The impact of acute exercise on factor VIII in young e-cigarette smokers

Andrew Allen

Follow this and additional works at: https://commons.lib.jmu.edu/masters202029

Part of the Exercise Science Commons

Recommended Citation
https://commons.lib.jmu.edu/masters202029/36

This Thesis is brought to you for free and open access by the The Graduate School at JMU Scholarly Commons. It has been accepted for inclusion in Masters Theses, 2020-current by an authorized administrator of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.
The Impact of Acute Exercise on Factor VIII in Young E-cigarette Smokers

Andrew R. Allen

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Science

Department of Kinesiology

May 2020

FACULTY COMMITTEE:

Committee Chair: Christopher J. Womack, Ph.D.

Committee Members/ Readers:

Stephanie P. Kurti, Ph.D.

Trent A. Hargens, Ph.D.
Acknowledgments

I would like to thank my Thesis Chair, Dr. Christopher Womack, for his continual support and guidance throughout this whole research process. Without his experience and resilience, this project would not have been possible.

I would like to thank my Committee Members, Dr. Stephanie Kurti and Dr. Trent Hargens for their diligent efforts in analyzing my documents and providing constructive feedback for me to enhance my thesis. Dr. Kurti’s genuine curiosity on e-cigarettes was a driving force behind this whole project being started.

I would like to thank my Research Partner, Chelsea Robinson. Her efforts were integral in starting up this project and keeping it running over the course of the past year. I will be forever grateful of all the work she took on and for her positive mindset throughout the whole process.

I would also like to thank the undergraduate students who volunteered their time to help us set-up and efficiently perform each visit with our participants.
Table of Contents

Acknowledgements............................................................................................................. ii
Table of Contents................................................................................................................ iii
List of Tables ........................................................................................................................ iv
List of Figures ........................................................................................................................ v
Abstract.................................................................................................................................. vi
I. Introduction ............................................................................................................................. 1
II. Methodology ........................................................................................................................... 8
III. Manuscript .......................................................................................................................... 11
IV. Appendices .......................................................................................................................... 30
   a. Appendix A ......................................................................................................................... 30
   b. Appendix B ......................................................................................................................... 34
   c. Appendix C ......................................................................................................................... 38
V. References ............................................................................................................................ 44
List of Tables

Table 1: Subject Demographics......................................................................................21
Table 2: Smoking Habits ...................................................................................................22
List of Figures

Figure 1: Pre and Post Exercise Values of Factor VIII .........................................................23
Abstract

Background: There is a strong association between factor VIII (FVIII) and presence of cardiovascular disease (CVD). Exercise is a known trigger for myocardial infarction, and the majority of ischemic events from physical stress occur due to an occlusive thrombus. FVIII increases acutely with exercise and remains elevated hours after exercise. Furthermore, FVIII is also higher in cigarette smokers, however, it has not been investigated in e-cigarette smokers. The purpose of this study is to identify potential differences in FVIII before exercise, after exercise, and its magnitude of change in e-cigarette users versus non-users.

Methods: Eighteen individuals (8 e-cigarette users, 3 women and 5 men, and 10 non-smokers, 7 women and 3 men; age, 18-32 years old) were recruited to participate in this research study. An incremental test to exhaustion ($\bar{v}$=12.20 ± 3.20 minutes) was performed on an electronically braked cycle ergometer (Lode Corival cpet; Groningen, Netherlands) to determine the peak rate of oxygen consumption ($VO_{2peak}$). Ten mL of blood was drawn into a citrate anticoagulant solution from an antecubital vein using clean venipuncture with minimal stasis both prior to and immediately following exercise testing. A commercially available ELISA kit was used to determine plasma concentrations of coagulation FVIII antigen.

Results: There was no significant difference between smokers ($N = 8, 4.35 \pm 2.09$ IU/ml) and nonsmokers ($N = 9, 5.02 \pm 4.87$ IU/ml, $p=0.72$) for resting FVIII antigen. There was a main effect for exercise in that the post-exercise values ($N=10, 7.76 \pm 1.40$ IU/ml) were larger than pre-exercise values ($N=10, 3.82 \pm 0.65$, $p=0.003$). However, there was no main effect for smoking and no smoking x exercise interaction (post-exercise smokers, $N=4, 6.67 \pm 1.46$, post-exercise non-smokers, $N= 7, 8.39 \pm 5.81$, $p=0.58$).
Conclusion: Our preliminary data suggests that e-cig use does not significantly affect FVIII or the FVIII response to exercise.
Chapter I

Introduction

Clinical Importance of Hemostasis

Cardiovascular disease (CVD) is the leading cause of death in the United States, manifesting itself through ischemic events like myocardial infarction and stroke (13). A majority of these events are caused by plaque ruptures that form a clot inside either the plaque or the lumen of a blood vessel, leading to blockage of blood flow in the artery. There is autopsy evidence of atherosclerotic plaques in people without any history of myocardial infarction (16,44), suggesting plaque rupture alone is not sufficient to cause an ischemic event and must occur in combination with prothrombotic conditions. Therefore, blood coagulation and clot dissolution (or fibrinolysis) are clinically relevant.

There is a strong association between coagulation and presence of CVD. In particular, fibrinogen is associated with CVD and related ischemic events. As a substrate converted to fibrin clot, fibrinogen promotes coagulation by enhancing platelet aggregation (38) and is linked with risk for CVD (20,46) coronary artery disease (CAD) (58), myocardial infarction (58,1), stroke (1), and mortality (1) in patients with CVD. Other important markers for blood coagulation include factors VII (FVII), and VIII (FVIII), von Willebrand Factor (vWF), and thrombin-antithrombin complex (TAT), a marker for thrombin formation. Like fibrinogen, all of these markers of coagulation potential are also associated with the presence of CVD (46), CAD (51), myocardial infarction (29), stroke (12), and mortality (12) in patients with CVD. Increased coagulation potential may also increase the progression of atherosclerosis, with higher rates of hypercoagulation in patients with peripheral artery disease (PAD)
(50). However, coagulation potential is not the only factor impacting risk of ischemic events.

