Fall 2016

Hemostatic responses to exercise in a polycythemia vera patient

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Hemostatic Responses to Exercise in a Polycythemia Vera Patient

An Honors Program Project Presented to
the Faculty of the Undergraduate
College of Health & Behavioral Sciences
James Madison University

by Allison Huschke

December 2016

Accepted by the faculty of the Department of Kinesiology, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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Reader: Michael J. Saunders, Ph.D., Director, Human Performance Laboratories, Kinesiology

Reader: Nicholas Luden, Ph.D., Graduate Director, Kinesiology

HONORS PROGRAM APPROVAL:

Bradley R. Newcomer, Ph.D., Director, Honors Program

PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at the Kinesiology Honors Symposium on
December 9, 2016.
Hemostatic Responses to Exercise in a Polycythemia Vera Patient

Allison Huschke

Undergraduate Honor’s Thesis

December 2016
Dedication

This thesis is dedicated to the Myeloproliferative Neoplasm Research Foundation as they continue to develop their research in pursuit of a cure. I would also like to dedicate this thesis to Dr. Erika Struble, MD for her excellent treatment and role in helping me to better understand Polycythemia Vera.
Acknowledgments

I would like to thank Dr. Chris Womack for the selfless dedication of his time as my research mentor. He provided me with the skills and knowledge needed to complete my thesis and I will be forever grateful for the opportunity he provided me. I would also like to thank my readers, Dr. Michael Saunders and Dr. Nicholas Luden, for their valuable time and support. The James Madison University Kinesiology Department has been an indispensible component of my college experience and has provided me with invaluable experience I will undoubtedly use as I further my education.
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Table 1. Mean (±SD) for all hemostatic variables for the Polycythemia Vera patient and the comparison group.
Abstract

PURPOSE: To assess the hemostatic responses to exercise in a patient with Polycythemia Vera (PV). METHODS: Six female runners (≥15 miles/week) completed a maximal treadmill test. One subject had PV while the other five subjects made up the comparison group. Blood samples were taken before and within two minutes after exercise. VO2max was also recorded. RESULTS: Pre-exercise Factor VIII and tPA-antigen were similar in the PV subject and comparison group. Factor VIII and tPA-antigen increased dramatically in the PV subject (+100%, +1000%) in relation to the comparison group (+22.9 ± 8.7%, +108 ± 78%) after exercise. Pre-exercise PAI-1 was lower in the PV subject and also decreased slightly more (-100%) in response to exercise than the comparison group (-43 ± 67%). CONCLUSIONS: Factor VIII, tPA-antigen, and PAI-1 responses to exercise are more dramatic in a Polycythemia Vera patient than in those without the disorder.
Chapter I: Introduction

Polycythemia Vera is a rare myeloproliferative neoplasm in which the bone marrow produces too many blood cells due to a mutation within the bone marrow. Red blood cells are mainly affected, however an overproduction of platelets and leukocytes may also be associated with this blood disorder. The most prevalent mutation is in the protein JAK2, which plays a role in cell growth. The JAK2 V617F mutation is seen in 95% of cases. Polycythemia is mainly diagnosed in males over age 60, however the disorder may occur at any age in both males and females. The incidence rate for this disorder in females aged 20-34 years is .04 per 100,000. Risks associated with Polycythemia include thrombotic events such as blood clots and ischemia, splenomegaly, and progression to other blood disorders. Simple treatments for mild cases consist of phlebotomies to maintain hematocrit between 40-45% and a daily 81 mg aspirin. Since this disorder is very uncommon, especially for young people, there is a lack of research on the subject.

Polycythemia Vera increases the risk for blood clots due to high hematocrit levels and a possible high number of platelets, which creates more viscous blood. Although high hematocrit levels have not been proven to be a direct cause for thrombotic events, previous studies have found strong correlations between hematocrit level and clot risk. The Tromsø study recruited 13714 women and 12394 men as subjects in 1994-5. After 12.5 years of follow-up, 447 venous thromboembolic events had occurred within the sample population. Compared to an age-adjusted hazard ratio of 1.0 for hematocrit <39% in women and <43% in men, the hazard ratio for unprovoked VTE with hematocrit ≥42% in women and ≥46% in men was 1.62 and 2.37, respectively. During exercise,
hematocrit levels increase due to a loss of plasma volume\textsuperscript{13}; therefore, risks associated with high hematocrit levels in those with Polycythemia Vera may be exacerbated if combined with exercise.

