Fall 2016

The hemostatic effects of acute exposure to colored cornstarch powder during a 5k run

Robert C. Allsbrook
James Madison University

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The Hemostatic Effects of Acute Exposure to Colored Cornstarch Powder During a 5k Run

An Honors Program Project Presented to
The Faculty of the Undergraduate
College of Health and Behavior Sciences
James Madison University

by Robert Cary Allsbrook

December 2016

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

FACULTY COMMITTEE:  HONORS PROGRAM APPROVAL:

___________________________________________
Project Advisor: Christopher Womack, Ph.D.,  Bradley R. Newcomer, Ph.D.,
Department Head, Exercise Science  Director, Honors Program

___________________________________________
Reader: Michael Saunders, Ph.D.,  Reader: Elizabeth Edwards, Ph.D.,
Director, Human Performance Laboratories  Executive Director, Morrison Bruce Center

PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at Godwin Hall, 336 on 12/9/16 at 10:00 a.m.
The Hemostatic Effects of Acute Exposure to Colored Cornstarch Powder During a 5k Run

Robert Cary Allsbrook

Undergraduate Honor’s Thesis

December 2016
# Table of Contents

Acknowledgements 
List of Tables 
List of Figures 
Abstract 
Chapter 1: Introduction 
Chapter 2: Methods 
Chapter 3: Manuscript 
Manuscript References 
Tables 
Figure Legend 
Figures 
Appendices
Acknowledgements

I would like to thank Dr. Womack for his continued help throughout this process. Dr. Womack helped to make this process engaging, understandable, and enjoyable. Without his support, I would have been unable to complete this process. I would also like to thank my readers, Dr. Saunders and Dr. Edwards for their help in the process. Dr. Saunders was the impetus for this process and both individuals have been a great source of support and knowledge throughout my undergraduate career at James Madison University. Lastly, I would like to thank Jonathan Moquin who completed the accompanying study titled *The physiologic effect of acute exposure to colored cornstarch powder during a 5k run.*
List of Tables

Table 1. Descriptive data for all recreationally trained subjects. 25
List of Figures

Figure 1. PAI-1 activity pre-exercise and post-exercise for the no-color and color run condition. 27

Figure 2. FVIII antigen pre-exercise and post-exercise for the no-color and color run conditions. 28

Figure 3. tPA activity pre-exercise and post-exercise for the no-color and color run conditions. 29
Abstract

PURPOSE: To examine the acute hemostatic effects of particulate matter (PM) in the form of colored corn starch powder during a 5 kilometer race. METHODS: 10 recreationally active adults completed two 5k runs, one with color and one without color. 10 mL blood samples were taken 6 hours prior to the trial and immediately following the trial. PAI-1 activity, FVIII antigen, and tPA activity were measured using an ELISA. RESULTS: No significant main effects or interaction effects (P<0.05) were observed among any of the variables although a trend (P = 0.082) was observed for increased PAI-1 activity during exercise in the color condition. CONCLUSIONS: There is a trend towards increased PAI-1 activity levels during exercise during color runs that could be due to increased inflammation. Furthermore, the lack of increased FVIII:ag and tPA activity suggests that the intensity of the exercise may not have been adequate and further research should be conducted in this area.
Chapter One

Expanded Introduction

Hemostatic imbalance can contribute to ischemic events resulting from cardiovascular disease (CVD). CVD typically develops gradually and can appear in the form of acute ischemic events, including, but not limited to, stroke, myocardial infarction, and transient cerebrovascular and cardiovascular ischemic events (36). In many of these events, the plaque ruptures and a thrombus is formed inside the blood vessel lumen, causing arterial occlusion. Autopsy studies have revealed ruptured atherosclerotic plaques in people without any prior history or evidence of a myocardial infarction, suggesting that plaque rupture must develop in prothrombotic conditions for an ischemic event to occur (9, 23).

While exercise has many clinical benefits, it also acutely increases risk for an ischemic event. The leading cause of death for individuals 40 years and older during exercise is coronary artery disease (25). Likewise, physical activity has been noted as the cause of one third of discernable myocardial infarctions (31). Moreover, physical stress has been seen to increase the incidence of ischemic events caused by an occlusive thrombus (14). These studies suggest that the coagulatory and fibrinolytic responses that occur during exercise may play an important role in the risk associated with acute physical exercise. Exercise has a profound impact on hemostasis, in part due to an increase in Factor VIII antigen and coagulation potential. While the exact manner in which exercise increases Factor VIII is not completely understood, various ideas have been proposed (13). Exercise may stimulate the release of newly synthesized Factor VIII and/or increase thrombin production, promoting Factor VIII formation (12, 19). Lastly, exercise may increase the adrenergic stimulation of receptors, leading to Factor VIII creation (6). tPA promotes the activation of plasmin from its inactive form, plasminogen. Plasmin is responsible for the degradation of fibrin clots (2, 3, 24, 27, 32). During exercise, both fibrinolysis and coagulation levels are elevated, as noted by increases in both tissue plasminogen...
activator (tPA) and plasminogen activator inhibitor (PAI-1), however upon the cessation of exercise fibrinolysis quickly returns to baseline while coagulation remains elevated for some time (10). This post-exercise imbalance favors increased coagulation potential, resulting in a pro-thrombotic environment.

