotine concentration and kept in our -80 F freezer in b/u vials in case the FedEx shipment to ClevelandHeart labs failed. This in-vitro model will allow us to find the pathways that might explain the pulmonary and vascular

phenotypes seen in our study. The effect of these inflammatory markers on the endothelial cells will be assessed using an arterial endothelial cell model, derived from induced pluripotent stem cells.

Plasma samples sent to Morgridge Institute for endothelial cell exposure and RNA extraction with be only identified by code ID number. The cells that Dr. Vereide's lab works with are an induced pluripotent stem cell model and is supervised by the Stem Cell Research Oversight (SCRO) committee (protocol SC-2015-0010). They use the ESC line H1 which is part of the NIH registry (https://grants.nih.gov/stem_cells/registry/current.htm?id=29)

Pre-challenge we will collect 16 ml of blood in Vacutainer CPT™ cell separation tubes containing sodium citrate (Becton Dickinson, Rutherford, NJ). Initial Plasma/PBMCs separation will be done locally at Dr Jarjour's UW lab. The processed samples will be shipped in bulk in dry ice and stored in a liquid nitrogen tank at Dr. Liu's lab in the Duke Molecular Physiology Institute for genomic studies. This will be done in a subgroup of participants (50 e-cig users, 25 smokers, and 25 controls). See CLUES PBMC protocol 10-29-2019.docx)

Additionally, pre-challenge samples will be brought to the UWHC outpatient lab for processing within 6 hours. Samples for CBC, HbA1c, hsCRP, and lipid panel will be obtained after 8 hours of fasting. The UWHC core lab is a CLIA-certified lab. Incidental findings (IF) forms will be sent if WBC count is >15K, Hgb <10 g/dL, HbA1c >7%, LDL-C≥190 mg/dL , TG≥400 mg/dL. All participants will receive a letter with their white blood cell count, hemoglobin concentration, fasting lipid panel, and hemoglobin A1c results.

Exhaled Carbon Monoxide Measurement

Breath carbon monoxide will be assessed using the commercially available Micro⁺Smokerlyzer® from Bedfont Scientific as laboratory biomarkers of use. CO tests will provide objective information on cigarette use, given that no CO is inhaled from e-cigs. It should be noted that the CO tests will be examined as biomarkers of abstinence for pulmonary function testing prior to Visits 2. This test has no anticipated IFs and the results are not reported to the participant.

Urine Cotinine (dip strips)

Commercial kits will be used to test urine samples for cotinine content. Urine will be tested at V1. This test has no anticipated IFs and the results are not reported to the participant.

Carotid Ultrasonography for IMT,

Please see CLUES Carotid Ultrasound MOP, CLUES CIMT Reading Protocol, CLUES CCA Grayscale Measurement Protocol and CLUES Incidental Findings and Alert MOP. These include images for wall thickness measurements, note presence or absence of plaques, measure carotid distensibility, and perform CCA gray scale analysis.

Brachial Artery Reactivity Testing for FMD

Please see CLUES FMD MOP. This test has no anticipated IFs and the results are not reported to the participant.

Measurement of HR and HRV

Please see CLUES Heart Rate Variability MOP and CLUES Incidental Findings and Alert MOP. HRV will be measured in the time and frequency domains following current guidelines using the Sphygmocor HRV module (actor Medical)

For the time domain, we will collect 3 HRV measures from 3-lead Holter ECG systems over a 10-minute interval after a 5-minute rest period, while lying supine for the brachial artery

ultrasound study: (i) standard deviation of all NN intervals (ms), (ii) square root of the mean sum of the squares of differences between NN intervals (ms), and (iii) percentage of differences between adjacent NN intervals that are >50ms (pNN50%). For the frequency domain, we will calculate 4 measures of the power spectrum: (i) very-low frequency (0.003-0.04 Hz), (ii) low frequency (LF, 0.04-0.15 Hz), (iii) high frequency (HF, 0.15-0.40 Hz) synchronous, and (iv) LF/HF ratio using the same recordings. A higher LF/HF ratio indicates low HRV and excess sympathetic drive. HRV tracing will be obtained during imaging for the brachial artery ultrasound study before and after acute challenge. The average HR will be used for all HR analyses. This test has no anticipated IFs and the results are not reported to the participant.