In addition to increasing blood viscosity by increasing hematocrit, exercise also independently alters hemostasis by acutely increasing coagulation potential and fibrinolysis.\textsuperscript{10} Markers of coagulation potential that increase with exercise include Thrombin anti-thrombin\textsuperscript{3}, Factor VIII\textsuperscript{2}, and platelet activation\textsuperscript{8}. Fibrinolytic potential is also increased during acute exercise as evidenced by increases in tissue plasminogen activator (tPA) activity\textsuperscript{11} and a decrease in the main circulating inhibitor of tPA, plasminogen activator inhibitor (PAI-1). tPA-antigen also increases in proportion to tPA activity during exercise\textsuperscript{15}. Although markers of fibrinolysis increase along with markers of coagulation potential, fibrinolysis decreases at a faster rate in the post-exercise period\textsuperscript{14}, which thereby increases potential for thrombosis. Thus, a multitude of variables can contribute to clotting risks during and after exercise that may lead to life-threatening ischemic events. This increased risk for the general population may be enhanced in a person with polycythemia vera. Since this disorder is typically seen in older male populations, little to no research exists on the complications of exercise in a young, active, female with polycythemia vera. This lack of research is most likely due to the minimal amount of available subjects and the reduced ability of the elderly with Polycythemia Vera to perform strenuous exercise.

A previous study simulated Polycythemia by infusion of erythrocytes (60% hematocrit) in 5 heat-acclimated male subjects and analyzed the effects of hydration and exercise on thermoregulation. Each subject completed four heat stress tests: pre and post-
infusion in a euhydrated state and pre and post-infusion in a hypohydrated state. The heat stress tests consisted of two trials of a 45-minute walk with a 15-minute rest in a hot (35 degrees C and 45% humidity) environment. Interestingly, simulated Polycythemia Vera resulted in a thermoregulatory advantage in both a euhydrated and hypohydrated state as post-infusion responses increased sweating rate, decreased core body temperature, and increased plasma volume. However, because this study simply increased RBC count in otherwise healthy people, the comparative exercise response in people who have the chronic form of Polycythemia is unknown. Polycythemia Vera may consist of elevated platelet counts in addition to high hematocrit and in turn may cause different hemostatic responses than solely erythrocyte injection. Exercise acutely increases platelet activation, which may be intensified with the presence of an increased platelet count. Therefore, the simulated Polycythemia study may not be representative of the chronic polycythemia population. Rather, this study supported the thermoregulatory advantages of blood doping. Thus, there is a paucity of research on exercise responses in patients with Polycythemia Vera.

Understanding the risks associated with Polycythemia is important in relation to exercise and other environments that increase thrombosis. Further information is necessary in determining the magnitude of these risks, as blood clots are a serious medical condition that can lead to other complications such as heart attacks and strokes. Since the increased risks with Polycythemia may be unavoidable, it is important to gather information on the responses to exercise. The purpose of this study is to create new information and analyze the effects of Polycythemia Vera on clotting factors after exercise in a young female diagnosed with Polycythemia compared to subjects without
the blood disorder. Further knowledge about this topic may lead to other remedies and prevention strategies in an effort to reduce the symptoms and risk factors associated with Polycythemia Vera.
Chapter II: Methods

Participants: The subjects in this study will consist of 10 college-aged, female runners (self-reporting >15 miles per week). The student researcher will be participating as the Polycythemia Vera subject, while the other subjects will be healthy adults without Polycythemia Vera. All subjects will be informed of the procedures and will give informed consent to participate. Subjects will be recruited by word of mouth around JMU and the greater Harrisonburg area. Participants will be at least 18 years of age. Deception will not be used among the participants in this study.

Exercise Testing: Prior to testing, all subjects will start this research study by being assigned an appropriate amount of fluid to ingest per day (42.9mL/kg of body weight) for a week. Following the week of controlled hydration, each subject will run an incremental treadmill test in a controlled environment between 7 and 9 a.m. The treadmill test will begin at 2.5 mph and increase 0.5 mph every minute until 6.0 mph; the speed will then remain constant as the grade increases by 3% every minute until volitional exhaustion. Oxygen uptake will be monitored using a metabolic cart and a heart rate monitor will be used to gather heart rate data.

