Spring 2019

The influence of body composition, cardiorespiratory fitness, and physical activity on serum GGT in college drinkers

Gillian Mackey

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The Influence of Body Composition, Cardiorespiratory Fitness, and Physical Activity on Serum GGT in College Drinkers

An Honors College Project Presented to
The Faculty of the Undergraduate
College of Kinesiology
James Madison University

Gillian O. Mackey
April 2019

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

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PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at the Kinesiology Honors Symposium on 18 April 2019.
Dedication

I dedicate this work to the memory of Frank Mather Archer II and Todd Allen Phillips.
Acknowledgements

Firstly, I would like to thank my faculty advisor, Dr. Christopher J. Womack, for his continuous support, patience, and guidance throughout this journey. His research experience and expertise were invaluable and working under him has increased my interest in research in my future endeavors. Beyond his intellectual assistance, his encouragement from the beginning instilled a confidence in me which I would not have found on my own, something for which I will be eternally grateful.

Second, I would like to thank Dr. Kurti and Dr. Irons for using their valuable time to read and provide feedback on my research project in all of its stages. Their comments were crucial in creating the final copy of this project.

Finally, I would like to thank my mom and dad. My parents pushed me to step out of my comfort zone and, without them, I would not have had this unique and humbling experience. I am grateful for their continued unending support, for without them, none of this would have been possible.
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Abstract

Purpose: To test the effect of body composition, cardiorespiratory fitness, and physical activity levels on serum gamma-glutamyltransferase (GGT) among college students who consume alcohol. Methods: Seven male and nine female subjects over 21 years old completed the Alcohol Use Disorders Identification (AUDIT), a maximal treadmill test to measure maximal oxygen uptake (VO$_{2\text{max}}$), a DEXA scan for body fat percentage, and a venous blood draw for serum GGT. In addition, subjects were assessed with respect to average daily moderate-to-vigorous physical activity (MVPA) via continuously worn (while awake) accelerometer for one week. Subjects were grouped based on AUDIT scores [$\geq 8$ = high alcohol intake ($n = 9$); $< 8$ = low alcohol intake ($n = 7$)] and compared with respect to outcome variables using a series of independent t-test. Correlations between serum GGT and other outcome variables were established using Pearson correlation coefficients in the higher AUDIT group. Results: No significant differences were found between lower and higher AUDIT groups with respect to average (± SD) VO$_{2\text{max}}$ (62.37 ± 11.09 vs. 62.02 ± 5.16 ml/kg FFM/min), average MVPA (45.15 ± 23.55 vs. 49.99 ± 16.82 minutes/day), body fat % (26.7 ± 8.0 vs. 22.9 ± 4.1 %) and serum GGT (13.93 ± 1.96 vs. 15.94 ± 3.33 U/L). For the higher AUDIT group, there was no correlation between serum GGT and VO$_{2\text{max}}$ or body fat %, but there was a trend towards a negative correlation between average daily MVPA and serum GGT ($r = -0.65$, p = 0.06). Conclusion: Findings suggest that chronic physical activity has a positive impact on serum GGT in individuals with high AUDIT scores. These findings warrant further investigation with larger sample size.

Keywords: Serum GGT, exercise and liver health, gamma-glutamyltransferase, physical activity and serum GGT, cardiorespiratory fitness.
Chapter I

Introduction

**Serum Gamma-Glutamyltransferase (GGT) and Alcohol Ingestion.** Gamma-glutamyltransferase (GGT) is a liver enzyme associated with neutralizing oxidative stress via the mediation of peptide transport and glutathione metabolism. Elevated GGT levels can indicate liver damage and disease due to the increased release or synthesis of GGT from damaged liver cells. Serum GGT levels to be elevated up to 10 times the normal levels in alcoholics due to the attenuated clearance of GGT when alcohol-induced liver damage is present. There is also a positive relationship between alcohol intake and serum GGT among individuals who do not have alcoholism. In a study by Alatalo et al., liver enzymes of alcohol-abstaining subjects and moderate drinkers were assessed, revealing elevated GGT levels in moderate drinkers in comparison to alcohol abstainers. Sharp et al. found similar results in patients at an alcohol and drug abuse hospital. GGT levels were elevated significantly in all groups that consumed alcohol, even those who only drank socially. Therefore, it appears that alcohol consumption of any kind is associated with liver damage as indicated by elevated serum GGT.