There is also a strong connection between decreased fibrinolysis and the presence of CVD. The main enzyme involved in fibrinolysis is tissue plasminogen activator (tPA), which catalyzes the conversion of plasminogen to plasmin, resulting in fibrinogen dissolving into fibrin dimer proteins. The most prominent inhibitor of tPA is plasminogen activator inhibitor-1 (PAI-1), which binds to tPA and inhibits its function. Increases in PAI-1 and decreases in tPA activity are both associated with CVD (37), CAD (47), ischemic events (58), stroke (34), and mortality (30). The degree of impairment in the fibrinolytic potential also impacts the progression of PAD, with significantly lower tPA activity in patients with severe claudication compared to those with mild claudication (37). Furthermore, impaired fibrinolysis is independently associated with risk factors for CVD, like body composition (36), hyperlipidemia (29,31) diabetes mellitus, body mass index, low-density lipoprotein (36).

**Coagulation Responses to Exercise**

Exercise is a known trigger for myocardial infarction in roughly one-third of patients who can identify a trigger (59). Furthermore, ischemic events resulting from physical stress occur due to an occlusive clot more frequently than events unrelated to physical stress (22). Thus, coagulation and fibrinolytic responses to exercise have important clinical implications.

Activated partial thromboplastin (APTT), a measure of the intrinsic and common pathway of coagulation, significantly decreases following exercise across sexes (7,25) and exercise modalities (52,60). However, this acute effect is not consistently seen on
prothrombin time (PT), with studies reporting both shortened (25) and unchanged (52) PTs after acute exercise. These inconclusive findings suggest that the extrinsic tissue factor pathway are not as involved in exercise-induced hemostatic responses (63).

FVIII activity (FVIII:c) and FVIII antigen (FVIII:ag) increase acutely with exercise and remain elevated hours after exercise (4,61), with the magnitude of both responses being dependent on exercise intensity (41). Other markers, including TAT, prothrombin fragments 1 and 2 and fibrinopeptide A are increased following 1-hour of high intensity exercise but not following a moderate-intensity exercise of the same duration (64). There are no acute effects of exercise on FVII, with no observed changes in FVII:c following maximal exercise (60,61), no change in FVII:ag, FVII:ag/FVII:c ratio, and plasma concentrations of circulating activated FVII following exhaustive exercise (63).

Although the specific mechanisms are uncertain, increased beta-adrenergic activity influences the coagulation response to exercise, as B-adrenergic blockade inhibits the exercise-induced increases in FVIII (14). Furthermore, platelet activation increases following an infusion of epinephrine or norepinephrine (26,39,40). Another mechanism influencing the coagulation response to exercise is endogenous NO production. When partially blocking NO production, the exercise induced response in the vWF and FVIII is inhibited (32). Despite markers for coagulation increasing following acute exercise, results for fibrinogen are inconclusive with evidence showing both increases (4), decreases (6), and no change (23) in fibrinogen levels following acute exercise.
**Fibrinolytic Responses to Exercise**

Overall, fibrinolysis increases as a result of acute exercise due to increases in tPA activity paired with reductions in PAI-1 activity. Mechanistically, these fibrinolytic responses during exercise are due to an increased release of tPA from vascular endothelial cells (41). This mechanism occurs through hypoglycemia as well as increases in 1-deamino-8-D-arginine-vasopressin, epinephrine, and thrombin; all of which occur during acute exercise (49). This increase in tPA may be large enough to effectively reduce PAI-1 activity as PAI-1 forms an inactive, irreversible bond with tPA.

These fibrinolytic responses are magnified with increases in intensity. Streiff and Bell (57) reported significant increases in fibrinolysis do not usually occur until individuals reach 50% of their max heart rate (HR). With there being a proposed threshold intensity of 80-90% max HR for exponential changes in fibrinolytic activity to occur (17). The threshold-like exercise response pattern for epinephrine could explain these findings in fibrinolytic activity (42). However, Womack et al. (65) reported fibrinolytic responses below lactate threshold, with intensities above lactate threshold eliciting greater fibrinolytic responses. Duration can influence the fibrinolytic responses, but to a lesser degree than does intensity, as Womack et al. observed higher intensity exercise at a duration of 20 minutes leads to greater fibrinolytic responses than does moderate intensity, equicaloric work at a moderate intensity.

**The Effects of Smoking/Nicotine on Hemostasis**

Both direct and second-hand exposure to cigarette smoking decreases Nitric Oxide (NO) availability by altering the expression and activity of the endothelial NO synthase enzyme (3). Mechanistically, cigarette smoke contains high amounts of free
radicals that lead to the reduced bioavailability of NO. NO plays both a direct and indirect role in thrombosis by inhibiting platelet adhesion and aggregation and increasing platelet recruitment (28,53). Impaired NO bioavailability results in secretion of thromboxane A2 from the platelets, thus enhancing platelet aggregation (33). Exposure to cigarette smoke increases vWF and fibrinogen, contributing to this process and enhancing thrombosis.

Higher plasma levels of fibrinogen and viscosity are the main contributors to higher coagulability found in smokers (43). Lifetime duration of smoking is a determinant of baseline fibrinogen levels; however, these levels quickly drop following smoking cessation. Cigarette smokers have significantly higher plasma fibrinogen than non-smokers (45,62,35), while pipe-cigar smokers show no significant difference from non-smokers (62). When these individuals were split into lighter (1-19 cigarettes/day) and heavier (20+ cigarettes/day) categories, heavier smokers had higher levels of fibrinogen (62). This increase is not unique to cigarette smoking, however, as hookah smokers have higher plasma fibrinogen in comparison to both non-smokers and cigarette smokers (54).

Fibrinolysis is also impaired by cigarette usage, as tPA production and release being significantly lower in smokers compared to nonsmokers despite no significant difference in PAI-1 activity (8). Pretorius et al. observed smokers exhibited a reduced tPA release compared to non-smokers when stimulated with bradykinin, a stimulant for tPA release (48).

There is no evidence that nicotine, the most integral ingredient found in cigarettes, has any effect on hemostasis. Oral and transdermal nicotine studies have found no
significant chronic influence on hemostatic markers in healthy adults (10). This would suggest the main cause for chronic hematological changes from smoking are caused by other ingredients embedded in the cigarette.

There is evidence from both epidemiological and experimental studies that particulate matter (PM), such as black carbon, nitrogen dioxide, and carbon monoxide, results in hypercoagulability and hypofibrinolysis. Plasma fibrinogen has been one of the most studied variables in both prospective cohort and experimental studies, with evidence suggesting increased fibrinogen levels from PM and ambient particles in concentrated doses (9, 27, 55). There is, however, no clear pattern for other pro-coagulant variables such as prothrombin time (PT), PAI-1, and tPA, which all have equivocal results (5, 11, 18, 19).

