

Title: *RESEC: RESpiratory effects of E-Cigarettes in obese youth*

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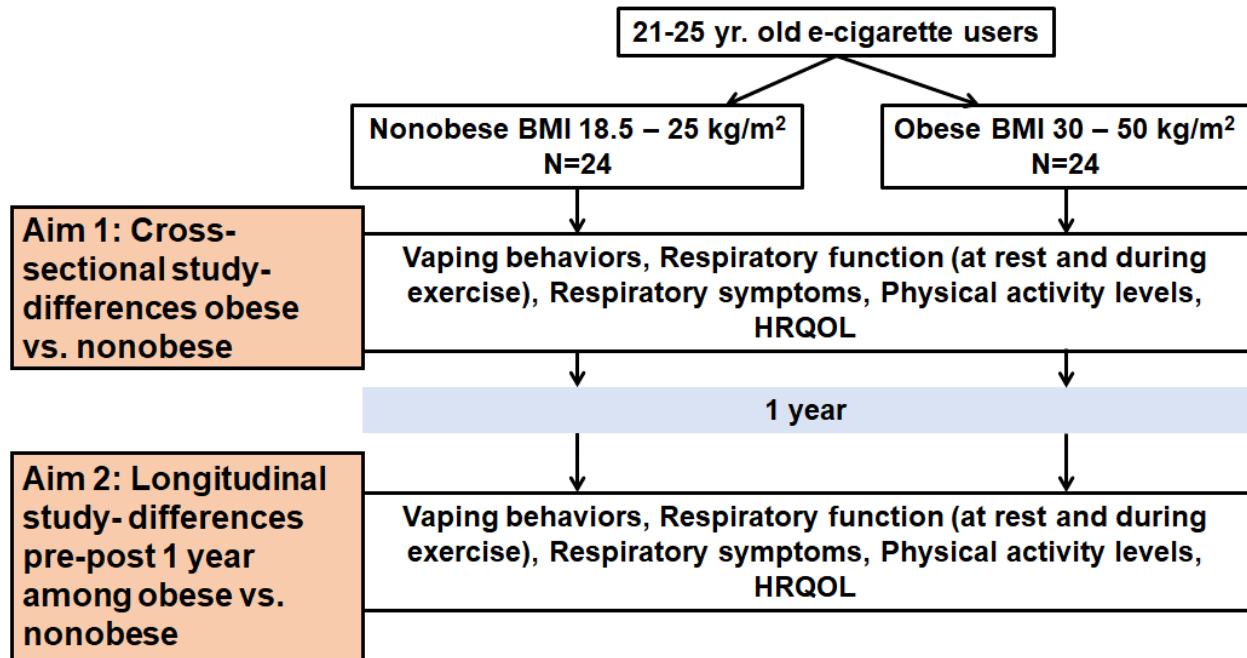
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Study Schema



Abstract

Obesity, physical inactivity, and e-cigarette use independently increase cancer risk and impact outcomes for patients with cancer. The prevalence of e-cigarette use is as high as 11% in American youth and almost four times higher among obese youth. With obese youth already more likely to be sedentary and have greater respiratory symptoms and disease compared with their lean counterparts, the additional risks that come with vaping could impact cancer risk significantly. NIH recognizes the need for studies to address gaps in our understanding of the impact of e-cigarette use on health outcomes especially in obese youth users (NOSI: NOT-OD-22-023). To address this need, we propose a project that will improve our understanding of the potential effects and interactions between obesity, respiratory symptoms that affect engagement in regular exercise, and e-cigarette use among 21-25-year-old obese youth.

Therefore, the central goal of the RESEC (RESpiratory effects of E-Cigarettes in obese youth) study is to investigate vaping behaviors, respiratory function, respiratory symptoms during exercise, and physical activity levels in e-cigarette users with and without obesity. We hypothesize that **obese youth users** will have increased e-cigarette use that will worsen respiratory function and symptoms and reduce physical activity levels. Our approach will involve a longitudinal study where vaping behaviors, respiratory function, respiratory symptoms, and physical activity levels will be assessed twice: baseline and 1 year, in 48 participants. The central goal of this proposal will be accomplished by pursuing two aims:

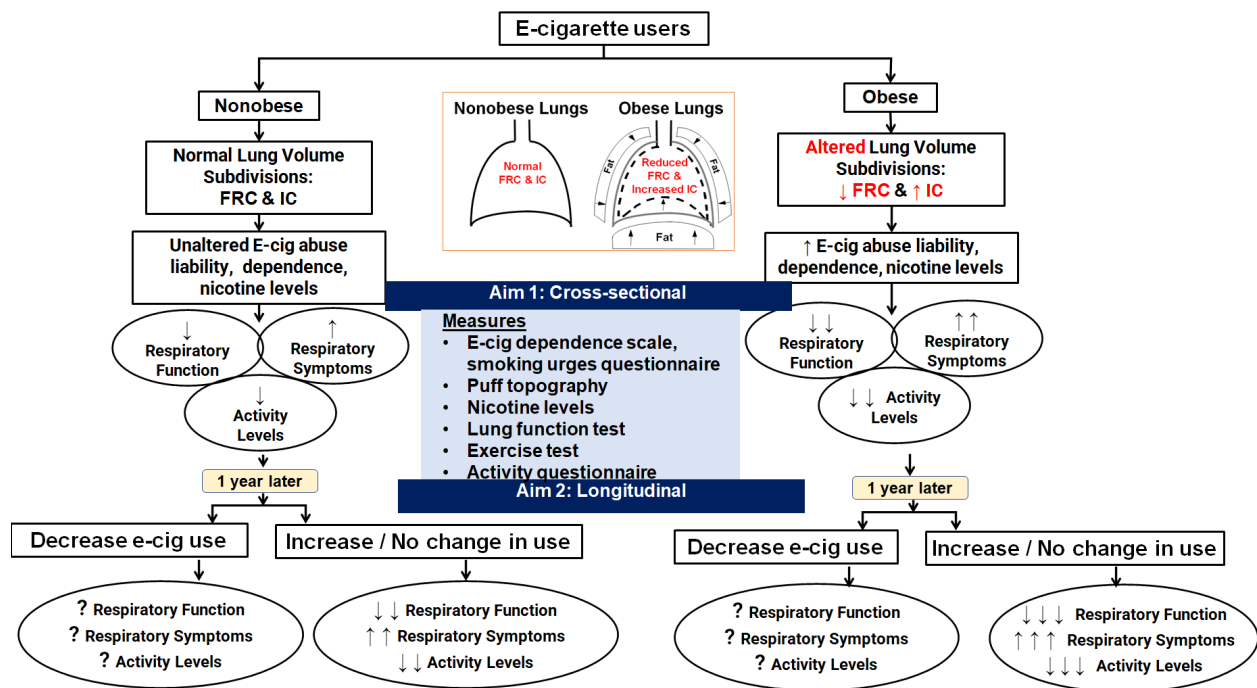
Aim 1: Determine the effects of vaping behaviors on respiratory function, respiratory symptoms, and physical activity levels in obese and nonobese youth e-cigarette users. We hypothesize that self-reported nicotine dependence will be associated with reduced respiratory function, greater respiratory symptoms, and reduced activity levels in obese compared with nonobese users. Smoking behaviors and nicotine dependence will be assessed using validated questionnaires

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and puff topography, respiratory function will be assessed using spirometry, diffusing capacity, and plethysmography, constant work-rate exercise testing will be used to assess respiratory symptoms, and physical activity levels will be assessed using validated questionnaires.

Aim 2: Determine the longitudinal effects of change in vaping behaviors on respiratory function, respiratory symptoms, and physical activity levels in obese and nonobese youth e-cigarette users. We hypothesize that continued and increased vaping will worsen respiratory function, respiratory symptoms, and physical activity levels in obese compared with nonobese youth e-cigarette users. Measurements in Specific Aim 1 will be repeated at 1 year to evaluate the effects of changes in vaping behaviors on outcome measures.

A conceptual model is provided below:



Impact: Our results could expand our collective understanding of the interactions between obesity, physical inactivity, and e-cigarette use among obese youth users. This foundational research is critical for informing policy recommendations because obese youth are at greater risk of developing cancer as well as cardiovascular and respiratory diseases, and tobacco experimentation and use typically begin during adolescence. Completion of this project will result in an NIH R01 grant submission in response to NOT-OD-22-023 by an early-stage investigator at the Center for Tobacco Research (Dr. Dharini Bhammar) in collaboration with an established tobacco scientist Dr. Theodore Wagener.

A. Significance

The prevalence of e-cigarette use is almost four times as high in obese youth when compared with their normal-weight counterparts^{2,3}. Obese youth report more respiratory symptoms, and respiratory pathologies increase with increasing body weight⁴. Making matters worse, e-cigarette use independently increases respiratory symptoms such as wheezing during exercise⁵. When obese youth become e-cigarette users, the risk of experiencing unpleasant respiratory symptoms especially during exercise increases and as a result the desire to engage in physical activity can plummet⁶. The

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combination of these three unhealthy risk factors- obesity, physical inactivity, and e-cigarette use- could exacerbate the risk of cancers in adulthood⁷⁻¹³, posing a serious long-term health risk for obese youth. Furthermore, respiratory symptoms associated with obesity and e-cigarette use could further reduce activity levels leading to greater weight gain, thus perpetuating a vicious cycle of obesity and inactivity. Thus, there is an urgent need to improve our understanding of the impact of e-cigarette use on respiratory outcomes and physical activity behaviors among obese youth with the goal of increasing cancer prevention.