Tonometry

Please see CLUES Tonometry MOP and CLUES Incidental Findings and Alert MOP. This test has no anticipated IFs and the results are not reported to the participant.

12-Lead Electrocardiogram (ECG) Protocol

Please see CLUES 12-Lead ECG MOP, CLUES ECG and Treadmill Stress Test Reporting MOP, and CLUES Incidental Findings and Alert MOP. Results are not reported to the participant unless an incidental finding of certain clinical significance is noted.

Treadmill Stress Test (TST) Protocol

Please see CLUES Treadmill Stress Test MOP, CLUES ECG and Treadmill Stress Test Reporting MOP, and CLUES Incidental Findings and Alert MOP. Peak METs and ischemia will be reported to the participant.

Pulmonary Function Test (PFT)

To assess acute effects of e-cig and common cigarette use as well as longitudinal effects of exposure on pulmonary health, participants will complete the pulmonary function tests. Participants will complete them before and after engaging in the smoking/vaping product use challenge described below. For all participants, pulmonary function will be assessed via spirometry conducted in accordance with the American Thoracic Society guidelines, using the NHANES-III methods. Maneuvers are repeated a maximum of 8 times or until 3 acceptable spirograms are obtained while the patient is standing. We will record FEV_1, FVC, FEV_1/FVC and FEF_{25-75}. These data will allow us to examine pulmonary function longitudinally as well as acute respiratory health in response to combustible cigarette and/or e-cig use and how that changes over time. For the assessment of acute lung function, we opted to allow participants to smoke/vape as much as desired using their own products for 20 minutes to mimic a more typical use pattern following extended abstinence. This test has no anticipated IFs. The FEV1 (%), FVC (%), and FEV1/FVC ratio will be reported to the participant.

Smoking/Vaping Product Use Challenge

During Visit 2 participants engage in a smoking/vaping task and additional assessments. Participants will be asked to refrain from smoking and vaping for 8 hours prior to their Visit 2. At the beginning of the visit, participants will provide a urine sample for isoprostane/creatinine ratio and complete a baseline pulmonary function test, FeNO and CO assessment. At the start of visit 2, participants will be asked to report: 1) the time of last e-cig and/or conventional cigarette use, 2) their withdrawal symptoms using the Wisconsin Smoking Withdrawal Scale (WSWS), 3) their beliefs for how well either conventional cigarettes or e-cigs would alleviate their withdrawal symptoms, and 4) their anticipation for using conventional cigarettes or e-cigs. Pre-challenge testing will be performed followed by exclusive smokers being allowed to smoke as much as they would like for 20 minutes. Exclusive E-cig users will be allowed to use their product/device as usual for 20' during the acute challenge. As this challenge occurs, participants will be videotaped to allow us to calculate number of puffs taken, they will be asked

to rate their pleasure and satisfaction experienced from smoking or vaping and their withdrawal symptoms. The video camera stores the session on a secure digital memory card. After a session has been recorded, the card will be uploaded to the CTRI secure server and the memory card will be erased. At the end of the night the camera and memory card will be stored in a locked cabinet. The recordings will be stored on the server until all recordings have been transcribed, at which point they will be deleted.

Analysis Plans

Power estimates assume β=0.2, α=0.05, and two-tailed tests. Brachial artery FMD is the major CVD index of the acute effects of product use. The single prior small study of product use on acute FMD change showed that amongst 20 smokers, smoking a cigarette produced an FMD response that decreased from 5.6% (SD=5.2%) to 2.8% (SD=3.6) within 30 minutes. The effects of cigarette use were even greater amongst the 20 nonsmokers. We expect that cigarettes will produce a significantly greater impact on FMD than E-cigs; with the pre-post mean values of 6.5% and 3.0% for exclusive cigarette users and 7.5% and 5.5% for exclusive E-cig users (with SD=4.0% for all time points and groups and a conservative pre-post FMD correlation of 0.60). This will yield more than adequate power (>0.80) to detect a Group x Time interaction with n=165. We also predict that those using E-cigs and/or cigarettes will show significantly greater challenge-induced reductions in FMD than will never-smokers who use neither product during the challenge (never-smoker means: pre=7.0% and post=7.0%); power will be >0.80 for contrasts of either E-cig or cigarette users with never-smokers (n=110). Thus, both products will reduce FMD acutely, but cigarettes will produce a greater decrease. Our experience with health studies with smokers shows that we experience virtually no attrition across samples when two proximal visits are scheduled amongst those who attend an Orientation session. We have modeled power for FMD vs. spirometry outcomes, since our data in hand from the EXHALE and WSHS-2 studies suggest that effect sizes and power will be even greater for the key spirometry indices; so FMD is a conservative test of power.