Exposure to high concentrations of air pollution in the form of particulate matter (PM) can lead to serious health implications. Particulate matter falls into various classifications, grouped according to how the particle behaves in air, its shape, and most importantly, diameter. Particles smaller than 10 μm in diameter are considered respirable particles and are further classified into three subcategories: coarse (2.5-10 μm), fine (1-2.5 μm), and ultrafine (<1 μm). The diameter of the particle is inversely related to the potential harm (7, 28).

Inhalation of large enough quantities of particulate matter could lead to inflammation of the lungs. When present, PM will be breathed in during normal inhalation. Larger particles (>10 μm) are commonly expelled via the defense systems of the lungs, causing no further harm, but smaller particles (<10 μm) are more often deposited into the pulmonary interstitial tissue. The deposition of respirable particles causes a failure of alveolar macrophages to phagocytose the smaller particles, allowing them to remain in the alveoli. This deposition commonly leads to an inflammatory response (29).

Inflammation is the normal bodily response to foreign substances or blood vessel damage and is often felt as pain or irritation in the afflicted area. During inflammation, platelets and leukocytes express tissue factor (TF), a thrombin activator. Thrombin cleaves fibrinogen to fibrin and activates factor XIII to form factor XIIIa, causing the cross-linkage of fibrin. Typically, fibrin restores structure and function to the injured tissue, resulting in decreased inflammation. However, excess deposition of fibrin in the interstitium of the lungs can cause a pro-inflammatory environment. Pulmonary inflammation then stimulates a pro-thrombotic environment since coagulation is initiated when TF is expressed (15, 18, 20, 22, 26, 30).
Factor VIII, one of the many proteins responsible for coagulation, levels are expected to increase during inflammation since inflammation typically leads to a hemostatic response. Fibrinolysis can be negatively impacted by acute inflammation as inflammation causes an increase in PAI-1(4). In a study by Wang et al. of 84 chronic obstructive pulmonary disease (COPD) patients and 51 healthy participants, PAI-1 levels were significantly higher in COPD patients compared to the control group, signifying that PAI-1 levels are related to systemic inflammation (33). Since PAI-1 is the major inhibitor of tPA, increased PAI-1 levels lead to increased potential for blood clotting (5, 8). The combination of these factors cause an increase in coagulation and a decrease in fibrinolytic activity during inflammation (16).

One event that is growing in global popularity combines both particulate matter exposure and exercise: the color run. Color runs are a form of 5k in which PM in the form of colored corn starch powder is thrown at participants. Thus, these races involve voluntary exposure to high levels of PM while running. The hemostatic response to exercise is well documented, but the potential additional effects of this voluntary exposure to PM are not well known. Color runs may have an additional effect on the acute inflammatory response to exercise, which may subsequently affect the hemostatic response.

**Purpose:**

The purpose of this study is to examine the acute hemostatic effects of particulate matter (PM) in the form of colored corn starch powder during a 5 kilometer race. We hypothesize that increased PM will result in increased levels of Factor VIII antigen, PAI-1 activity, and tPA antigen while decreasing tPA activity.
Chapter Two

Methods

Subjects

Twenty recreationally active adults between the ages of 18 and 50 will be recruited from James Madison University for participation in the present study via mass emails to students, faculty and employees of the university as well as placement of advertisement fliers at the university’s recreation center. Subjects must meet the following criteria for inclusion in the study: no history of chronic hypertension, cardiovascular disease, pulmonary disease or allergies; no history of respiratory illness within the past six weeks; nonsmoker for at least the previous five years; and no medications prescribed by a physician that could alter blood pressure. Additionally, subjects will be instructed to abstain from caffeine ingestion and vigorous physical activity for at least 24 hours prior to testing. Prior to participation, subjects will be informed both verbally and in writing of the study procedures as well as any potential risks and benefits associated with participation after which subjects will be requested to sign a statement of informed consent if they elect to proceed. Study procedures will be approved by the James Madison University Institutional Review Board prior to the onset of the study.

Experimental Design

Subjects will be preliminarily screened in the James Madison University Human Performance Laboratory at which time subject screening and risk factor assessment will be performed (Attachments 1 & 2). Subjects will also be provided with an explanation of experimental design and procedures at this time and informed consent will be requested.