The past decade of my career as an exercise physiologist has been focused on investigating the respiratory effects of obesity. My research has shown that obesity imposes a significant mechanical burden on the respiratory system resulting in constrained and inefficient breathing, especially during exercise¹⁴⁻¹⁸. Obese individuals are sedentary partly because their experience during exercise is less than positive⁶. In a misguided attempt to fit in with their peers or perhaps to lose weight, obese youth begin to engage in vaping behaviors early-on and at high rates¹⁹. Obesity exerts a mechanical load on the thoracic cavity through pressures exerted from chest wall fat on the rib cage and from abdominal fat on the diaphragm²⁰⁻²⁵. A measurable effect of this load is exhibited as a reduction in the functional residual capacity (FRC; see **Figures 1 and 2**)²⁴⁻²⁷. FRC is the volume of air that is in the lung when the diaphragm is in a relaxed position at the end of a normal breath. The TLC of an individual is the total volume of air that is in the lungs when the person breathes in as deeply as possible. TLC minus FRC equals the inspiratory capacity (IC) of the individual, which is how much capacity they have to breathe air in. In a study of N=92 children, we found that IC was on average 360 mL higher and FRC was 10%TLC lower in those with obesity compared with those without obesity. This 360mL higher IC, if recruited during vaping, could be a mechanistic explanation for why inhaled toxicants are more easily deposited in the lungs of individuals with obesity compared with those without. Thus, unlike their leaner counterparts, obese youth also have a much greater capacity to inhale or “breathe in” (i.e., inspiratory capacity) because the excess weight on their chest wall pushes their breathing to much lower lung volumes (i.e., a lower FRC). Having a greater inspiratory capacity could make it easier for obese e-cigarette users to inhale much larger volumes of e-cigarette aerosol. Also, having a lower functional residual capacity can lead to premature airway closure during expiration²⁸, which would not allow e-cigarette vapors to be completely expired. Thus, through the mechanism of altered lung volumes, obese individuals could be exposed to more toxicants and addictive nicotine, which

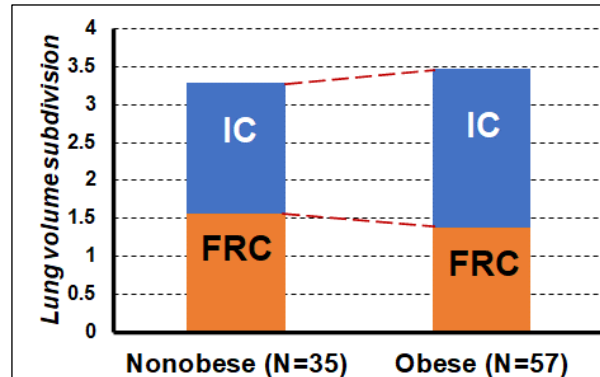


Figure 1: IC is higher and FRC is lower in obese individuals. Preliminary data from Dr. Bhammar's project (NCT: 03376880).

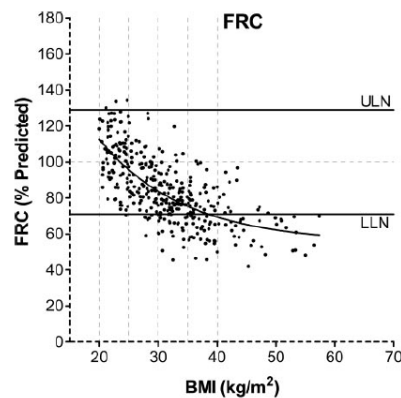


Figure 2: FRC as a percent of predicted is lower with increasing body mass index (BMI). Published work: Jones 2006¹

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could lead to worse respiratory outcomes and greater nicotine dependence; however, this basic physiological mechanism has never been investigated in obese youth vapers. There is a 29% higher rate of alveolar fractional deposition of fine particulate matter in the lungs of obese children (who breathe more) compared with nonobese²⁹. The knowledge regarding how obesity may impact vaping behaviors (e.g., greater inhalation volume) and the impact of vaping on respiratory function, respiratory symptoms during exercise, and physical activity levels among obese youth users is scarce as is longitudinal data on how changes in use patterns can affect respiratory health outcomes and activity levels. Investigating these important questions is critical to inform policy decisions and recommendations that could specifically target obese e-cigarette users.

B. Research Design and Methods

B.1. Design Overview

This is an observational study. In the cross-sectional portion of this project, baseline data from obese youth e-cigarette users will be compared non-obese youth e-cigarette users. Measurements completed during the cross-sectional portion of the project will be repeated at 1 year to evaluate longitudinal effects of changes in vaping behaviors on respiratory function, respiratory symptoms, and physical activity levels. All participants will be 21-25 years old, regular e-cigarette users (at least daily use over the past 3 months), and free from any disease that may increase risks during exercise testing³⁰. Nonobese will be defined as a body mass index (BMI) = 18.5–25 kg/m² and obesity as BMI = 30–45 kg/m² (i.e., mild-to-moderate obesity). 48 participants (24 with obesity and equal distribution of males and females) will be recruited.