Blood sampling, assays: Prior to the treadmill test, participants will rest in a semi-recumbant position for 15 minutes. Following this rest period, 10 mL of blood will be drawn from an antecubital vein using assumed venipuncture. All blood samples will be obtained by either Dr. Womack or a student/faculty member that’s been appropriately trained via procedures approved by the Human Performance Laboratory. The protocol for becoming trained and approved for venipuncture is listed in Appendix C. Samples
will be obtained in an identical manner (except for the 15 minute rest period) immediately post-exercise. Hematocrit and hemoglobin levels will be measured from the whole blood samples using a Hemocue automated analyzer. The remainder of the blood samples will be centrifuged immediately for 20 minutes at 1,500xg and 4°C to obtain platelet-poor plasma, and then frozen and stored at -20°C until assayed. Plasma concentrations of factor VIII levels, tPA-antigen, and PAI-1 activity will be determined using commercially available ELISA kits (VisuLize® Ontario, Canada; Eagle Bioscience, Inc, Nashua, New Hampshire) according to manufacturer specifications.

*Risks/Benefits: *With strenuous activity, there is potential for muscle or joint soreness, and a slight risk of more serious muscular or cardiovascular injury such as muscle strain or sprain, ligament damage, heart attack or stroke. The overall risk of strenuous activity (including both low- and high-risk participants) is approximately six cardiac events per 10,000 tests, or 0.06%. The risk of a complication requiring hospitalization (including non-cardiac problems) is ≤ 0.2%. Risks of blood drawing may include discomfort, bruising, and, in rare instances, infection, lightheadedness, and fainting. In order to mitigate these exercise related risks, participants will be screened with a health-history questionnaire. Only low risk participants, based on the American College of Sports Medicine guidelines, will be included in the study. Furthermore, all faculty and students engaged in exercise testing in the Human Performance Laboratory are required to have current CPR training. Any evidence of an acute cardiovascular event will prompt the research team to activate emergency medical services by calling 911.
Alternatives to participating in the study: All participants will be informed that they have the right to withdraw from the study at any time without penalty.

Benefits of Research: Due to the limited existing research on Polycythemia Vera, this study may provide initial information for further research on the risks of exercise in this specific population. Participants will also be given information regarding their cardiorespiratory fitness from the exercise test.

Research will take place at the Human Performance Lab in Godwin Hall located on the campus of James Madison University. Research will be completed within the Fall semester of 2016 (by December 16th, 2016, after receiving IRB approval).

Data Collection: Data will originally be collected on paper data sheets and then will be manually entered into a computer spreadsheet. Data, including a flash drive with the spreadsheet, will be locked in a file cabinet in Dr. Womack’s office. Dr. Womack and I will be the only people to have access to the data. All consent forms will be kept in a separate file in a locked file cabinet in Dr. Womack’s office. Dr. Womack will be the only one with access to said file cabinet. All subjects will be given a subject # which will be used as an identifier on all data sheets during the study. The key for the ID numbers will be kept in the same file as the consent forms. Data will be destroyed by Dr. Womack within a year after my completed honor’s thesis.
**Reporting Procedures:** The audience to be reached in the report of this study is the Honor’s thesis committee and the Honor’s college. Research will be presented to the Department of Kinesiology faculty and students and a possible regional/local exercise science conference.

The subjects will receive feedback from the study via email.

**Data Analysis**

Due to the limited sample size, this study will be treated as a pilot study and will be analyzed without statistical comparisons. Hematocrit and clotting factors will be compared between each subject before and after exercise. The differences in clotting factors between the control subjects will then be compared to the differences in the Polycythemia Vera subject.

**Experience of the Researcher (and advisor, if student):**

Dr. Christopher J Womack, PhD will be advising me through my first research experience with this study. Dr. Womack has over 25 years of research experience and has authored or co-authored over 50 papers in peer-reviewed exercise science journals. He is a Fellow of the American College of Sports Medicine, the governing body that establishes Guidelines for Exercise Testing and Prescription.
Chapter III: Manuscript

Introduction

Polycythemia Vera is a rare myeloproliferative neoplasm in which the bone marrow produces too many blood cells due to a mutation within the bone marrow. Red blood cells are mainly affected, however an overproduction of platelets and leukocytes may also be associated with this blood disorder. The incidence rate for this disorder in females aged 20-34 years is 0.04 per 100,000.\(^6\) Risks associated with Polycythemia include thrombotic events such as blood clots and ischemia, splenomegaly, and progression to other blood disorders.\(^{21}\) Simple treatments for mild cases consist of phlebotomies to maintain hematocrit between 40-45% and a daily 81 mg aspirin.\(^{29}\) Since this disorder is very uncommon, especially for young people, there is a lack of research on the subject.