**College Students and Alcohol Ingestion.** Among college-aged individuals in America, repeated binge-drinking and above moderate intake of alcohol is prevalent. Knight et al. describe binge/heavy episodic drinking as having five or more drinks in a single occasion for men and four or more drinks in a single occasion for women (in the two weeks prior to measurement). After evaluating a large population of college students, Knight et al. found that 80.9% of students consumed alcohol of any amount and only 19.1% abstained. Of those who consumed alcohol, 21.5% were described as occasional heavy episodic drinkers and 22.7% were described as frequent episodic drinkers. According to a national survey by Johnson et al. in 2015, 32% of
college students reported having five or more drinks in one sitting on one or more occasions in the two weeks prior to survey and 13.0% of college students reported having 10 or more drinks in one sitting on one or more occasions in the two weeks prior to survey. These drinking habits can result in liver damage as indicated by elevated liver enzymes such as GGT.

**Other Causes of Serum GGT Elevation.** In addition to liver damage and alcohol consumption, high serum GGT levels have also been associated with pancreatic disease, biliary disease, and the intake of drugs such as cocaine, antibiotics and anabolic steroids. In a study by Lee *et al.*, GGT levels above 40 U/L were found to be tightly linked to incidence of type II diabetes, especially in diabetic subjects between 45 and 55 years old and those with a BMI between 27 and 32 kg/m$^2$. These potential contributors to serum GGT elevation are not expected in a healthy, college-aged population.

Serum GGT levels may also be elevated in the presence of several cardiovascular disease risk factors including smoking, older age, sedentary lifestyle, high BMI, and high blood pressure. Celik *et al.* found that serum GGT in alcohol-abstaining young adults with pre-hypertension was positively associated with decreased arterial elasticity, a contributing factor to hypertension. These findings suggest that serum GGT contributes to hypertension independently, but is not elevated due to hypertension.

Blood lipid levels (HDL and LDL) are also with GGT levels. Patel *et al.* discovered that young adults with persistently high GGT levels had significantly higher average LDL levels and significantly lower HDL levels than subjects with low GGT levels. The relationship between serum GGT and LDL levels also seems to contribute to atherosclerosis. Paolicchi *et al.* studied the blood lipid and liver enzyme levels in patients with coronary atherosclerosis and found that the amount of GGT bound to LDL was directly proportional to serum GGT levels in all subjects,
regardless of alcohol intake. The complex that forms when GGT binds LDL contributes to LDL oxidation, causing damage to the arterial wall. This damage results in plaque build-up in the arteries, ultimately contributing to atherosclerosis. Based on Paolicchi et al.’s findings, it appears that GGT is also independently related to increased LDL oxidation and atherosclerosis.

**Exercise Training and Serum GGT.** Considering exercise has antioxidant effects, it is possible that habitual training might affect liver health as indicated by altered serum GGT levels. Little research has been conducted on the effects of exercise training on liver health and serum GGT levels in human subjects with liver disease; however, results from a recent study by Linden et al. found that the increased mitochondrial content in the liver associated with high aerobic fitness protected against liver damage in aerobically fit rats. Additionally, Linden et al. found that aerobically fit rats that had continuous access to ethanol over 8 weeks were resistant to liver damage. The findings suggest that aerobic training protects against alcohol-induced liver damage in rats due to direct increases in mitochondrial health and decreases in mitochondrial beta-oxidation, ultimately leading to improved liver function, decreased apoptosis, increased anti-apoptosis processes, and decreased inflammation. The present study aims to investigate the presence of the protective effects of fitness among humans who consume alcohol.