B.2. Power calculation

With 20 participants, we would be able to assess differences in the proposed mechanism (the capacity to inhale or inspiratory capacity) between obese and nonobese participants at an effect size Cohen's *d* of 1.38, alpha of 0.05 and power of 0.83 from published estimates³¹. By recruiting 48 participants, we will have a sufficient sample to detect sex differences and differences in other outcomes measures between obese and nonobese for measures with an effect size of 0.90, alpha 0.05, and power of 0.80, assuming a 15% rate of attrition and a final sample of 42 participants. With the caveat that our longitudinal component is "free-living" rather than a behavioral intervention, we seek to detect changes in outcome variables based on changes in vaping behaviors (increase vs. decrease). Based on previous data showing a significant improvement in respiratory function and respiratory symptoms after smoking cessation with a total sample of 21 adults³², we anticipate detecting changes in outcome variables based with our total sample of 42. However, we note that the longitudinal aim of this study is exploratory and is expected to guide a final sample calculation for a larger grant application.

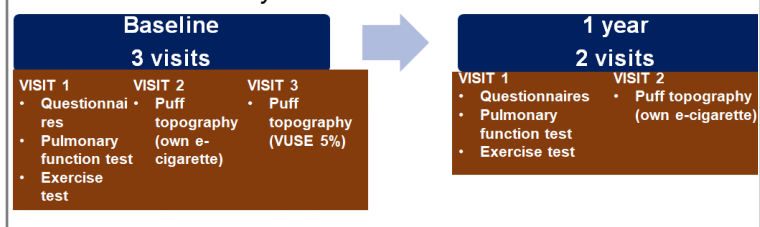
B.2. Study Procedures

Figure 3 shows the basic measures that we will collect at baseline for Aim 1 (cross-sectional aim) and at 1 year (for Aim 2- longitudinal aim). We will recruit participants from the OSU campus and the public.

B.2.1. Pre-visit instructions

Participants will be told to avoid vigorous exercise for 24h prior and to avoid eating or drinking anything except for water for 2h prior to each study visit. Participants will be required to report at least 12 hours abstinent from nicotine before each study visit. Prior to the start of the first laboratory visit, twelve-hour tobacco abstinence will be assessed via self-report and confirmed with exhaled carbon monoxide testing (eCO_≤

Figure 3: Participants will complete measurements on three visits at baseline and 2 visit at 1y



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10ppm). A research assistant will explain the study to the participant and answer any questions they may have. The participant will give an exhaled breath sample into the handheld CO monitor to determine if they are eligible to participate in the laboratory vaping session. If the participant's exhaled CO concentration ≤ 10 ppm, they are deemed eligible to participate further, and will be asked to read the informed consent form.

B.2.2. Informed consent

Study staff will read the consent with the subject and answer any questions. After all questions are answered, the participant will be asked to sign the consent form to participate in the study. A copy of the signed consent will be provided to the participant.

B.2.3. Pregnancy exclusion

Pregnancy exclusion will be confirmed with a urine test.

B.2.4. Questionnaires

Participants will complete a medical history and demographics questionnaire and a physical activity readiness questionnaire (PAR-Q) to identify factors that increase risks of exercise³³, a 24 h health history questionnaire to establish health, activity, medications, sleep, etc over the past 24 hours, background measures (Sociodemographic measures, socioenvironmental measures), Tobacco use history (including a Product Use questionnaire that obtains information on the first tobacco product tried by the participant and detailed information on all tobacco use, the Timeline Followback (TLFB) interview– week, month, lifetime, and the Sensory E-Cigarette Expectancies Scale (SEES)³⁴), the modified Cigarette Dependence Scale³⁵, the International Physical Activity Questionnaire or IPAQ for adults that assesses the physical activities (vigorous, moderate, and walking) in MET-min/wk and sitting time³⁶, the Dyspnea-12 (a 12-question survey assessing breathing difficulty) and the RAND-36 (a 36-item instrument used to assess health-related QOL³⁷- it includes 8 subscales: physical functioning, role limitations due to physical health, role limitations due to emotional problems, energy/fatigue, emotional well-being, social functioning, pain, and general health with each receiving a score from 0 to 100). A surveillance product counseling questionnaire will be administered at the 1-year follow-up visit to assess change in use behaviors. Study staff will remain available to answer questions and guide participants while they fill these questionnaires.

Timing & administration of questionnaires: To reduce patient burden, some questionnaires including sociodemographic and socio environmental measures, tobacco use history, Timeline followback, SEES, modified cigarette dependence scale, IPAQ, Dyspnea-12, and RAND36 may be completed on either the first or the second visit (or fourth or fifth visit for the post-1 year testing). The demographics, medical history and PAR-Q will always be conducted on the first visit because we need this information to assess safety for the exercise test and we need demographic information to calculate reference values during the pulmonary function test. For surveys that we have set-up in REDCap- they will either be done in the lab on paper or one of our computers or a link will be shared with the participant to complete on their mobile or tablet devices.

B.2.5. Anthropometrics

Height will be measured using a stadiometer and weight will be measured using a weighing scale. Circumferences including waist and hip will be measured using a tape measure.