Although high hematocrit levels have not been proven to be a direct cause for thrombotic events, previous studies have found strong correlations between hematocrit level and clot risk.\(^{27}\) During exercise, hematocrit levels increase due to a loss of plasma volume\(^{20}\); therefore, risks associated with high hematocrit levels in those with Polycythemia Vera may be exacerbated if combined with exercise. In addition to increasing blood viscosity by increasing hematocrit, exercise also independently alters hemostasis by acutely increasing coagulation potential and fibrinolysis.\(^{16}\) Markers of coagulation potential that increase with exercise include Thrombin anti-thrombin\(^{4}\), Factor VIII\(^2\), and platelet activation.\(^{12}\) Fibrinolytic, or clot lysis, potential is also increased during acute exercise as evidenced by increases in tissue plasminogen activator (tPA).
activity\textsuperscript{18} and a decrease in the main circulating inhibitor of tPA, plasminogen activator inhibitor (PAI-1). tPA-antigen also increases in proportion to tPA activity during exercise.\textsuperscript{25} Although markers of fibrinolysis increase along with markers of coagulation potential, fibrinolysis decreases at a faster rate in the post-exercise period\textsuperscript{24}, which thereby increases potential for thrombosis. Thus, a multitude of variables can contribute to clotting risks during and after exercise that may lead to life-threatening ischemic events. This increased risk for the general population may be enhanced in a person with Polycythemia Vera due to the elevated number of platelets activated by exercise.\textsuperscript{14} Since this disorder is typically seen in older male populations, little to no research exists on the complications of exercise in a young, active, female with Polycythemia Vera. This lack of research is most likely due to the minimal amount of available subjects and the reduced ability of the elderly with Polycythemia Vera to perform strenuous exercise.

A previous study simulated Polycythemia Vera by infusion of erythrocytes (60% hematocrit) in five heat-acclimated male subjects. Interestingly, simulated Polycythemia Vera resulted in a thermoregulatory advantage in both a euhydrated and hypohydrated state as post-infusion responses increased sweating rate, decreased core body temperature, and increased plasma volume.\textsuperscript{26} However, because this study simply increased RBC count in otherwise healthy people, the comparative exercise response in people who have the chronic form of Polycythemia is unknown. Polycythemia Vera may consist of elevated platelet counts in addition to high hematocrit and in turn may cause different hemostatic responses than solely erythrocyte injection. Exercise acutely increases platelet activation, which may be intensified with the presence of an increased
platelet count. Therefore, the simulated Polycythemia study may not be representative of the chronic Polycythemia population.

Understanding the risks associated with Polycythemia is important in relation to exercise and other environments that increase thrombosis. Further information is necessary to determine the magnitude of these risks, as blood clots are a serious medical condition that can lead to other complications such as heart attacks and strokes.\textsuperscript{12} Since the increased risks with Polycythemia may be unavoidable, it is important to gather information on the responses to exercise. The purpose of this study is to create new information and analyze the effects of Polycythemia Vera on clotting factors after exercise in a young female diagnosed with Polycythemia compared to subjects without the blood disorder. Further knowledge about this topic may lead to other remedies and prevention strategies in an effort to reduce the symptoms and risk factors associated with Polycythemia Vera.
Methods

Participants: The subjects in this study consisted of 6 college-aged, female runners (self-reporting >15 miles per week). The student researcher participated as the Polycythemia Vera subject, while the other subjects in the control group were healthy adults without Polycythemia Vera. The control group had a mean height, weight, and VO2max of 168.4 ± 7.9 cm, 67.2 ± 11.0 kg, and 43.2 ± 4.3 ml/kg/min respectively. Mean age for the control group was 21 years. The Polycythemia Vera subject’s height, weight, and VO2max were 165.1 cm, 55.8 kg, and 60 ml/kg/min. The Polycythemia Vera subject was 21 years old. All subjects were informed of the procedures and gave informed consent to participate. Subjects were recruited by word of mouth around JMU and the greater Harrisonburg area.

Exercise Testing: Prior to testing, all subjects were assigned an appropriate amount of fluid to ingest per day (42.9mL/kg of body weight) for a week. Following the week of controlled hydration, each subject ran an incremental treadmill test in a controlled environment between 8 and 10 a.m. The treadmill test began at 2.5 mph and increased 0.5 mph every minute until 6.0 mph; the speed then remained constant as the grade increased by 3% every minute until volitional exhaustion. Oxygen uptake was monitored using a Moxus metabolic cart and a heart rate monitor was used to gather heart rate data. All subjects were fasted for a minimum of 10 hours prior to testing and abstained from alcohol 24 hours prior to testing.