Very few studies have analyzed associations between individual PM constituents and changes in biomarkers of hemostasis. However, particle mass influences the hemostatic response, with no statistically significant changes from pre-exposure for prothrombin, fibrinogen, FVII, FVIX, vWF, PAI-1, plasminogen, tPA, or fibrin d-dimer at 1 hour or at 20 hours after exposure to coarse air particles and a statistically significant increase in all of those variables after exposure to fine air particles (56). These fine particles are transmitted in concentrated doses while smoking cigarettes. However, the main ingredients in Juuls, propylene glycol and glycerine, have not been examined in humans for their hematologic effects.

**Purpose**

The purpose of this study is to identify potential differences in hemostatic variables both before and after exercise in e-cigarette users versus non-users.
Hypothesis

Resting differences of hemostasis will persist in e-cigarette users immediately following acute, maximal exercise.
Chapter II

Methodology

Subjects

Forty individuals will be recruited to participate in this research study (20 e-cigarette users and 20 non-smokers). Subjects will be college-aged (18-35 years of age), healthy, and never diagnosed with pulmonary, metabolic, cardiovascular or renal disease. The subjects will be matched for physical activity (pa) level between experimental (e-cigarette users) and the control group (non-smokers) using the long version of the International Physical Activity Questionnaire (IPAQ). E-cigarette users will be defined as actively smoking e-cigarettes at least 4 days a week. Non-smokers will be defined as not having smoked any form of tobacco or marijuana over the past 6 months. Participants will be recruited through on campus flyers, a bulk e-mail request to all James Madison University faculty and students, and from surrounding areas in the Harrisonburg community. Subjects will be excluded if they have any signs or symptoms of cardiovascular or metabolic disease, or are asymptomatic but have previously been diagnosed with disease. Subjects will also be excluded if they are on any medication that could alter their hemostatic variables. Transdermal nicotine or chewing tobacco use will be permitted, but the Tobacco Use Questionnaire will be used to screen for and exclude combustible tobacco and marijuana usage and baseline testing will be performed to confirm subjects’ exhaled carbon monoxide (eCO) < 4 parts per million (ppm). All methods received IRB approval and informed consent will be obtained from all subjects prior to testing.
Study Design

All testing will be done before 10:00 am, to control for diurnal influences on the variables under examination (2). Subjects will abstain from caffeine and alcohol for 24 hours before each visit, and have no food or drink other than water for 12 hours before their visit. In addition, subjects will be required to refrain from smoking for 3 hours and exercise for 24 h before their laboratory visit. Lastly, subjects will be excluded from participation if they have any flu or fever symptoms within one week of their lab sessions.

Blood Sampling Assays

Subjects will assume a semi-recumbent position prior to and following exercise testing, 10 mL of blood will be drawn into a citrate anticoagulant solution from an antecubital vein using clean venipuncture with minimal stasis. Blood samples will be immediately centrifuged for 20 min at 1500g and 4°C to obtain platelet-poor plasma. Plasma aliquots will be frozen and stored at -80 degrees C until assayed. Commercially available ELISA kits will be used to determine plasma concentrations of coagulation FVIII antigen (VisuLize; Ontario, Canada), fibrinogen (Eagle Bioscience, Inc; Nashua, NH), active tPA (Eagle Bioscience, Inc; Nashua, NH), tPA antigen (Eagle Bioscience, Inc), and active PAI-1(Eagle Bioscience, Inc).

Incremental Exercise Test

An incremental test to exhaustion (12-15 minutes) will be performed on an electronically braked cycle ergometer (Lode Corival cpet; Groningen, Netherlands) to determine the peak rate of oxygen consumption (VO$_{2\text{peak}}$). Resting metabolic measurements will be taken for 3 minutes prior to starting the test. Participants will then begin to warm-
up for approximately 2 minutes at a work rate of 60 watts, always keeping the cadence above 50 revolutions per minute (rpm). Work rate will increase by 10 watts per minute for females and 20 watts per minute for males. Ventilatory and metabolic data will be recorded via a Vmax metabolic measuring system (Carefusion; San Diego, CA). Heart rate will be recorded during the test with a PolarLink heart rate monitor and chest strap. The test will be terminated when the subject reaches volitional exhaustion or when they can no longer maintain 50 rpm. Blood samples will be obtained again within two minutes of exercise cessation (15).

Accelerometer

Individuals will be asked to wear an Actigraph GT3X accelerometer for seven days to objectively measure their moderate-to-vigorous physical activity (MVPA). The participants will wear the accelerometers at all times, except while sleeping and showering. Freedson cut points will be used to quantify the amount of physical activity participants obtain per week (21). Participants will also be asked to keep a log of non-wear time and report when they put the accelerometer on in the morning and when they take the accelerometer off at night. Subjects must wear the accelerometer for a minimum of 13 hours a day for the data to count (24).

Statistical Analysis

FVIII antigen, fibrinogen, active tPA, tPA antigen, and active PAI-1 will be analyzed using repeated measures analysis of variance with exercise as the within-subjects factor and smoking status as the between-subjects factor. Post hoc comparisons will be made using Fisher’s LSD test. A priori statistical significance will be set at $P < 0.05$. 
Chapter III

Manuscript

The Impact of Acute Exercise on Factor VIII in Young E-cigarette Smokers

Authors: Andrew R. Allen, Stephanie P. Kurti, Trent A. Hargens, Christopher J. Womack

Institution: James Madison University, Harrisonburg, Virginia 22807

Contacts: Andrew R. Allen, allenar@dukes.jmu.edu
          Christopher J. Womack, womackcx@jmu.edu
          Stephanie P. Kurti, kurtisp@jmu.edu
          Trent A. Hargens, hargenta@jmu.edu

Address of Correspondence
Christopher J. Womack, Ph.D.
Department of Kinesiology
James Madison University
Harrisonburg VA, 22807
Phone: (540) 568-6145
Email: womackcx@jmu.edu
Abstract

Background: There is a strong association between factor VIII (FVIII) and presence of cardiovascular disease (CVD). Exercise is a known trigger for myocardial infarction, and the majority of ischemic events from physical stress occur due to an occlusive thrombus. FVIII increases acutely with exercise and remains elevated hours after exercise. Furthermore, FVIII is also higher in cigarette smokers, however, it has not been investigated in e-cigarette smokers. The purpose of this study is to identify potential differences in FVIII before exercise, after exercise, and its magnitude of change in e-cigarette users versus non-users.