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B.2.6. Pulmonary function testing (PFT)

PFT will be done in accordance with American Thoracic Society guidelines³⁸⁻⁴¹ and will include: 1) spirometry to assess forced vital capacity, mid-expiratory flows, and forced expiratory volume in 1 second, 2) plethysmography to assess lung volumes and airway resistance, 3) diffusing capacity to assess any alveolar epithelial damage, 4) maximal voluntary ventilation in 12 seconds to estimate maximal ventilatory capacity.

B.2.7. Exercise testing

Exercise testing will include three consecutive constant work rate exercise tests at 40W (6 minutes), 60W (4 minutes), and 80W (4 minutes) for females and 60W (6 minutes), 80W (4 minutes), and 100W (4 minutes) for males on an electronically braked cycle ergometer. Participants will be wearing a mask or nose-clip and mouthpiece so we can measure their breathing. Participants will be fitted with a single-use forehead sensor so we can monitor oxygen saturation and heart rate. Before exercise participants will complete spirometry to measure forced vital capacity and slow vital capacity. During exercise, expired gases, ventilation, operating lung volumes (using periodic inspiratory capacity maneuvers), and breathing pattern will be measured using a metabolic cart¹⁸. Ratings of perceived breathlessness and exertion will be assessed every 2 minutes during exercise and a multidimensional dyspnea profile will be completed after exercise for a detailed look at respiratory symptoms^{14, 42-46}. Spirometry measurements will be completed @ 5min and @ 15 min after exercise is ended to capture immediate and delayed changes in airway functioning.

B.2.8. Puff topography sessions

At baseline, there will be two sessions where we will assess puff topography: 1) Session where participant uses their own device (for assessing longitudinal changes in puffing behavior), and 2) Session where participant uses a VUSE 5% nicotine device (for cross-sectional comparisons between obese and nonobese participants). After 1 year, only the session where participant uses their own device will be completed.

During each vaping session, e-cig puff topography will be measured to produce measures of puff count, puff duration, inter-puff-interval, puff flow rate, average puff volume, and total puff volume⁴⁷. Puff topography will be assessed with a validated device (SPA-D). Puff topography will be assessed during a vaping session, which will include a standardized, 5-minute, 10-puff vaping bout (30 seconds between each puff) followed by 30 minutes of ad libitum vaping. E-liquid consumption will be determined by weighing (mg) the participant e-liquid pod before and after the vaping session.

After visit 2 where participants vape using their own device, participants will take home a study e-cig device and 1 weighed pod pre-filled with study e-liquid (the participant was randomized to at the end of visit 1) to practice (20 puffs per day with the study device and until the participant feels comfortable) using the device before visit 2. Practice use with the study e-cig device and pre-filled pod will be verified based on data downloaded (via eScribe) from the device during visit 3.

Before each vaping session, subjects will complete weight measurements, a 24-hour health history questionnaire, and a respiratory health questionnaire. Before and after each vaping session we will complete spirometry, lung volumes and airway resistance measurements to assess any acute changes in airway functioning. Subjects will also complete the Tiffany-Drobes Questionnaire of Smoking Urges⁴⁸ survey before and after each vaping session.

B.2.9. Plan to contact participants between annual study visits

Every 3 months, we will contact participants via email to thank them for their continued participation. We will ask two questions: 1) about any change to their vaping habits, and 2) about any plan to move in the coming 3 months.

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If the participant notes a plan to move in the coming 3 months, we will try and schedule their annual visit a little early so we can test them before they move (testing them up to 4 months earlier than their annual visit will be allowed).

Reminder plan: If after three emails (one initial and 2 follow-up) we don't get the surveys completed, we will call the participant (up to 3 times) and try and get the survey completed over the phone. If these attempts are unsuccessful, we will try again 3 months later. If all attempts remain unsuccessful for 1 year or the participants requests to withdraw from the study, the participant will be withdrawn.

B.2.6. Urine analysis. Urine pregnancy testing will be done before study procedures begin. The collected urine samples will also be aliquoted into 5-mL cryovials, and each vial will be labeled with a unique ID encoding the study, subject, sample collection time-point, and the type of assay. Aliquots will be frozen at -20 °C until analyses are conducted. The urine samples will be pretreated with glucuronidase and total cotinine and 3-hydroxycotinine in urine will be quantified using a validated gas chromatography–mass spectrometry method. Urinary creatinine will be analyzed to adjust biomarker levels for urine dilution.

Measures that will be collected are included in Table 1.

Table 1: Study Measures					
Visit	1	2	3	After 1 year	
				4	5
Background Measures					
Height	X			X	
Weight	X	X	X	X	X
Circumferences	X			X	
24 h health history questionnaire	X	X	X	X	X
Medical history questionnaire	X			X	
Physical activity readiness questionnaire	X			X	
Sociodemographic and socioenvironmental measures*	X			X	
Tobacco Use History & Sensory E-Cigarette Expectancies Scale (SEES)*	X			X	
E-Cig Dependence scale (modified Cigarette Dependence Scale)*	X			X	
Timeline Followback (week, month, lifetime)*	X			X	
Physical activity levels (IPAQ)*	X			X	
Dyspnea 12*	X			X	
Health-related quality of life (RAND-36)*	X			X	
Respiratory health questionnaire		X	X		X
Surveillance product counseling to check changes in behavior				X	
E-cig Abuse Liability					
Participant e-cigarette: Puff Topography (puff count, puff duration, inter-puff interval, puff flow rate, average puff volume, total puff volume)		X			X
VUSE e-cigarette: Puff Topography (puff count, puff duration, inter-puff interval, puff flow rate, average puff volume, total puff volume)			X		