**Summary.** In conclusion, elevated serum GGT levels are associated with the intake of alcohol and liver damage. Elevated serum GGT levels can also be caused by pancreatic disease, type II diabetes, cocaine use, anabolic steroid use, biliary disease, and antibiotic intake. Serum GGT levels are associated with many risk factors of cardiovascular disease and have been found to be independently associated with oxidized LDL and hypertension. Alcohol use, specifically binge-drinking, appears to be prevalent among college-aged individuals and can cause liver damage. It is not yet known whether fitness levels and/or chronic physical activity levels influences liver
function in college-aged individuals who consume alcohol, but high aerobic capacity has been shown to improve liver function and protect against alcohol-induced liver damage in rats. The present cross-sectional study aims to evaluate whether serum GGT levels are affected by body composition, cardiorespiratory fitness, and/or physical activity in college-aged individuals who drink alcohol. We hypothesize that serum GGT levels will be positively associated with alcohol intake and body fat percentage and negatively associated with chronic physical activity levels and cardiorespiratory fitness.
Chapter II

Methods

Subjects. College-aged subjects from James Madison University will be studied. All subjects will be 21 years old or older and self-report consuming at least four drinks per month. All subjects will complete the Alcohol Use Disorders Identification (AUDIT) to assess alcohol intake and drinking related behavior, which has been validated in college students. A copy of this survey is included in Appendix B. The World Health Organization has noted an AUDIT score of eight or more as indicative of hazardous and harmful alcohol use. Therefore, we will subdivide our sample into subjects with lower (AUDIT > 8) and higher (AUDIT ≤ 8) scores. Informed consent will be given to each subject prior to data collection (Appendix A).

Physical Activity Assessment. To determine the subjects’ physical activity levels, each participant will wear an Actigraph accelerometer around the hip for seven days and will be removed during showering and sleeping. For inclusion of the data, the accelerometer has to record at least 10 hours of data on at least four of the seven days. From the valid days, the average minutes per day spent in moderate-to-vigorous physical activity (MVPA) will be obtained. The use of an accelerometer to determine the physical activity levels of college students has been validated previously in the literature.

Cardiorespiratory Fitness. The cardiorespiratory fitness of all subjects will be evaluated during a graded exercise test to volitional exhaustion on a treadmill to evaluate maximum oxygen consumption (VO_{2max}). The test will begin at 3.0 mph and will increase by 0.5 mph each minute until 6.0 mph is reached. After this point, grade will increase by 3% per minute until volitional exhaustion. Oxygen uptake (VO_{2}) will be continuously assessed using a Sensormedics (Yorba Linda, CA) metabolic cart. VO_{2max} will be defined as the largest 30-second average in VO_{2} that
was achieved during the test. To ensure that a true VO₂max is achieved, a verification stage will be implemented at the end of the test. After 5 minutes of walking subjects will begin the test again at the speed and grade that immediately preceded their final stage and will continue until volitional fatigue.

**Body Composition.** Height will be determined using a stadiometer and weight and body fat percentage will be determined using a Lunar (GE Healthcare, Chicago IL) dual energy x-ray absorptiometry (DEXA) machine. All subjects will have a full body scan performed.

**Serum GGT Assessment.** To determine the subjects’ serum GGT levels, venous blood draws will be obtained the morning following a 10-hour fast. Water consumption will be allowed during the fast. Blood will be drawn from an antecubital vein into a 10 mL Vacutainer (Becton Dickenson, Franklin Lakes, NJ) serum blood collection tube and will be allowed to clot for 30 minutes. The blood sample will then be centrifuged at 2000 x g for 10 minutes in a refrigerated centrifuge. The resulting serum will be stored at −80°C until analysis. Serum GGT will be assayed in duplicate using a commercially available colorimetric assay (Sigma-Aldrich, St. Louis, MO).