Blood sampling, assays: Prior to the treadmill test, participants rested in a semi-recumbant position for 15 minutes. Following this rest period, 10 mL of blood was drawn from an antecubital vein using assumed venipuncture. Samples were obtained in
an identical manner (except for the 15 minute rest period) immediately post-exercise. Hematocrit and hemoglobin levels were measured from the whole blood samples using a Hemocue automated analyzer. The remainder of the blood samples were centrifuged immediately for 20 minutes at 1,500xg and 4°C to obtain platelet-poor plasma, and then frozen and stored at -20°C until assayed. Plasma concentrations of factor VIII levels, tPA-antigen, and PAI-1 activity were determined using commercially available ELISA kits (VisuLize® Ontario, Canada; Eagle Bioscience, Inc, Nashua, New Hampshire) according to manufacturer specifications.
Results

The comparison group completed the maximal treadmill test with a mean VO2max of 43.2 ± 4.3 ml/kg/min while the Polycythemia Vera (PV) subject achieved a VO2max of 60 ml/kg/min. Pre- and Post-exercise hemostatic variables for the PV patient and the comparison group are displayed in Table 1. The comparison group had a mean pre-exercise hematocrit and hemoglobin of 40.5 ± .8% and 13.38 ± .97 g/dl respectively. Pre-exercise hematocrit and hemoglobin in the PV patient was 44.5% and 15.15 g/dl. Hematocrit in both groups increased in response to exercise similarly. Hemoglobin in the PV subject increased less (+6.9%) than the comparison group (+11.51 ± 1.42%) after exercise. The PV subject’s pre-exercise Factor VIII (0.64) was similar to the comparison group pre-exercise (0.70 ± 0.11). However, the PV subject had a larger increase (+100%) than the comparison group following exercise (+22.9 ± 8.7%). Pre-exercise tPA-antigen was similar in the PV subject (1.1) and the comparison group (1.00 ± 0.31). Post-exercise tPA-antigen in the PV subject increased dramatically (+1000%) relative to the comparison group (+108 ± 78%). Pre-exercise PAI-1 was lower in the PV subject (0.56) than the comparison group (3.21 ± 2.22). Post-exercise PAI-1 in the PV subject had a larger decrease (-100%) than subjects in the comparison group (-43 ± 67%).
Table 1. Mean (±SD) for all hemostatic variables for the Polycythemia Vera patient and the comparison group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Polycythemia Vera Patient</th>
<th>Comparison Group (mean ± SD)</th>
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<tbody>
<tr>
<td><strong>Factor VIII</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre-Exercise</td>
<td>.64</td>
<td>.70 ± .11</td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>1.28</td>
<td>.86 ± .19</td>
</tr>
<tr>
<td>% change with exercise</td>
<td>100%</td>
<td>22.9 ± 8.7%</td>
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<tr>
<td><strong>tPA-antigen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>1.13</td>
<td>1.00 ± .31</td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>12.50</td>
<td>2.08 ± .86</td>
</tr>
<tr>
<td>% change with exercise</td>
<td>1000%</td>
<td>108 ± 78%</td>
</tr>
<tr>
<td><strong>PAI-1</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre-Exercise</td>
<td>0.55</td>
<td>3.21 ± 2.22</td>
</tr>
<tr>
<td>Post-Exercise</td>
<td>0</td>
<td>1.83 ± 1.60</td>
</tr>
<tr>
<td>% change with exercise</td>
<td>-100%</td>
<td>-43 ± 67%</td>
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<tr>
<td><strong>Hematocrit</strong></td>
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<tr>
<td>Pre-Exercise</td>
<td>44.5</td>
<td>40.5 ± .8</td>
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<tr>
<td>Post-Exercise</td>
<td>47.5</td>
<td>43.8 ± 2.2</td>
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<tr>
<td>% change with exercise</td>
<td>6.7%</td>
<td>8.15 ± .57%</td>
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<tr>
<td><strong>Hemoglobin</strong></td>
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<tr>
<td>Pre-Exercise</td>
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<td>13.38 ± .97</td>
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<tr>
<td>Post-Exercise</td>
<td>16.2</td>
<td>14.92 ± .76</td>
</tr>
<tr>
<td>% change with exercise</td>
<td>6.9%</td>
<td>11.51 ± 1.42%</td>
</tr>
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Discussion