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E-liquid consumption (pre and post weighing of e-cigarette pods)		X	X		X
Tiffany-Drobes Questionnaire of Smoking Urges: Brief Form		X	X		X
Nicotine levels					
Exhaled carbon monoxide testing (eCO _≤ 10ppm indicates 12h abstinence)	X	X	X	X	X
Total cotinine (urine)	X	X	X	X	X
Nicotine metabolite ratio (urine)	X	X	X	X	X
Urinary creatinine (urine)	X	X	X	X	X
Respiratory Effects					
Spirometry	X	X	X	X	X
Lung volumes (plethysmography)	X	X	X	X	X
Diffusing capacity (DLCO)	X			X	
Airway resistance (plethysmography)	X	X	X	X	X
Maximum Voluntary Ventilation (MVV)	X			X	
Oxygen uptake during exercise	X			X	
Ventilation, breathing pattern, lung volumes during exercise	X			X	
SpO ₂ , heart rate during exercise	X			X	
Ratings of perceived exertion during exercise	X			X	
Ratings of perceived breathlessness and multidimensional dyspnea profile during exercise	X			X	
Spirometry 5 and 15min post exercise	X			X	

*some of these questionnaires may be done on the second visit instead of the first to reduce patient burden. At 1 year follow-up, some of these maybe done on the fifth visit instead of the fourth to reduce patient burden.

B.3. Recruitment Feasibility and Retention

B.3.1. Recruitment

We intend to recruit 48 e-cig users from the community over a 12-month period and have them complete five study visits (3 before and 2 after 1 year); therefore, we need 4-5 participants to be recruited and complete study procedures per month.

We are confident our recruitment approach, laboratory facilities, and equipment redundancy will result in a sufficient participant processing rate, given our successful completion of other tobacco-related research of similar design⁴⁹. Based on 2020 Ohio BRFSS data, there are ~53,000 residents of Franklin County ages 21-24 years. Of these, ~6% use e-cigarettes and 18% are obese. We expect that ~572 people are eligible to be part of the 'obesity' arm of this study throughout Franklin County (ignoring the other inclusion/exclusion for now). Recruiting 20 to the obesity arm, requires enrollment of ~3% of the eligible population in the area. E-cig users will be recruited from advertisements at OSU and neighboring college campuses, and through a variety of media outlets and the internet, including Study Search, as well as community events. Participants from other studies who have agreed to be contacted regarding other study opportunities will also be contacted. Staff from those studies will prepare contact letters/emails and call participants on behalf of this study. Interested participants will be referred to this study for screening. We will plan to be extremely responsive to interested participants and schedule them quickly to minimize losses. We recognize that it may be easier to recruit normal weight participants compared with obese participants, so we will be attentive to how many patients we

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enroll in each group. Participants will access the screening questionnaire using a public survey link generated by REDCap or they will be screened over the phone. Based on my previous studies, I anticipate a 15% attrition rate. However, if the attrition rate is higher, then we will recruit a few more participants until we meet our recruitment goals. Thus, we need to recruit 48 participants to have 42 complete the study.

B.3.2. Inclusion criteria

1) a current e-cigarette user (≥ 1 vaping bout daily) for at least the past 3 months, 2) 21-25 years old, 3) willing to abstain from all tobacco and nicotine for at least 12 hours prior to lab sessions, 4) willing to complete five lab visits lasting up to 4 hours each, 5) able to read and speak English, 6) willing to provide informed consent.

B.3.3. Exclusion criteria

1) self-reported diagnosis of lung disease including asthma or cystic fibrosis, 2) history of cardiac event or distress within the past 3 months, 3) history of metabolic disease including thyroid disease or diabetes, 4) history of orthopedic or neuromuscular problems that preclude exercise, 5) currently pregnant (determined using urine pregnancy test), planning to become pregnant, or breastfeeding, 6) use of other tobacco products >10 days in the past month, 7) current marijuana use >10 times per month, 8) currently engaging in a vaping cessation attempt, 9) currently engaged in an exercise training program involving 300min or more (moderate intensity aerobic activity) or 150 min or more (vigorous intensity aerobic activity) per week, 10) plans to leave the Columbus or central Ohio region within the next year.

B.3.4. Screening procedures

Participants' eligibility will be determined over the phone or via an online screening form in REDCap. Those who are eligible and willing to participate will be invited to sign an informed consent and complete their baseline visit in a private participant room at the Ohio State University. All participants will be given adequate time to review the informed consent with a trained research staff to help answer any questions that may arise during the consent process. Additionally, a copy of the informed consent will be given to all participants. A pregnancy test will be completed at the initial visit as well as before starting all the in-lab visits to ensure that the participant is not pregnant.