Potential Risks

The risks associated with this study (breach of confidentiality, brief tobacco withdrawal, blood draw discomfort, stress test effort, and brief discomfort during pulmonary function tests) are minimal compared to the potential benefits to the participants as they gain information on their health that might influence their decisions to quit using these products and compared to the potential benefits that the knowledge gained from this study might have in informing clinical practice and public policy.

Physical Risks

Participants will be allowed to smoke and/or vape their own products ad libitum, following assessment, which will alleviate all withdrawal symptoms. Please refer to the CLUES Emergency-Safety Procedures MOP.

- We will not permit women to who are or may be pregnant to enroll, since smoking cigarettes and using e-cigarettes are hazardous to the health of pregnant women and fetuses. Of course, the participants already chose to smoke or vape, but we do not want to subject them to an acute product inhalation challenge for non-therapeutic study. Also, pregnancy significantly alters all of the vascular measures we are obtaining. We will ask participants to confirm that they have had two consecutive regularly timed menstrual periods and to agree to abstain from becoming pregnant by using acceptable contraception during the conduct of this observational study. We will warn them verbally and in writing about the risks of smoking and/or vaping during pregnancy. Since all participants have chosen to smoke or vape prior to their participation and we are not asking them to smoke or vape more than they typically would, we do not think pregnancy testing is necessary to confirm the absence of pregnancy status.

- Drawing blood is associated with temporary pain at the needle insertion site. It also can be associated with bruising, fainting, or, in rare circumstances, a skin infection.
- Carotid and brachial artery ultrasound tests are safe, noninvasive, and not painful. There are no known adverse effects related to diagnostic medical ultrasound. The two ultrasound tests lasts 20-25 minutes. A participant may become tired or have a stiff neck during the ultrasound of the neck.
- Skin might be a little red after the ECG electrodes used for the 12-lead ECG, ultrasound tests, and stress test.
- Spirometry is a routine breathing test that sometimes can cause temporary coughing, shortness of breath, or feeling lightheaded; these symptoms almost always pass within a few seconds.
- Exercise testing is routinely done in patients with heart disease but has some risks. Participants will undergo the stress test only if they do not have contraindications and are clinically stable (see UW AIRP Treadmill Stress Test MOP). Participants will be screened prior to the test by an exercise specialist for contraindications to stress testing, using the CLUES Treadmill Stress Test MOP. During the exercise test, the participant may notice fatigue, chest discomfort, or shortness of breath. These are signs that may indicate that the test needs to be stopped. Other possible side effects of the exercise test are abnormally high or low blood pressure, fainting, abnormal heart beats (too slow, too fast or ineffective), and very rarely a heart attack. About 1 in every 10,000 patients with heart disease dies during an exercise test though we have stricter screening procedures and exclude patients at higher CVD risk (see CLUES Treadmill Stress Test MOP). We have not had a serious complication from as tress test in the UW AIRP since we started performing them in 2005 (>2000 tests performed). Prolonged chest pain or serious heart rhythm problems happen in about 4 of every 10,000 patients. Up to 2% of patients with heart failure having an exercise test have had a serious abnormal heart rhythm, but none cause immediate death.

Loss of Confidentiality Risks

Confidentiality of participant data and information will be accomplished by using participant numbers as unique identifiers, allowing us to keep participant data separate from identifying information. Data generated from the study participant will be stored in secure databases under protections and procedures consistent with the guidelines and regulations of the UW School of Medicine and Public Health (UW-SMPH). All data are behind UW-SMPH and UW Department of Medicine firewalls. Outside access is available only via an encrypted connection to the Department of Medicine Citrix server located at the UW Clinical Science Center in Madison. The servers at the UW-CTRI Madison office are physically secured in a locked room. Data backups are created nightly and stored in a locked safe. Significant safeguards have been implemented to protect data including virus and adware protection, firewall, access controls and encryption when appropriate such as wireless and remote access. All UW-CTRI and UW-AIRP staff members have completed HIPAA/human subjects training and are aware of the sensitivity of study-related data. The UW SMPH has developed school-wide data security policies and procedures and these were implemented in 2009. UW-CTRI data security policies and procedures conform to those of the SMPH. UW-CTRI and the UW-AIRP web based database (maintained by the DOM IT group) use an enterprise-level database that supports audit trails such as access, change logging, and more sophisticated access control for managing and tracking user access privileges. No identifying data other than a participant ID number is entered on any data form. Any data collected on paper are entered into the computer immediately upon receipt at the UW-CTRI or the UW AIRP and the paper document will be stored securely at the UW-CTRI Madison office or UW AIRP lab. Consent forms are obtained in paper copy; these forms contain the participant name and signature and are stored securely at the UW AIRP lab. After an acute challenge session has been recorded on videotape, the memory card will be uploaded to the UW-CTRI secure server and the memory