Methods: Eighteen individuals (8 e-cigarette users, 3 women and 5 men, and 10 non-smokers, 7 women and 3 men; age, 18-32 years old) were recruited to participate in this research study. An incremental test to exhaustion (̄x=12.20 ± 3.20 minutes) was performed on an electronically braked cycle ergometer (Lode Corival cpet; Groningen, Netherlands) to determine the peak rate of oxygen consumption (VO2peak). Ten mL of blood was drawn into a citrate anticoagulant solution from an antecubital vein using clean venipuncture with minimal stasis both prior to and immediately following exercise testing. A commercially available ELISA kit was used to determine plasma concentrations of coagulation FVIII antigen.

Results: There was no significant difference between smokers (N = 8, 4.35 ± 2.09 IU/ml) and nonsmokers (N = 9, 5.02 ± 4.87 IU/ml, p=0.72) for resting FVIII antigen. There was a main effect for exercise in that the post-exercise values (N=10, 7.76 ±1.40 IU/ml) were larger than pre-exercise values (N=10, 3.82 ± 0.65, p=0.003). However, there was no main effect for smoking and no smoking x exercise interaction (post-exercise smokers, N=4, 6.67 ± 1.46, post-exercise non-smokers, N= 7, 8.39 ± 5.81, p=0.58).
**Conclusion**: Our preliminary data suggests that e-cig use does not significantly affect FVIII or the FVIII response to exercise.
Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States, manifesting itself through ischemic events such as myocardial infarction and stroke (9). There is a strong association between coagulation and presence of CVD. In particular, fibrinogen and factor VIII (FVIII), promote coagulation and are linked with risk for CVD (12,24), coronary artery disease (CAD) (26,29), myocardial infarction (1,16,29), stroke (1,8), and mortality (1,8) in patients with CVD.

Exercise is a known trigger for myocardial infarction (30), and the majority of ischemic events from physical stress occur due to an occlusive thrombus (13). FVIII increases acutely with exercise and remains elevated hours after exercise (3,31). FVIII is also elevated in cigarette smokers (17), with CVD risk increasing as fibrinogen levels increase (18). This may be due to increased inflammation from inhaling particulate matter (PM) as inhalation of wood smoke results in acute increases in FVIII (5) and the inflammatory cytokine IL-6 has been linked to FVIII (33). In contrast, oral and transdermal nicotine use does not chronically influence hemostatic markers in healthy adults (7). This suggests the main cause for chronic hematological changes from smoking are caused by other PM embedded in the cigarette, such as black carbon, nitrogen dioxide, and carbon monoxide which are all transmitted in concentrated doses in cigarettes. The evidence also suggests increased fibrinogen levels from PM and ambient particles in concentrated doses (6,15,28).

Recently, e-cigarette smoking has surged in young adults; increasing by 78% among high school students from 2017 to 2018 (11). However, the main ingredients in Juuls®, propylene glycol and glycerine, have not been examined in humans for their hematologic effects. Furthermore, the hematologic response before and after exercise in
e-cigarette smokers has not been clearly identified. The purpose of this study is to identify potential differences in FVIII both before and after exercise in e-cigarette users versus non-users.

**Methodology**

**Subjects**

Eighteen individuals were recruited to participate in this research study (8 e-cigarette users and 10 non-smokers, see Table 1 for demographic characteristics). Subjects were free from known pulmonary, metabolic, cardiovascular or renal disease. E-cigarette users were defined as actively smoking e-cigarettes at least 4 days a week. Non-smokers were defined as not having smoked any form of tobacco or marijuana over the past 6 months. Participants were recruited through on campus flyers, a bulk e-mail request to all James Madison University faculty and students, and from the surrounding Harrisonburg community. Subjects were also excluded if they were on any medication that could alter hemostatic variables (such as aspirin or anti-coagulants). Transdermal nicotine or chewing tobacco use was permitted in the smoking group, but the Tobacco Use Questionnaire was used to screen for and exclude combustible tobacco and marijuana usage and baseline testing was performed to confirm subjects’ exhaled carbon monoxide (eCO) < 4 parts per million (ppm). All methods received IRB approval and informed consent was obtained from all subjects prior to testing.

**Study Design**

All testing was done before 10:00 am, to control for diurnal influences on hemostasis (2). Subjects abstained from caffeine and alcohol for 24 hours before each visit and had no food or drink other than water for 12 hours before their visit. In addition, subjects were required to refrain from smoking for 3 hours and exercise for 24 hours...
before their laboratory visit. Lastly, subjects were excluded from participation if they had any flu or fever symptoms within one week of their lab sessions.

**Blood Sampling Assays**

Subjects assumed a semi-recumbent position prior to and following exercise testing. Ten mL of blood was drawn into a citrate anticoagulant solution from an antecubital vein using clean venipuncture with minimal stasis. Blood samples were immediately centrifuged for 20 min at 1500g and 4°C to obtain platelet-poor plasma. Plasma aliquots were frozen and stored at -80 degrees C until assayed. A commercially available ELISA kit was used to determine plasma concentrations of coagulation FVIII antigen (VisuLize; Ontario, Canada). For the FVIII antigen assay, several samples were higher than the highest standard used in the ELISA plate. Despite the manufacturer’s recommendations, we calculated FVIII for these samples from the standard curve that was generated. We successfully obtained pre-exercise blood samples for 8 smokers and 9 nonsmokers and post exercise blood samples for 4 smokers and 7 nonsmokers.

**Incremental Exercise Test**

An incremental test to exhaustion (12-15 minutes) was performed on an electronically braked cycle ergometer (Lode Corival cpet; Groningen, Netherlands) to determine the peak rate of oxygen consumption (VO$_{2peak}$). Resting metabolic measurements were taken for three minutes prior to starting the test. Participants then began to warm-up for approximately two minutes at a work rate of 60 watts, always keeping the cadence above 50 revolutions per minute (rpm). Work rate was increased by 10 watts per minute for females and 20 watts per minute for males. Ventilatory and metabolic data was recorded via a Vmax metabolic measuring system (Carefusion; San Diego, CA). Heart rate was recorded during the test with a PolarLink heart rate monitor.
and chest strap. The test was terminated when subjects reached volitional exhaustion or when they could no longer maintain 50 rpm. Blood samples were obtained again within two minutes of exercise cessation (10).