Hematocrit

As expected, the Polycythemia Vera subject started with higher pre-exercise hematocrit levels than the control group. Hematocrit increased in the Polycythemia subject at a similar degree to the control group in response to exercise. While artificially increasing hematocrit (e.g. blood doping) has resulted in enhanced aerobic performance, naturally occurring elevated hematocrit levels may have a negative effect on aerobic fitness.\(^5\,^6\) A study of 77 soccer players in France separated each player into specific quintiles based on their hematocrit. The first quintile contained those with the lowest hematocrit (38.7 ± .8) while the 5\(^{th}\) quintile contained players with the highest hematocrit (45.5 ± .3). The 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) quintiles contained players with a mean hematocrit of 42.3 ± .2. The players in the 1\(^{st}\) quintile proved to have a higher aerobic fitness level than the players in every other quintile, thus suggesting an inverse relationship between hematocrit and fitness. This inverse relationship may be attributable to a hemodilution effect associated with training. Ferritin also has a negative correlation with hematocrit, which could be a factor contributing to a lower aerobic fitness level in those with high hematocrit.\(^5\) Patients with Polycythemia Vera typically have lower ferritin levels\(^30\) and therefore, the higher VO2max achieved by the Polycythemia Vera subject may not be due to a high hematocrit.
Factor VIII

Pre-exercise Factor VIII levels for both the Polycythemia Vera subject and control group were similar. The Polycythemia Vera subject’s post-exercise levels exhibited a larger increase than the control group. This may be explained by the higher intensity and duration of exercise achieved by the Polycythemia Vera subject.\(^1\) There is also speculation of a chronically activated coagulation cascade in patients with Polycythemia Vera, specifically the intrinsic pathway.\(^8\) Factor VIII is an intrinsic pathway factor that originates from platelets.\(^3\) With the combination of a chronically activated intrinsic pathway and elevated levels of platelets associated with Polycythemia Vera, an increased production or activation of Factor VIII could be associated with these Polycythemia Vera complications.

tPA antigen

tPA-antigen in the Polycythemia Vera subject was similar to the control group before exercise. Post-exercise tPA-antigen, however, increased dramatically in the Polycythemia Vera subject compared to the control group. Although tPA is positively correlated with exercise intensity\(^28\) and may explain a portion of the greater increase, the large difference between groups would suggest other mechanisms contributing to the marked increase observed in the Polycythemia Vera patient.

Along with increased red blood cells, Polycythemia Vera may also be accompanied with elevated platelet levels. In Polycythemia Vera patients with the JAK2V617F mutation, platelet thrombin generation potential was significantly elevated compared to control groups.\(^23\) Exercise also enhances thrombin generation, which can
lead to platelet activation. With an increased presence of thrombin and activated platelets, clot formation may increase due to the conversion of fibrinogen to fibrin. Since fibrin is necessary for the creation of plasmin by tissue plasminogen activator, tPA-antigen may be more susceptible to increase during exercise in Polycythemia Vera patients.

**PAI-1**

Pre-exercise PAI-1 in the Polycythemia Vera subject was lower than the control group, which contradicts previous findings of elevated PAI-1 in Polycythemia Vera subjects. PAI-1 can also be significantly influenced by BMI. Although the protocol did not collect BMI specifically, the Polycythemia Vera subject had the lowest BMI calculated from height and weight. In agreement with previous research, a lower BMI correlates with lower PAI-1.

**Implications**

Since high Factor VIII levels may increase risk for thrombotic events, a low PAI-1 level may be important in attenuating these risks. The high tPA-antigen response to exercise in the Polycythemia Vera subject may represent increased fibrinolytic response to offset the increased coagulation potential evidenced during exercise. High Factor VIII in response to exercise increases the risk for thrombosis and other related events, however the large increase in post-exercise tPA-antigen may be able to attenuate these risks. Since fibrinolysis tends to decrease at a faster rate than coagulation potential, Polycythemia Vera patients may be at an increased risk for thrombotic events after
exercise due to a larger production of Factor VIII, however the dramatically high tPA-antigen response may have an acute protective affect during and immediately after exercise. Extensive research must be conducted before these findings can be generalized to the whole Polycythemia Vera population. Future research is also necessary on this disorder in order to find effective treatment during exercise. The current treatment for mild Polycythemia Vera is a daily 81mg aspirin and phlebotomy p.r.n., however previous research indicates aspirin may not be effective in the context of exercise.\textsuperscript{13}

**Limitations:**

There are many limitations to these findings since this study only included one Polycythemia Vera subject. Perhaps researching subjects with similar VO2max and BMI values may have led to more comparable results. The mean results in the control group also do not have any statistical significance due to the small sample size.
Manuscript References

## Appendices

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Consent to Participate in Research

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Allison Huschke and Christopher J. Womack, Ph.D. from James Madison University. The purpose of this study is to determine if a person with Polycythemia Vera is more likely to have higher clotting potential after exercise than a healthy adult without the blood disorder.