B.3.5. Retention and Compensation

Participants will receive \$75 for completing visit 1, \$50 for visit 2, \$50 for visit 3, \$75 for visit 4, and \$50 for visit 5, for a total of \$300 for completing the full study. Payments will be prorated with details included in the consent form. Compensation will be provided at the end of each study visit. Consistent with our previous studies, payments will be made using the Greenphire ClinCard to increase accountability and facilitate ease of payment. We will facilitate visits by using additional retention strategies (e.g., reminder calls/texts/emails) and/or offering weekend visits. Participants will receive reminder calls in addition to email or text reminders. Reminders will be sent by text or email based on a participant's preferred method of contact.

B.4. Protocol Adherence and Quality Control

B.4.1. Data Management

Data will be collected directly from the participant by a research assistant and/or research nurse. Data will include participant responses to computer-based survey questionnaires,

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exhaled breath CO, urine sample, physiological (respiratory function at rest and metabolic and respiratory responses during exercise) effects, cardiovascular measures (heart rate during exercise), perceptual responses during exercise, and puff topography.

B.4.2. Quality Assurance

All research staff will have completed Human Subjects training. Standard operating procedures (SOP) will be developed and all staff will be trained to ensure adherence to the SOP. As is standard practice for our team's current studies, each visit will have its own checklist of specific measures to be completed and the order in which they are to be administered. To reduce data entry errors, participants will enter data into secured computer-based questionnaires. All specimens collected for biomarker analysis will be given individualized bar codes. All key on-site personnel will meet face-to-face weekly throughout the entire study. During these meetings, recruitment, enrollment, data collection, data monitoring results, and any concerns/issues will be discussed.

B.5. Statistical Analysis Plan

Normality will be determined using a Shapiro Wilk test. Data will be transformed or nonparametric statistics will be utilized for non-normal data. Our study statistician Dr. Menglin Xu, will advise on data management and conduct statistical analysis. Statistical comparisons between obese vs. nonobese groups at baseline (cross-sectional Aim 1) will be completed with unpaired t-tests. Pearson correlations will be used to test associations between continuous variables. Statistical analysis for longitudinal Aim 2 will be conducted using a two-way mixed ANOVA (group: obese/nonobese × change in behavior category: increase/no change vs. decrease/cessation). Exploratory analysis to investigate the effect of sex will be performed using a 2-way ANOVA. All tests will be two-tailed and P will be 0.05.

C. Protection of human subjects

C.1. Recruitment and Informed Consent

At first contact, all participants will be screened according to the study's inclusion/exclusion criteria. Those who are eligible will be given a brief verbal overview of the study and invited to participate. Informed consent (including a description of the nature, purpose, risks, and benefits of the study) of the participant will take place through both oral and written explanation of the study. The voluntary nature of the study and the participant's right to withdraw at any time will be stressed during the consent process. A copy of the informed consent will be provided to the participant in written form at the time of consent for them to keep. Informed consent will be collected by IRB approved study personnel. Recruitment script and materials, consent forms, and all study procedures will be approved by the OSU Institutional Review Board. All participants will provide informed written consent before any study data is collected.

C.2. Potential Risks and Protections against Risk

There are minimal risks associated with this protocol. The protocol requires e-cig users to undergo 12 hours of tobacco/nicotine abstinence on five occasions (3 at baseline and 2 at 1 year). These e-cig users will complete 3 vaping sessions – two sessions at baseline and 1 at 1 year. For two of these sessions, participants will vape using their own e-cigarettes. For one session (visit 3 at baseline), all participants will use the VUSE 5% nicotine e-cigarette. All participants are already regular users of e-cigarettes and will only be asked to use what will be a

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commercially available e-cigarette device. Questionnaires and urine collection procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) risk of using e-cigarettes, b) risk of pulmonary function testing, c) risk of exercise testing, and c) loss of confidentiality or privacy.

C.2.1. Risk of Using ECs

Risks

The risk of side effects and adverse events are very low. The VUSE device we are using is sold online, and at e-cigarette specialty stores and convenience stores nationwide, without a prescription. The participants personal e-cigarette devices are already being used regularly by participants and they should be aware of the effects of their own devices.

Protections

Nevertheless, all participants will be screened for general medical precautions (pregnancy, cardiovascular disease) and monitored for adverse events during the study period. Study personnel will assess for adverse events via self-report at all follow-up visits. Any serious adverse events will be reported to the OSU IRB. We will withdraw participants who have a serious adverse event, or become pregnant, begin to breastfeed, or have a cardiovascular or pulmonary event during the study. The most likely adverse (potential for nicotine overdose) event is anticipated to be rare (<5% in our team's previous studies) and mild (nausea, headache, disrupted sleep), and will be handled quickly (i.e., advice to participant to reduce or stop e-cig use). Lab studies of toxin exposure suggest that e-cigs incur no greater risk to health than do conventional cigarettes. Indeed, e-cigarettes generally show lower levels of harmful and potentially harmful constituents. To date, e-cigarette studies discussing adverse events report mild and tolerable side effects that generally resolved completely over time with continued use. In four randomized clinical trials, no serious adverse events were reported and the e-cigarette group and the nicotine patch group had comparable levels of adverse events in two studies. The most common were mouth irritation, throat irritation, dry cough and headache. Following the completion of baseline visits 1-3 for this study, we will encourage all participants to quit their use of e-cigarettes.