**Statistical Analysis**

We performed an independent t-test to compare resting FVIII antigen for the two groups. For the exercise response, we performed a repeated measures analysis of variance with exercise (pre, post) as the within-subjects factor and smoking status (smoker, non-smoker) as the between-subjects factor. A priori statistical significance was set at P < 0.05.

**Results**

For the baseline comparison of FVIII antigen, there was no significant difference between smokers (N = 8, 4.35 ± 2.09 IU/ml) and nonsmokers (N = 9, 5.02 ± 4.87 IU/ml, p=0.72). There was a main effect for exercise in that the post-exercise values (N=10, 7.76 ±1.40 IU/ml) were larger than pre-exercise values (N=10, 3.82 ± 0.65, p=0.003). However, there was no main effect for smoking and no smoking x exercise interaction (Figure 1). Average smoking habits are reported in Table 2.

**Discussion**

These data suggest that e-cigarette smoking does not affect resting or post-exercise FVIII antigen. Although there is no comparable resting data on the coagulation response in e-cigarettes smokers, our data does contrast what has been found in cigarette smokers at rest, who exhibit higher levels of FVIII (144.63 IU/dL) and significantly higher levels of plasma fibrinogen (249.4 ± 1.93 mg/dL) compared to non-smokers (141.72 IU/dL, p=0.173, 244.4 ± 1.12 mg/dL, p<0.05) (17,32,19). These findings may be influenced by smoking volume, however, as hypercoagulability, measured by platelet
aggregation, is significantly higher in healthy adults who have smoked greater than 15 pack years, compared to those who have smoked 5-15 pack years (27). Furthermore, those who smoke 20+ cigarettes a day, as compared to those who smoke 1-19 cigarettes a day, have significantly higher fibrinogen levels (32). Our e-cigarette smokers had only been smoking 21.49 ± 15 months for an average of 22.38 ± 10.95 days a month (Table 2). Thus, the volume and/or duration of smoking may have not been sufficient to substantially impact FVIII.

There is no conclusive evidence in the literature that smoking of any kind has an impact on blood coagulation following exercise. In a male monozygotic twins study with one twin smoking cigarettes for 20 pack years and the other a nonsmoker, fibrinogen tended to be increased among cigarette smokers following exercise but FVIII was not significantly different between the two groups (20). This FVIII finding is consistent with our results, but does not lead to a clear conclusion on the impact e-cigarettes or smoking in general has on blood coagulation, due to our small sample size. We did, however, observe a main exercise effect on blood coagulation, as FVIII was elevated following exercise in both groups. This is consistent with the literature, as FVIII activity and FVIII antigen increase to a similar degree from acute exercise (3,31).

The known health risks of traditional cigarettes have been well documented, while the risks of e-cigarettes still remain to be less understood. This has potentially contributed to increased e-cigarette usage over the past decade. Although e-cigarettes have been marketed as being a safer alternative to conventional cigarettes, e-cigarettes still release nicotine and concentrated substances that increase the risk of a cardiovascular event (21). Our study provides initial findings for a lack of hemostatic changes with e-cigarette usage. Further evaluation of the impacts e-cigarettes have on hemostasis and other
markers of cardiovascular health still need to be performed, however, as our study only measured FVIII antigen. Other hemostatic markers, like fibrinogen, have been seen to be positively associated with smoking status (4). Furthermore, when examining the effects of e-vapor on hemostasis, there is an e-vapor induced increase in platelet activation that was independent of nicotine (14). This suggests that fine particulate matter from e-cigarettes can lead to increased coagulability. E-cigarettes also lead to the largest levels of high sensitive c-reactive protein when compared to both nonsmokers and cigarette smokers (22), indicating an increased level of oxidative stress and inflammation similarly found in smokers (33) which can further increase risk for CVD. The correlation between this increase in inflammation found in e-cigarette users and any changes in hemostatic markers has yet to be examined. However, the nicotine (23), aerosol (25), and particulate matter (25) in e-cigarettes have all been found to influence individual risks of CVD.

Our study did have some major limitations that could have impacted our findings. Definitive conclusions cannot be made because of our low sample size and the inappropriate analysis of FVIII in samples that exceed the highest standard ELISA kit. This makes our findings preliminary, however, they can potentially be used for sample size calculations for future studies evaluating the effect size of e-cigarette use on hemostasis. Further limitations include the non-standardization of both smoking duration and smoking vessel, making it difficult to draw specific conclusions from our participants. However, the diversity in our e-cigarette smoking pool is more representative of the smoking population as a whole who typically smoke various brands and have varying durations of active smoking. Finally, we did not investigate the different in e-cigarette compositions that can potentially alter hemostasis. To better understand the potential mechanisms e-cigarettes have on CVD risk and to further
identify differences between e-cigarettes and conventional cigarettes, future studies should isolate the compounds and report their effects.

In conclusion, our preliminary data suggests that e-cig use does not significantly affect FVIII or the FVIII response to exercise.
<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>NS</th>
<th>S</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (n=3)</td>
<td>Women (n=7)</td>
<td>Men (n=5)</td>
<td>Men (n=3)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>20.7 ± 0.6*</td>
<td>18.6 ± 0.8</td>
<td>20.8 ± 3.5</td>
<td>22.7 ± 8.1</td>
</tr>
<tr>
<td>Height (in)</td>
<td>65.0 ± 3.0</td>
<td>65.6 ± 1.7</td>
<td>68.4 ± 2.5</td>
<td>70.7 ± 2.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.5 ± 4.2</td>
<td>69.1 ± 14.1</td>
<td>67.4 ± 11.8</td>
<td>74.6 ± 9.8</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.5 ± 3.5</td>
<td>24.7 ± 4.6</td>
<td>22.4 ± 5.0</td>
<td>23.0 ± 1.7</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>76.3 ± 3.3</td>
<td>79.5 ± 11.8</td>
<td>79.3 ± 10.9</td>
<td>79.7 ± 2.8</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>27.8 ± 8.0</td>
<td>32.9 ± 6.6</td>
<td>19.9 ± 9.9</td>
<td>16.4 ± 6.5</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>43.7 ± 1.8</td>
<td>42.9 ± 6.5</td>
<td>50.6 ± 7.2</td>
<td>60.2 ± 11.4</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>17.2 ± 6.2</td>
<td>22.5 ± 8.5</td>
<td>14.6 ± 7.1</td>
<td>11.6 ± 4.1</td>
</tr>
</tbody>
</table>