**Statistical Analysis.** To minimize gender differences, VO₂max will be expressed relative to lean body mass (ml/kg ffm/min). Body fat %, VO₂max (ml/kg FFM/min), Average MVPA, and serum GGT will be compared between low and high AUDIT groups using an independent t-test. For the higher AUDIT group, Pearson correlation coefficients will be used to establish the association between serum GGT and: Body fat %, VO₂max, and average MVPA. A priori statistical significance will be set at P <0.05.
Chapter III

Manuscript

Introduction

Gamma-glutamyltransferase (GGT) is a hepatic enzyme associated with neutralizing oxidative stress via the mediation of peptide transport and glutathione metabolism. Elevated GGT levels can indicate liver damage and disease due to the increased release or synthesis of GGT from damaged liver cells. Elevated serum GGT levels have been observed in individuals who drink alcohol in any amount including social drinkers, moderate drinkers, heavy drinkers, and alcoholics. Among college-aged individuals in the US, repeated binge-drinking and above moderate intake of alcohol is prevalent. Studies suggest that over 80% of college students consume alcohol, and many engage in occasional heavy drinking or frequent heavy drinking. Such drinking habits may result in liver damage as indicated by elevated liver enzymes such as serum GGT.

Because chronic exercise has antioxidant properties, it is possible that habitual training status might affect liver health as indicated by lowered serum GGT levels. A previous study concluded that the increased mitochondrial content in the liver associated with high aerobic fitness protected against liver damage in rats treated with ethanol. This suggests that aerobic training protects against alcohol-induced liver damage in rats due to direct increases in mitochondrial health and decreases in mitochondrial beta-oxidation, ultimately leading to improved liver function, decreased apoptosis, increased anti-apoptosis processes, and decreased inflammation; however, this possibility has not been evaluated in humans. The present study evaluated whether serum GGT levels are affected by body composition, cardiorespiratory fitness, and/or physical activity in college-aged individuals who drink alcohol.
Methods

Participants. Sixteen healthy, college-aged subjects from James Madison University (seven males and nine females) were studied. All subjects were 21 years old or over and self-reported consuming at least four drinks per month. All subjects completed the Alcohol Use Disorders Identification (AUDIT) to assess alcohol intake and drinking related behavior, which has been validated in college students. A copy of this survey is included in Appendix B. The World Health Organization has noted an AUDIT score of eight or more as indicative of hazardous and harmful alcohol use. Therefore, we subdivided our sample into subjects with lower [AUDIT > 8 (n = 7)] and higher [AUDIT ≤ 8 (n = 9)] scores. Descriptive characteristics for both groups are shown in table 1. A copy of the informed consent given to each subject prior to data collection is located in Appendix A.

Body Composition. Height was determined using a stadiometer and weight and body fat percentage was determined using a Lunar (GE Healthcare, Chicago IL) dual energy x-ray absorptiometry (DEXA) machine.

Cardiorespiratory Fitness. The cardiopulmonary fitness of all subjects was evaluated during a graded exercise test to volitional exhaustion on a treadmill to evaluate maximum oxygen consumption (VO$_2$max). The test began at 3.0 mph and was increased by 0.5 mph each minute until 6.0 mph was reached. After this point, grade was increased by 3% per minute until volitional exhaustion. To ensure that a true VO$_2$max was achieved, a verification stage was implemented at the end of the test. After 5 minutes of walking on the treadmill at 3.0 mph, subjects began the test again at the speed and grade that immediately preceded their final stage and continued until volitional fatigue. Oxygen uptake (VO$_2$) was continuously assessed using a CareFusion Vmax Encore (Yorba Linda, CA) metabolic cart. VO$_2$max was defined as the largest
30-second average in VO\textsubscript{2} that was achieved during the test.

**Physical Activity Assessment.** To determine the subjects’ physical activity levels, each participant wore an Actigraph accelerometer around the hip for seven days. The accelerometer was only removed during showering and sleeping. For inclusion of the data, the accelerometer had to record at least 10 hours of data on at least four of the seven days. From the valid days, the average minutes per day spent in moderate-to-vigorous physical activity (MVPA) was obtained. The use of an accelerometer to determine the physical activity levels of college students has been validated in previous literature\textsuperscript{10}.

**Serum GGT Assessment.** To determine the subjects’ serum GGT levels, venous blood draws were obtained the morning following a 10-hour fast. Blood was drawn from an antecubital vein into a 10 mL Vacutainer (Becton Dickenson, Franklin Lakes, NJ) serum blood collection tube and was allowed to clot for 30 minutes. The blood samples were then centrifuged at 2000 x g for 10 minutes in a refrigerated centrifuge. The resulting serum was stored at −80°C until analysis. Serum GGT was assayed in duplicate using a commercially available colorimetric assay (Biomatik, Wilmington, DE).