card will be erased. At the end of the night the camera and memory card will be stored in a locked cabinet. The recordings will be stored on the server until all recordings have been transcribed, at which point they will be deleted. No publications or presentations resulting from this research program will contain any identifying information about individual participants. Conduct of study events for EMA reporting, data collection, and tracking of progress is guided by a customized database as we have used previously developed with the assistance of the UW DoIT team. Data are entered into Qualtrics™, a computerized assessment resource with built-in quality assurance and security features (e.g., not permitting skipped or out-of-range values). Data are stored securely and accessed in accord with all UW, state, and federal regulations.
Samples sent to Duke University for genetic testing are de-identified. Genetic information will not be provided to participants. The samples will be coded (with the link kept at the UW) and leftover samples will be disposed at Duke (not returned to the UW).

Potential Benefits of the Proposed Research to the Participants and Others

Although participants may experience a benefit from participating in this study, there is no direct benefit guaranteed to participants. The potential benefits for smokers participating in this study include learning information about their health. As in our past research, we will provide individuals with their test results and other information about their health because it is appropriate clinical practice and helps reduce attrition. Such information will include white cell count, hemoglobin concentration, lipid profile, spirometry results, and results of exercise stress test.

Provisions in Place to Address any Unanticipated Problems or Complications

The study will use the UW-CTRI DSMC chaired by Dr. James Cleary, leader of the Cancer Control Program of the UW Comprehensive Cancer Center. Dr. Cleary is an experienced physician and clinical trial researcher with no involvement in any of this project's research activities. Dr. Cleary is joined on the DSMC by Dr. James Sosman and Dr. Burke Richmond. Dr. Sosman is Associate Professor of Medicine and Medical Director of the HIV/AIDS Comprehensive Care Program at UW Hospital and Clinics who has previously collaborated on clinical trials of smoking cessation with UW-CTRI. Dr. Richmond is a family physician and otolaryngologist who has served on independent DSMBCs for Phase II and III trials at UW-CTRI. Neither has direct involvement with any of the proposed research. The Principal Investigators will report to the DSMC.

This study involves neither medication nor treatment of any sort, therefore the occurrence of any unanticipated health events which are severe, unanticipated, and possibly related to study protocol should be uncommon. Any serious unanticipated health event that study staff become aware of will be queried and reported even if it appears that the serious unanticipated health event is unrelated to study participation. The Principal Investigators will be responsible for the accurate documentation, investigation and follow-up of all study-related unanticipated health events. Unanticipated health event assessment, recording, reporting and investigation will be accomplished through staff training, structured/standardized reports of untoward occurrences/events, and regular monitoring by other study investigators. The DSMC requires that investigators notify NIH and the University of Wisconsin IRB in a timely manner (consistent with IRB and NIH policies) of the occurrence of any SAE or any AE which is severe, unexpected, and possibly related to study protocol. Unanticipated AEs, including less serious problems that merit reporting to the DSMC because they are severe, unexpected, and possibly related to study participation. Any SAE will be queried and reported even if it appears that the serious adverse event is unrelated to study participation. The Principal Investigators will also be responsible for the accurate documentation, investigation and follow-up of all study-related adverse events. Additionally, the IRB will receive an annual report of all serious unanticipated health events and unanticipated health events.

Importance of the Knowledge to be Gained

The results from this research will provide important insight into acute and chronic cardiovascular and pulmonary effects and exposure biomarkers of e-cig use compared to, cigarette smokers and quitters. This information will aid clinicians, scientist, and regulatory bodies to make informed decisions about clinical practice, future research, and public health policy.