*p=0.003 when compared to Non-Smoking Women
<table>
<thead>
<tr>
<th>Smoking Habit</th>
<th>S (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months Smoking (Lifetime)</td>
<td>21.49 ± 15</td>
</tr>
<tr>
<td>Days Smoking (Past 30 days)</td>
<td>22.38 ± 10.95</td>
</tr>
<tr>
<td>Smoking Sessions Per Day</td>
<td>16.75 ± 12.10</td>
</tr>
</tbody>
</table>
Figure 1. Pre and Post-Exercise Values of Factor VIII (FVIII) Antigen in Smokers and Nonsmokers
Manuscript References


Figure Legend

Table 1. Subject Demographics between Women and Men in Smokers and Non-smokers

*-Significantly different than non-smoking women, p=0.003

Table 2. Average Smoking Habits of E-Cigarette Smokers

Figure 1. Pre and Post-Exercise Values of Factor VIII (FVIII) Antigen in Smokers and Nonsmokers
Chapter IV
Appendices
Appendix A

Informed Consent

Project Title: The impact of varying physical activity levels on airway inflammation, airway hyperresponsiveness and hemostasis in e-cigarette smokers

Consent to Participate in Research

Identification of Investigators and Purpose of Study

You are being asked to participate in a research study conducted by Drs. Stephanie Kurti and Christopher Womack from James Madison University. To participate, you must be an electronic cigarette user or nonsmoker with no previous history of cardiovascular, renal, and metabolic disease. The purpose of this study is to determine if varying physical activity levels in e-cigarette users impacts airway inflammation, airway hyperresponsiveness, and hemostasis. Previous research has shown that e-cigarette use results in lower lung function compared to nonsmokers, but the degree of lung function is much lesser compared to exclusive conventional tobacco cigarette users. Moreover, exclusive conventional tobacco cigarette use increases blood coagulation and decreases fibrinolysis compared to nonsmokers, but no investigation has looked at e-cigarette use. Therefore our aim is to determine whether e-cigarette use is causing damage to the airways before pulmonary function decline is observed as well the impact it has on hemostatic variables at varying physical activity levels. This study will contribute to the knowledge of both practitioners and clinicians, and may provide an important public health message in providing recommendations for physical activity levels that would have a protective effect in those who smoke e-cigarettes and/or conventional tobacco cigarettes chronically. Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Research Procedures

This study consists of questionnaires, a body composition scan, tracking physical activity, one VO\textsubscript{2} peak tests, pulmonary function tests, exhaled gas measurements, and hypertonic saline challenges which will be administered to individual participants in the Human Performance Laboratory at James Madison University.

The specific procedures during each visit are included here:
Initial visit: On the first visit to the laboratory, a researcher will go over informed consent with you as well as begin the process of completing all questionnaires including the international physical activity questionnaire, and the Physical Activity Readiness questionnaire-Plus, and tobacco use questionnaire.

After completion of the questionnaires, we will obtain your height, weight, waist circumference, and DEXA scan measurements to assess your body composition. You will then perform an incremental exercise test to exhaustion on a cycle ergometer.
Ventilatory, metabolic and heart rate data will be recorded for the entirety of the test until you reach volitional fatigue at which the test will be terminated. Before and after the exercise testing session, you will perform standard pulmonary function tests 2 minutes and 10 minutes after the bout of exercise. Upon completion of the exercise session, you will be given an accelerometer to wear for one week, which you will return on your second visit.

**Incremental exercise test to exhaustion:** You will ride on an electronically braked cycle ergometer approximately 12-15 minutes to determine \( \text{VO}_{2\text{peak}} \). Your resting metabolic measurements will be taken for 3 minutes prior to starting the test. You will begin the test with a 2 minute warm-up, pedalling at 50 revolutions per minute at work rate of 50 watts. Work rate will increase each minute by 10 watts for females and 20 watts for males. Your ventilatory and metabolic data will be recorded through breath by breath analysis. Your heart rate will be recorded during and throughout the test with a heart rate monitor and chest strap. A pulse oximeter will be secured to your left earlobe to estimate arterial oxygen saturation. After each stage of the test, you will be asked to report your RPE (rating of perceived exertion) as well as how difficult breathing feels to you (sensation of dyspnea). The \( \text{VO}_{2\text{peak}} \) test will be terminated when you can no longer continue exercise or maintain 50 revolutions/minute pedal frequency and have reached volitional fatigue. You may end the test whenever you want if you feel uncomfortable. Initial blood samples will be repeated at the end of the exercise test, within two minutes of ending the exercise.

**Pulmonary function tests:** You will perform a maximum flow-volume loop where you maximally inhale, forcefully exhale for 6 seconds and then maximally inhale into a spirometer while wearing a nose-clip. This will record forced expiratory volume in one-second, forced vital capacity, forced expiratory flow between 25 and 75% of forced vital capacity, and peak expiratory flow. Then using impulse oscillometry, you will breathe normally on a mouthpiece while the machine records resistance in your airway. Finally you will be asked to take a brief, maximal inhale at the end of a normal breath for every stage of the test. This is an experimental test to determine whether you are experiencing expiratory flow limitation during exercise. It is a simple breathing maneuver that poses no risk to you.

**Accelerometry:** You will be asked to wear an accelerometer for 7 days around your waist to track your physical activity. A researcher will guide you on how to appropriately place the accelerometer on.

**Second and third visit:** You will come into the laboratory in the morning after an overnight fast at which your blood pressure will be taken and 10 mL of your blood will be collected via a serial venipuncture in the antecubital vein. Then you will exhale into a nitric oxide analyzer for 6 seconds two times to measure your exhaled nitric oxide as a measure of airway inflammation. You will then perform standard pulmonary function testing via the maximum flow volume loop. Following this, you will perform both the hypertonic saline challenge and hypertonic saline challenge with deep inspirations each on different days for 20 minutes, and then perform pulmonary function testing immediately after.

**Hypertonic saline challenge session:** You will breathe in 25% hypertonic saline (salt and water) from an over-the-counter ultrasonic nebulizer that converts the salt solution from a liquid to a mist. You will breathe normally on the nebulizer for 20 minutes while seated comfortably and wearing a nose clip.
**Hypertonic saline challenge with deep inspiration session:** You will complete the exact same protocol as the hypertonic saline challenge session plus perform 5 deep inspirations immediately after the hypertonic saline challenge and prior to the pulmonary function tests.

**Risks**
Participation in this study does have some small risks. The risk of any serious event during this study is minimal. Possible risks include:

**Hypertonic saline challenge:** You may experience irritation, coughing, wheezing, and/or bronchoconstriction (may be like asthma-like symptom in severe cases) after inhaling this saline solution.