**Statistical Analysis.** To minimize gender differences, VO\textsubscript{2\text{max}} was expressed relative to lean body mass (ml/kg ffm/min). Body fat %, VO\textsubscript{2\text{max}} (ml/kg FFM/min), Average MVPA, and serum GGT were compared between alcohol intake groups using an independent t-test. For the higher AUDIT group, Pearson correlation coefficients were used to establish the association between serum GGT and: Body fat %, VO\textsubscript{2\text{max}}, and average MVPA. A priori statistical significance was set at P <0.05.
Results

There were no significant differences between the higher and lower AUDIT groups with respect to serum GGT, body fat %, VO$_{2\text{max}}$, and MVPA, as shown in Table 2. Within the higher AUDIT group, there was no significant relationship between VO$_{2\text{max}}$ and serum GGT ($r = -0.38$, $p = 0.31$) or body fat % and serum GGT ($r = -0.52$, $p = 0.16$). There was a trend towards a correlation between average daily MVPA and serum GGT ($r = -0.65$, $p = 0.06$, Figure 1).

Discussion

In the present study, serum GGT levels in the lower and higher AUDIT groups were not significantly different. The serum GGT levels in both the lower and higher AUDIT group reflected female serum GGT levels in the 50$^{\text{th}}$ to the 75$^{\text{th}}$ percentile and male serum GGT levels in the 25$^{\text{th}}$ to the 50$^{\text{th}}$ percentiles $^{11}$. The similar GGT levels between groups conflicts with findings from Sharp et al. and Alatalo et al. in which serum GGT was positively associated with alcohol intake $^{3,4}$.

It is possible that the similarities in serum GGT between the AUDIT groups were the result of similar body fat % between the two groups. A study by Jong Choi in which the serum activities of several liver enzymes were measured from 732 subjects found that the serum GGT levels in both men and women with high fatness were significantly higher than those with low fatness $^{12}$. Because the body fat percentages of the two AUDIT groups in the present study were not significantly different, this could have driven the similar serum GGT values observed between the two groups. Additionally, the VO$_{2\text{max}}$ levels between the two groups were not different. Devries et al. evaluated VO$_{\text{peak}}$ and serum GGT levels of 20 obese and 21 lean men and women before and after 12 weeks of endurance training. VO$_{\text{peak}}$ and serum GGT in the lean and obese male groups were significantly increased and decreased respectively after training and
these effects were independent of weight loss. The subjects used for the present study all had relatively high VO$_{2\text{max}}$ values, regardless of alcohol intake. Thus, it is possible that the high fitness levels in the high AUDIT group were protective against elevated serum GGT levels.

Correlation between serum GGT and fitness related variables was confined to the higher AUDIT group to isolate the effects of fitness in individuals with potentially harmful/hazardous drinking habits. We observed no correlation within the high AUDIT group between serum GGT and body fat % or VO$_{2\text{max}}$. There was a negative (though not significant) correlation ($p = 0.06$) between serum GGT and MVPA which suggests that chronic physical activity levels may combat some of the negative effects of alcohol on the liver independent of having high cardiorespiratory fitness; however, further study is needed to fully examine this relationship. A post-hoc sample size calculation revealed that 16 subjects in the higher AUDIT group would be necessary to obtain statistical significance.

One limitation of the present study was that the two alcohol intake groups were imbalanced with respect to gender. Previous literature has shown that the baseline serum GGT levels of men and women are different such that a gender effect should be examined in this context. Future studies should use a large enough sample to capture gender effects. It is likely that having more males and fewer females in the lower and higher AUDIT groups, respectively, would increase serum GGT in the former and decrease serum GGT in the latter. This would tend to make our groups even more similar with respect to serum GGT. This is the first known study to evaluate the effects of fitness related variables on serum GGT. Future cross-sectional studies comparing active and inactive groups would also help elucidate the potential effects of chronic physical activity and physical fitness. Finally, longitudinal training studies should be done to elucidate the role of activity and/or cardiorespiratory fitness on serum GGT.
In conclusion, in the present study, there were no differences found in serum GGT, % body fat, VO$_{2\text{max}}$, and average MVPA between the high and low AUDIT groups. There was a trend towards a correlation between serum GGT and average MVPA within the high AUDIT group that encourages more data collection for this project.
Manuscript References


   doi:10.11604/pamj.2009.3.17.125


   doi:10.3390/biom5043295


Table 1. Mean ± SD of age, height, weight, and ratio of males to females in the lower (n = 7) and higher (n = 9) AUDIT groups.