Potential Risks & Benefits

If you choose to participate in this study, you will perform one maximal treadmill test. With strenuous exercise, the investigator perceives a potential for muscle or joint soreness, and a slight risk of more serious muscular or cardiovascular injury such as muscle strain or sprain, ligament damage, heart attack or stroke. In healthy individuals, the risk of death during vigorous exercise has been estimated at 1 death per year for every 18,000 individuals. In order to mitigate these exercise related risks, participants will be screened with a health-history questionnaire. Only low risk participants, based on the American College of Sports Medicine guidelines, will be included in the study. Furthermore, all faculty and students engaged in exercise testing in the Human Performance Laboratory are required to have current CPR training. Any evidence of an acute cardiovascular event will prompt the research team to activate emergency medical services by calling 911.

Potential benefits from participation in this study include:

VO$_{2\max}$ results and the opportunity to help increase research conducted on Polycythemia Vera.

Research Procedures

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. This study consists of one week of controlled hydration as well as one maximal treadmill test. You will also be subjected to blood tests both before and after each trial. Your heart rate will be monitored by a monitor that wraps around your chest.

Blood Sampling: We will obtain about 10 ml of blood (about 2 teaspoons) prior to and immediately after the treadmill test in order to determine the potential of your blood to coagulate. These blood samples will be obtained from an arm vein.

Confidentiality
The results of this study will be presented to the Department of Kinesiology faculty and students and a possible regional/local exercise science conference. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. However, you can ask that your data be removed from the study at any point prior to presentation and publication. 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 researchers. Data will be destroyed within a year after research is completed. Final results will be made available to you upon request.

**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. Your right to withdraw includes the right to request that your blood samples be discarded at any time.

**Questions**

You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact Allison Huschke at huschkam@dukes.jmu.edu, or Christopher J. Womack, Ph.D. at womackcx@jmu.edu or 540-568-6515.

**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.

<table>
<thead>
<tr>
<th>Name of Participant (Printed)</th>
<th>Name of Researcher(s) (Printed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of Participant (Signed)</td>
<td>Name of Researcher(s) (Signed)</td>
</tr>
<tr>
<td>Date</td>
<td>Date</td>
</tr>
</tbody>
</table>
For questions about your rights as a research subject, you may contact the chair of JMU’s Institutional Review Board (IRB). Dr. David Cockley, (540) 568-2834, cocklede@jmu.edu.
James Madison University
Department of Kinesiology
Health Status Questionnaire

Instructions: Complete each question accurately. All information provided is confidential.

Part I: General Information

1. Subject #

2. Local Phone Email:
____________________________________

3. Gender (circle one) Male Female

4. Date of Birth (Month/ Day/ Year)

Part II: Medical History

5. Circle any that died of heart attack before age 50: Father Mother Brother Sister Grandparent

6. Date of last medical exam: _____________ Last physical fitness test:
________________________

7. Circle operations you have had: Back Heart Kidney Eyes Joint Neck Ears Hernia
   Lung Other ________________

8. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

   Alcoholism Diabetes Kidney Problems
   Anemia (sickle cell) Emphysema Mental Illness
   Anemia (other) Epilepsy Muscular Injury
   Asthma Eye Problems Neck Strain
   Back Strain Gout Obesity
   Bleeding trait Hearing Loss Orthopedic Injuries
   Bronchitis, chronic Heart Problem Phlebitis
   Cancer High Blood Pressure Rheumatoid arthritis
   Cirrhosis, liver Hypoglycemia Stroke
   Concussion Hyperglycemia Thyroid problem
   Congenital defect Infectious Mononucleosis Ulcer
   Other ____________________
9. Circle all medications taken in the last six months:

<table>
<thead>
<tr>
<th>Blood thinner</th>
<th>Epilepsy medication</th>
<th>Nitroglycerin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic pill</td>
<td>Heart-rhythm medication</td>
<td>Other ________________</td>
</tr>
<tr>
<td>Digitalis</td>
<td>High-blood pressure medication</td>
<td>Insulin</td>
</tr>
</tbody>
</table>

10. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

5 = Very often  4 = Fairly often  3 = Sometimes  2 = Infrequently  1 = Practically never

a. cough up blood  f. chest pain
   1  2  3  4  5  1  2  3  4  5

b. abdominal pain  g. swollen joints
   1  2  3  4  5  1  2  3  4  5

c. low back pain  h. feel faint
   1  2  3  4  5  1  2  3  4  5

d. leg pain  i. dizziness
   1  2  3  4  5  1  2  3  4  5

e. arm or shoulder pain  j. breathless on slight exertion
   1  2  3  4  5  1  2  3  4  5

**Part III: Health Related Behavior**

11. Do you smoke?  Yes No

12. If you are a smoker, indicate the number of smoked per day:

Cigarettes:

   40 or more  20-39  10-19  1-9

Cigars or pipes only:

   5 or more or any inhaled  less than 5, none inhaled

13. Do you exercise regularly? Yes No
14. How many times in a week do you spend at least 30 minutes in moderate to strenuous/vigorous exercise?

1  2  3  4  5  6  7  days per week

15. Can you walk 4 miles briskly without fatigue? Yes No

16. Can you jog 3 miles continuously at a moderate pace without discomfort? Yes No

17. Weight now: __________ lb. One year ago: __________ lb  Age 21: __________ lb
PROCEDURES/POLICIES FOR VENOUS BLOOD DRAWS

Laboratory faculty, instructors and researchers (including research assistants) should complete the following training actions prior to conducting this procedure:

a) Complete all standard lab safety procedures (review CHBS Lab Safety Plan and the HPL Safety Protocols and Responsibilities, and complete the Acknowledgement of Safety Training Form and HPL Safety Protocols Test)
b) Compete bloodborne pathogen training (http://www.jmu.edu/bbp/index.shtml)
c) Review the written procedures below
d) Receive hands-on training from a trained faculty member (or graduate student who has completed training previously), including at least three consecutive successful procedures by the trainee
e) Complete/sign page 2 of this form, and provide a hard-copy to the Lab Director (Dr. Mike Saunders)

Procedures

1) Prepare all materials ahead of time: needle, plastic Vacutainer holder (screw needle into holder ahead of time, and leave cover on needle), rubber tourniquet, alcohol swabs, gauze, Vacutainer tubes.

2) Exam gloves are to be worn at all times

3) Subject will have the needle inserted in an antecubital vein while sitting in a partially reclined chair.

4) Apply tourniquet firmly to arm around the lower bicep area.

5) Palpate the area on the inside of the elbow joint to find a prominent antecubital vein.

6) Prepare the insertion area by wiping thoroughly with an alcohol swab, then allow ~30 seconds for any remaining alcohol to evaporate.

7) Remove cover from needle and insert the needle (beveled edge facing up) through the skin into the antecubital vein, along the center of the longitudinal axis of the vein.

8) Once the needle is in the vein, hold the plastic needle holder steady, and press the Vacutainer firmly into the plastic holder (puncturing the seal on the Vacutainer, and initiating blood collection).

9) Remove tourniquet as the Vacutainer is filling.
10) Once the desired amount of blood is obtained, remove the Vacutainer from the holder (if you are filling more than one tube, insert a second tube as described in # 8).

11) To remove the needle from the vein, quickly withdraw the needle in a direction directly opposite to insertion. Immediately apply sterile gauze to the area and apply pressure.

12) Have the subject continue to apply pressure to the area for at least 2 minutes to cease bleeding and prevent bruising.

13) Apply a band-aid over top of the gauze.

14) Waste removal:
   a. All sharps (needles, catheter, any glass, hard plastic, etc) must be disposed in a red plastic sharps container (Biohazard).
   b. All non-sharps materials that have been in contact (or potentially in contact) with blood (gauze, gloves, etc.) must be disposed in a red Biohazard bag.
   c. Unsoiled paper wrappers etc. can be disposed in regular trash bins.
   d. Check that all waste has been removed, and the area is entirely clear of any waste/blood.
   e. If any blood has spilled in the area (i.e. on chairs, floor, equipment), clean thoroughly with germicidal bleach (i.e. Clorox®) and paper towels.
   f. If you are supervising students who are performing this technique (i.e. for a lab class or study), it is your responsibility to double-check the area is clean before leaving the area.

Date of Training Completion:______________

By signing below, the Trainee is acknowledging that they have completed items a, b, c, and d on page 1 of this form. The Trainer is acknowledging that they have provided hands-on training of this procedure for the Trainee, and have witnessed the trainee perform the procedures successfully on at least three consecutive occasions.

Trainee (individual completing the training):
<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

Trainer (individual providing the training):

| Name | Signature | Date |
References