**Venipuncture:** You may experience mild bruising, risk of transfer of blood borne pathogens, infection, and/or skin irritation when blood is taken intravenously.

**DEXA:** The DEXA scan entails a low dose of radiation equivalent to approximately one transatlantic flight (0.015 mSv= millisievert). While there is no validated questionnaire to define extensive exposure, radiation exposure is cumulative over the lifespan (200 DEXA scans is equal to the cumulative exposure of living at sea level for a year (3 mSv). DEXA scans carry minimal X-ray exposure. To minimize exposure, the DEXA scan will only be performed once. All body composition assessment will be performed according to the American College of Sports Medicine guidelines for body composition assessment.

**Benefits**
By participating in this study, you will learn about your current pulmonary health and function (airway inflammation, airway hyperresponsive, forced expiratory flow in one-second, forced vital capacity, forced expiratory flow at 25-75% of vital capacity), VO\textsubscript{2}peak, and body composition. Your data will be provided to you upon completion of your participation in the study. If an emergency arises and you must dropout, you may still receive your data.

Society will benefit from more knowledge about how e-cigarette usage may influence adverse vascular and pulmonary outcomes, as well as how lifestyle may influence these outcomes.

**Confidentiality**
The results of this project will be coded in such a way that the respondent’s identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the JMU researchers. Upon completion of the study, all information that matches up individual respondents with their answers will be destroyed.

**Participation & Withdrawal**
Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.
Questions about the study
If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Researcher’s Name: Dr. Stephanie Kurti
Email Address: kurtisp@jmu.edu
Department of Kinesiology
James Madison University
Cell-Phone number: 630-205-6363
Telephone: 540-568-3947

Researcher’s Name: Dr. Christopher Womack
Email Address: womackcx@jmu.edu
Department of Kinesiology
James Madison University
Telephone: 540-658-6145

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

____________________________________
Name of Participant (Printed)

____________________________________
Name of Participant (Signed)    Date

____________________________________
Name of Researcher (Signed)    Date
Appendix B

The Physical Activity Readiness Questionnaire

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Has your doctor ever said that you have a heart condition OR high blood pressure?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? PLEASE LIST CONDITION(S) HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) Are you currently taking prescribed medications for a chronic medical condition? PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active. PLEASE LIST CONDITION(S) HERE:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7) Has your doctor ever said that you should only do medically supervised physical activity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

☑ If you answered NO to all of the questions above, you are cleared for physical activity. Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

⚠️ If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.
2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?
   If the above condition(s) is/are present, answer questions 1a-1c
   (Answer NO if you are not currently taking medications or other treatments)
   1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
   YES □ NO □
   1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolisthesis/pars defect (a crack in the bony ring on the back of the spinal column)?
   YES □ NO □
   1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months?
   YES □ NO □

2. Do you currently have Cancer of any kind?
   If the above condition(s) is/are present, answer questions 2a-2b
   (Answer NO if you are not currently taking medications or other treatments)
   2a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?
   YES □ NO □
   2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?
   YES □ NO □

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm
   If the above condition(s) is/are present, answer questions 3a-3d
   (Answer NO if you are not currently taking medications or other treatments)
   3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
   YES □ NO □
   3b. Do you have an irregular heart beat that requires medical management?
   (e.g., atrial fibrillation, premature ventricular contraction)
   YES □ NO □
   3c. Do you have chronic heart failure?
   YES □ NO □
   3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?
   YES □ NO □

4. Do you have High Blood Pressure?
   If the above condition(s) is/are present, answer questions 4a-4b
   (Answer NO if you are not currently taking medications or other treatments)
   4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
   YES □ NO □
   4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication?
   (Answer YES if you do not know your resting blood pressure)
   YES □ NO □

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes
   If the above condition(s) is/are present, answer questions 5a-5e
   5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?
   YES □ NO □
   5b. Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.
   YES □ NO □
   5c. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, or the sensation in your toes and feet?
   YES □ NO □
   5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?
   YES □ NO □
   5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?
   YES □ NO □
6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer’s, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome
   If the above condition(s) is/are present, answer questions 6a-6b
   If NO go to question 7

   6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES □ NO □

   6b. Do you have Down Syndrome AND back problems affecting nerves or muscles?
       YES □ NO □

7. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure
   If the above condition(s) is/are present, answer questions 7a-7d
   If NO go to question 8

   7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES □ NO □

   7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?
       YES □ NO □

   7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?
       YES □ NO □

   7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?
       YES □ NO □

8. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia
   If the above condition(s) is/are present, answer questions 8a-8c
   If NO go to question 9

   8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES □ NO □

   8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?
       YES □ NO □

   8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?
       YES □ NO □

9. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event
   If the above condition(s) is/are present, answer questions 9a-9c
   If NO go to question 10

   9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
       (Answer NO if you are not currently taking medications or other treatments)
       YES □ NO □

   9b. Do you have any impairment in walking or mobility?
       YES □ NO □

   9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?
       YES □ NO □

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
    If you have other medical conditions, answer questions 10a-10c
    If NO read the Page 4 recommendations

   10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months or have you had a diagnosed concussion within the last 12 months?
       YES □ NO □

   10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?
       YES □ NO □

   10c. Do you currently live with two or more medical conditions?
       YES □ NO □

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE:

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.
2017 PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the ePARmed X+ at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME ___________________________ DATE ___________________________
SIGNATURE ___________________________ WITNESS ___________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER

For more information, please contact www.eparmedx.com

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Werburtion with Dr. Norman Gedhil, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Citation for PAR-Q+

Key References

Copyright © 2017 PAR-Q+ Collaboration 4 / 4 01-01-2017
Appendix C

Tobacco Use Questionnaire

VCTRS-Project 2, Study 2

Subject Identifier:  
Date:  
Interviewer Initials:  
Visit: 0 0

Completed by: 0 Participant  Interviewer

**Tobacco Use Questionnaire**

"Now I am going to ask you some questions about your tobacco use history."

The following questions are about your past cigarette use.

How old were you when you first started smoking cigarettes regularly?

_____ years old

1. Have you smoked at least 5 cigarettes per day for the past year?  
   - [ ] Yes  
   - [ ] No

2. Right now, how many days/week do you smoke cigarettes?
   _____ Days/week

3. On the days you smoke, on average how many cigarettes do you smoke?
   _____ Cigarettes

4. What is your usual brand of cigarette (the type you smoke most often)?
   
   INTERVIEWER NOTE: If subject rolls own cigarettes please list the tobacco brand on the line above.