<table>
<thead>
<tr>
<th></th>
<th>AUDIT &lt; 8</th>
<th>AUDIT ≥ 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>21.29 ± 0.18</td>
<td>21.65 ± 0.242</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.81 ± 3.44</td>
<td>172.88 ± 3.08</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.43 ± 3.31</td>
<td>74.45 ± 3.96</td>
</tr>
<tr>
<td>Males/Females</td>
<td>2/5</td>
<td>5/4</td>
</tr>
</tbody>
</table>

Table 2. Mean ± SD of serum GGT, body fat %, VO$_{2\text{max}}$, and average moderate-to-vigorous physical activity (MVPA) for the lower and higher AUDIT score groups.

<table>
<thead>
<tr>
<th></th>
<th>AUDIT &lt; 8</th>
<th>AUDIT ≥ 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GGT (U/L)</td>
<td>13.93 ± 1.96</td>
<td>15.94 ± 3.33</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>26.70 ± 7.96</td>
<td>22.86 ± 4.05</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (mL/kgFFM/min)</td>
<td>62.37 ± 11.09</td>
<td>62.02 ± 5.16</td>
</tr>
<tr>
<td>MVPA (mins/day)</td>
<td>45.15 ± 23.55</td>
<td>49.99 ± 16.82</td>
</tr>
</tbody>
</table>

Figure 1. Relationship between average MVPA per day and serum GGT in individuals with an AUDIT score ≥ 8. (r = -0.65, p =0.06)
Appendix A

Informed Consent Form
Consent to Participate in Research

Identification of Investigators & Purpose of Study
You are being asked to participate in a research study conducted by Gillian Mackey, Jessica Irons, Danielle Valenti and Chris Womack from James Madison University. The purpose of this study is to determine if physical activity and/or physical fitness impacts early signs of liver damage from alcohol consumption. This study will contribute to completion of Gillian Mackey’s Honors Thesis.

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. In addition, you will be asked to complete a health history questionnaire that includes your known history of disease, medical procedures, and medications. This study consists of two visits to the Human Performance Laboratory in Godwin Hall, Room 209. For the first visit, you will complete a survey that allows us to obtain information on your alcohol intake. Additionally, you will perform a treadmill test that allows us to determine the maximal amount of oxygen that your body is capable of using (VO$_{2\text{max}}$). This is a good evaluation of your cardiovascular system’s ability to supply blood to your working muscles. For this visit, you will be asked to refrain from eating or drinking anything except water for three hours prior to the test. Following your first visit, you will receive a physical activity tracking device that you will wear over a seven-day period. You will be given access to $10 if you wear the device (as evidenced by our electronic data collection) 80% (or more) of the time during this seven-day period. In addition, once you return the device you will be entered into a drawing to win a $100 prize. You will return it to the laboratory one week later for your second visit, which will involve assessment of your body fat percentage and a blood draw. For this visit, you will be asked to refrain from eating or drinking anything except water for 10 hours prior to reporting to the lab. You will also be asked to refrain from consuming alcoholic beverages for 24 hours prior to reporting.

$VO_{2\text{max}}$ Test (1st visit): To determine your cardiorespiratory endurance, you will begin walking on a treadmill at 2.5 miles/hour. The speed of the treadmill will increase every minute until you reach 6.0 miles/hour. After that, the elevation (grade) of the treadmill will increase by 3% per minute until you indicate that you can no longer continue. After a 5-minute rest, you will resume the test at the intensity that preceded your highest intensity achieved during the test. We will continue the test in the same manner until you indicate that you can no longer continue. Throughout the test, you will be breathing through a mouthpiece so that we can collect and analyze your expired air for oxygen content. You will also wear a strap around your chest so that we can monitor your heart rate.