Cessation resources: We will provide participants with cessation resources including

- OSU quit smoking and tobacco use flyer (From the OSU cessation website)
- List of cessation providers in Columbus from the Columbus Public Health website

Additional resources that are included in the IRB submission and will be provided to participants as needed:

- Handout of free clinics in Columbus
- Mental health resources handout
- Nicotine overdose pamphlet
- Pregnancy information handout

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C.2.2. Risk of pulmonary function testing

Risks

There are no known significant risks of performing pulmonary function tests. Some participants may experience transient discomfort, cough, or feel lightheaded with repeated forced expirations during spirometry.

Protections

We will give a break for participants who feel discomfort during spirometry. For participants who are unable to tolerate efforts, we will discuss the importance of the measurements and remove them from the study for inability to complete these measurements successfully.

C.2.3. Risk of exercise testing

Risks

During exercise, participants will experience discomforts similar to those associated with any type of moderate-intensity exercise such as leg discomfort, discomfort from sitting on a cycle ergometer seat, or shortness of breath. Exercise may also cause bronchoconstriction of the airways, but the risk is very low for participants without a history of asthma. There is a slight risk of abnormal blood pressure, fainting, disorder of heartbeat, and in rare instances, heart attack with exercise. These risks occur in 1 in every 40,000 tests when the exercise is done till maximal effort. However, the exercise intensity in this study is submaximal, so the risks should be lower.

Protections

Participants will not be left unattended during any study procedures. Every effort will be made to minimize risks through a preliminary interview and screening process, and by observations made during testing. Participants will be asked to stop exercising if personnel notice any problems and a participant request to stop will always be honored. The personnel involved in testing are experienced with such testing and trained in cardio-pulmonary resuscitation.

C.2.4. Loss of Confidentiality and Privacy

Risks

There is a risk of breach of confidentiality or a loss of privacy associated with taking part in the study.

Protections

Protection against loss of confidentiality and privacy will be maintained by assigning ID numbers to subjects to de-identify data that is stored in any database, disguising or redacting identifying information, and keeping data locked in file drawers and in a secure, password-protected database server. Only study research assistants and the PI will have the information that connects participant's name and ID number. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., CITI trainings). Identifying information will not be reported in any publication.

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C.3. Potential Benefits of the Proposed Research

Whereas no assurance can be made to an individual participant that s/he will personally benefit from this research, the experience should be beneficial. The immediate benefits of this research are scientific in nature, which in the long-term should benefit society as a whole because basic physiological knowledge about how obesity-related increases in inspiratory capacity can impact vaping behaviors could be leveraged to develop educational vaping prevention materials targeting obese youth. The study will also benefit e-cig users, as a group, by providing information as to the abuse liability of other e-cig/e-liquid products; and serve as evidence to inform regulatory action that improves public health. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

D. Data and Safety Monitoring Plan

D.1. Plan

Data will be analyzed initially after 20 participants are accrued, to ensure electronic data capture systems employed (i.e., REDCap) are accurately capturing data and to ensure the format and completeness of all data collected.

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. All Serious Adverse Events are to be submitted to the IRB.

D.2. Adverse Events

All adverse events including serious or unexpected adverse events as defined in 21 CFR 312.32 will be reported to the IRB. The IRB requires that fatalities and all other unanticipated problems involving risk to subjects be reported within 10 business days if they are related to the study.

Any changes or amendments to the protocol made in response to adverse events/SAEs (or independent of AEs/SAEs) will be requested in writing to the IRB, which will then grant or deny permission to make the requested change in protocol. The IRB will be notified about any significant changes to the protocol. Changes that significantly alter the scope of the research or the ability of the research to achieve its specific aims will be submitted to the IRB for approval prior to implementation.

Adverse events will be assessed by study staff at each visit via participant self-report. All adverse events will be reported to the OSU IRB. We will monitor for risk of smoking/vaping by screening participants for general medical precautions (pregnancy, cardiovascular disease). Any adverse events, breaks of confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel and Dr. Bhammar. Dr. Bhammar will be responsible for completing an Adverse Events Form should an event occur. Dr. Bhammar will report Serious Adverse Events to the OSU IRB within 24 hours of having received notice of the event. Dr. Bhammar will gather any information needed to investigate the event and to determine subsequent action. Any subsequent action will be documented and reported to the OSU IRB. Adverse event reports will be reviewed annually with the OSU IRB to ensure

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participant safety.

Collection of Adverse Events

The collection of adverse events will be on a self-report basis and logged within an electronic data capture system (REDCap) or collected using standardized paper forms and will only be identified with the study ID of the participant.

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