   INTERVIEWER NOTE: Be specific about the type of cigarette! For example: Marlboro Reds regular length, American Spirit Yellows regular length, Newport Menthol 100s green box

5. Is your usual cigarette brand menthol or non-menthol?
   - [ ] Menthol  
   - [ ] Non-Menthol  
   - [ ] Both (ex. Camel Crush, Marlboro NXT)  
   - [ ] No Usual Type

   i. Would you prefer to be assigned to smoke a menthol or non-menthol cigarette for the duration of the study?  
      IMPORTANT: This information will be used by the VCTRS website for Randomization.

      - [ ] Menthol  
      - [ ] Non-Menthol

6. How long have you smoked this brand?
   _____ days
   _____ weeks
   _____ months
   _____ years

7. Did participant indicate rolling their own cigarettes on question 4?
   - [ ] No, skip to question 10  
   - [ ] Yes, answer questions 8-9

8. Do you also smoke machine-manufactured cigarettes?
   - [ ] No, skip to question 10  
   - [ ] Yes, what is your usual brand of machine manufactured cigarettes?
9. What portion of your cigarettes are roll your own?
   [ ] Few (less than 25%)
   [ ] Many (25-49%)
   [ ] About half (50%)
   [ ] Most (51-75%)
   [ ] Almost All (75-99%)

10. How many times have you tried to quit smoking completely?
   _______ Times

11. What is the longest amount of time you were ever able to quit?
    _______ days
    _______ weeks
    _______ months
    _______ years

12. How long ago was the last time you tried to quit?
    _______ days
    _______ weeks
    _______ months
    _______ years

13. When you tried to quit in the past, did you ever use a medication to help?
    [ ] No  [ ] Yes

14. When you tried to quit in the past, did you ever use counseling to help?
    [ ] No  [ ] Yes

15. Have you ever stopped smoking for an entire day?
    [ ] No  [ ] Yes
    a. The last time you stopped, did you notice any of the following symptoms?
If the participant answers **yes** to question 15, ask questions A-I.

<table>
<thead>
<tr>
<th>If the participant answers yes to question 15, ask questions A-I.</th>
<th>The last time you stopped, did you notice any of the following symptoms?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Anger/frustration</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>B. Anxiety</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>C. Depression</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>D. Difficulty Concentrating</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>E. Insomnia</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>F. Irritability</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>G. Restlessness</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>H. Increased appetite or weight gain</td>
<td>☐ No  ☐ Yes  ☐ Refused</td>
</tr>
<tr>
<td>Other</td>
<td>☐ No  ☐ Yes, please describe________________________________________</td>
</tr>
<tr>
<td></td>
<td>☐ Refused</td>
</tr>
</tbody>
</table>
16. If you had any of those 16 symptoms, were any of them severe enough to impair your ability to function?
   [] No  [] Yes

17. Do you plan to stop smoking cigarettes for good? (Circle only one)...
   1. I have no plans to stop smoking.
   2. I plan to stop smoking in the next 7 days
   3. I plan to stop smoking in the next 30 days
   4. I plan to stop smoking in the next 6 months
   5. I plan to stop smoking in the next year
   6. I plan to stop smoking more than 1 year from now
   7. I plan to stop smoking, but I am unsure when

18. Have you ever tried to cut down on the number of cigarettes you smoke per day that was not part of a quit attempt?
   [] No  [] Yes

19. If you did, how many cigarettes did you cut out per day?
   i. _______ Cigarettes

20. If you did, how long were you able to cut down?
   i. _______ days
   ii. _______ weeks
   iii. _______ months
   iv. _______ years

The following questions are about your past e-cigarette use.

21. Have you used an e-cigarette/vape, such as NJoy, Blu, or Smoking Everywhere, even one or two times?
   [] Yes  [] No

22. Do you now use e-cigarettes?
   [] Every day  [] Some days  [] Not at all
VCTRS-Project 2, Study 2

Subject Identifier: K- Date: / / Interviewer Initials: Visit: 0 0

Completed by: O Participant ● Interviewer

23. On how many of the past 30 days did you use an e-cigarette?

24. On the days that you vape, how many times do you use your e-cigarette?

25. On the days that you use e-cigarettes, how soon after you wake up do you typically take your first puff of the day?

26. How old were you when you first used an e-cigarette, even one or two times?

27. How old were you when you first started using e-cigarettes fairly regularly?

28. What is your usual brand of e-cigarette/vape? (The type you smoke most often)

29. What is your usual flavor of e-cigarette/vape? (the type you smoke most often)

30. About how long did you use / have you been using your regular brand of e-cigarette?

31. Have you completely quit using e-cigarettes?

☐ Yes ☐ No

32. Does the e-cig you usually use contain nicotine?

☐ Yes ☐ No
### VCTRS-Project 2, Study 2

**Subject Identifier:** K-

**Date:** / / 

**Interviewer Initials:**

**Visit:** 0 0

**Completed by:** 
- Participant
- Interviewer

---

**OTHER TOBACCO PRODUCTS – ALL RESPONDENTS**

*The next questions are about the use of tobacco products other than cigarettes.* For definitions of tobacco products see "Definitions of Tobacco Products’ sheet."

21. Enter response in Part I, and if YES, answer Parts II and III

<table>
<thead>
<tr>
<th>Part I: Have you EVER used any of the following EVEN ONE TIME?</th>
<th>Part II: How often did you use X when you were using it the most?</th>
<th>Part III: How many of the past 30 days did you use the following?</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Yes (Ask Parts II and III)</td>
<td>Every Day</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>a. Cigar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Cigarillo such as Black and Mild or Swisher Sweets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Little Cigar such as Winchester, Cheyenne or Remington</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Pipe filled with tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Chewing tobacco such as Redman, Levi Garrett or Beechnut</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Snuff such as Skoal, Skoal Bandits or Copenhagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. Snus such as Camel snus or Marlboro snus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. E-cigarettes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Hookah</td>
<td></td>
<td></td>
</tr>
<tr>
<td>j. Dissolvables such as Ariva or Stonewall Compressed Tobacco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k. Bidis or Clove Cigarettes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter V

References


13. *Circulation*. 2019;139:e56-e528. DOI: 10.1161/CIR.0000000000000659