DEXA Scan (2nd visit): Your body fat percentage will be determined by a dual-energy x-ray absorptiometry (DEXA) scan. This will involve lying on a cushioned table while the machine scans your entire body with a low-level x-ray.

Blood Draw (2nd visit): After sitting in a chair for 15 minutes, we will obtain blood samples from an arm vein. A total of 15 ml (about 1 tablespoon) of blood will be collected. These blood samples will be used to assess levels of a marker of liver damage, your blood lipid and cholesterol levels and markers of your blood’s tendency to clot.

Time Required
Your participation will require two sessions over the course of a week. It is estimated that each session will take about 45 minutes each for a total of 90 minutes for the entire study.

Potential Risks
The investigator perceives the following are possible risks arising from your involvement with this study. Venous blood draws involve the risk of pain, bruising and, in rare instances, infection. All of our investigators that draw blood for our research studies are trained to minimize these risks. Research on risk of exercise testing has suggested that approximately six cardiac events occur for every 10,000 exercise tests. The risk of death is even less, with a rate of approximately one death per 1,000,000 tests. This is likely to be even less in college-aged individuals. In the
unlikely event of an event, at least one investigator will be CPR-trained at every test. The DEXA test involves exposure to low-level radiation. This exposure is approximately the same as the exposure from a flight across the United States.

Potential Benefits
Potential benefits from participation in this study include feedback on your current level of cardiorespiratory fitness and body composition. In addition to your actual scores, you will be given established norms for both fitness-related variables. The overall benefit of the study may include furthering knowledge about how physical activity and physical fitness may impact the effects of alcohol. Participants enrolled in PSYC 101 or PSYC 160 at the time of participation will receive 3-credit hours for participation that will be allocated via the Psychology Participant Pool (Sona Systems). You will also have the opportunity to earn $10 for wearing an activity device for 7 days as instructed and included in a drawing to win an additional $100 prize. The exact odds of winning the $100 prize are unknown until we know how many participants enroll in our study; however, participants will only be entered into the drawing ONCE if they wear the device for 80% (or more) of the time as instructed AND return the device as requested.

Confidentiality
The results of this research will be presented at relevant regional and national/international conferences. Our findings will also be published in relevant research journals and/or books in the field of exercise science. 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. 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 researcher. 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:

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James Madison University
mackeygo@dukes.jmu.edu

Christopher Womack
Department of Kinesiology
James Madison University
womackcx@jmu.edu

Telephone: (540) 568-6515

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

Alcohol Use Disorders Identification Test (AUDIT)
Alcohol Use Disorders Identification Test (AUDIT)

1. How often do you have a drink containing alcohol?

2. How many drinks containing alcohol do you have on a typical day when you are drinking?

3. How often do you have six or more drinks on one occasion?

4. How often during the last year have you found that you were not able to stop drinking once you had started?

5. How often during the last year have you failed to do what was normally expected from you because of drinking?

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?

7. How often during the last year have you had a feeling of guilt or remorse after drinking?

8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?

9. Have you or someone else been injured as a result of your drinking?

10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?
Appendix C

\( \text{VO}_{2\text{max}} \) Data Sheet
Subject #- Temp-

Date- Rh-

Height (cm)- Pb-

Weight (kg)- Region %Fat- BMD t-score-

Age-

<table>
<thead>
<tr>
<th>Time</th>
<th>Speed (mph)</th>
<th>Elevation (%)</th>
<th>HR</th>
<th>RPE</th>
<th>VO₂ (L/min)</th>
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Exercise test duration:

Difference between VO₂ in last 2 full stages:

Validation Stage

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<th>Speed</th>
<th>Elevation</th>
<th>VO₂</th>
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Validation Stage duration:

VO₂max from validation stage? (Y/N):

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<th>MAX VO₂ (L/min)</th>
<th>MAX RQ</th>
<th>MAX RPE</th>
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