Subjects who meet the entry criteria will perform a graded exercise test on a treadmill to assess VO$_{2\text{max}}$ and complete familiarization trials for pulmonary function testing and recording of dietary intake (Attachment 3). Following this preliminary session, each subject will then complete two
exercise trials separated by a minimum of 7 days. The first trial will consist of subjects completing a 5k run around an outdoor loop of 400 meters. The second trial will consist of subjects completing a simulated color-run by performing a 5k run on the same course while being exposed to particulate matter in the form of a colored cornstarch powder (CP) every kilometer. Trials will be randomly counterbalanced and split times will be used to insure consistent pacing between trials for each subject. Ambient environmental conditions will be monitored for consistency between trials with acceptable ambient temperatures during exercise of 7-25°C, average wind speed less than 10 mph and relative humidity below 70%. In addition, repeated trials will occur in consistent temperatures for each subject (within ± 5 °C).

Particulate Exposure

Particulate matter will be administered by 8-10 volunteers placed on both sides of the track covering approximately 20 feet of the course. CP will be released into the air as subjects complete each kilometer with a double dose released at finish line of the course, to simulate conditions during color runs. Exact amounts of CP released at each interval remain to be determined, though an average .6-1.0 lb per person is considered typical for most races (17).

Blood Sampling

Prior to sampling, subjects will assume a consistent, semi-recumbent position. Venous blood will be sampled from an antecubital vein 6 hours prior to exercise, immediately following the exercise trial, and 24 hours post-exercise (± 4 hr, held constant for each subject). The pre and 24-hour post blood draws will occur in the morning, following a 12-hour overnight fast. Subjects will be instructed to maintain a consistent diet and abstain from alcohol and tobacco during interval between blood draws. Compliance will be verified using a self-recorded dietary intake record. Baseline venipuncture
will occur in a laboratory setting following 15 minutes of supine rest. Initial post-exercise venipuncture will occur in the field prior to PFT. Final post-exercise measurement will occur in laboratory setting following 15 minutes of supine rest. Following sterile preparation with alcohol, approximately 10mL of whole blood will be obtained in sodium citrate collection tubes. Samples will be centrifuged at 10,000 rpm for 20 minutes at 4°C to separate serum from plasma, both of which will be stored at -80°C for later analysis.

Fibrinolytic potential will be determined by assaying for tissue plasminogen activator (tPA) activity and antigen and plasminogen activator inhibitor-1 (PAI-1) activity. tPA activity will be measured using an amidolytic activity assay (Biopool International; Ventura, Calif.). Factor VIII antigen (FVIII:ag), tPA activity, and PAI-1 activity will be measured by an enzyme-linked immunosorbent activity assay (ELISA) (American Diagnostica, Greenwich, Conn., and Biopool International respectively).

Dietary and Exercise Controls

Subjects will be instructed to avoid heavy physical activity for at least 24 hours prior to each exercise trial as well as over the seven-day period between trials. Subjects will also be instructed to abstain from ingestion of any tobacco or caffeine products for at least 24 hours prior to both trials. Dietary consistency during 24-hour interval between blood draws should be limited to low fat meals with no ingestion of tobacco or caffeine products.

Statistical Analysis:

Repeated Measures Analysis of Variance will be used to assess main effects of exercise (pre vs. post) and condition (color vs. no color), as well as the interaction between these two effects. Post-hoc
tests will be performed using t-tests to detect changes in Factor VIII antigen, tPA activity, and PAI-1 activity. A priori significance will be set at P < 0.05.
Chapter Three: Manuscript

Introduction

While exercise has many benefits, it also acutely increases risk for an ischemic event. The leading cause of death for individuals 40 years and older during exercise is coronary artery disease (25). Likewise, physical activity has been noted as the cause of one third of discernable myocardial infarctions (31). Moreover, physical stress has been seen to increase the incidence of ischemic events caused by an occlusive thrombus (14). These studies suggest that the coagulatory and fibrinolytic responses that occur during exercise may play an important role in the risk associated with acute physical exercise.

Exposure to high concentrations of particulate matter (PM) can potentially affect the inflammatory response to exercise. Particles smaller than 10 um in diameter are considered respirable particles and are often deposited into the interstitial tissue of the lung, causing a failure of alveolar macrophages to phagocytose these particles. This deposition commonly leads to an inflammatory response (29). Factor VIII, one of the many proteins responsible for coagulation, levels are expected to increase during inflammation since inflammation typically leads to a hemostatic response (34). The process of fibrinolysis is responsible for the prevention of excess clot formation. A major enzyme responsible for fibrinolysis is tissue plasminogen activator (tPA). tPA promotes the activation of plasmin from its inactive form, plasminogen. Plasmin is responsible for the degradation of fibrin clots (2, 3, 24, 27, 32). Fibrinolysis can be negatively impacted by acute inflammation. Inflammation causes an increase in plasminogen activator inhibitor (PAI-1), the major circulating inhibitor of tPA (5, 8). The combination of these factors could cause an increase in coagulation and a decrease in fibrinolytic activity in response inflammation (16). Thus, exercising in an environment filled with particulate matter could potentially lead to an even greater inflammatory and hemostatic response.
One event that is growing in global popularity combines both particulate matter exposure and exercise: the color run. Color runs are a form of 5k in which colored corn starch powder (PM) is thrown at participants. These races involve voluntary exposure to high levels of PM while running. The hemostatic response to exercise is well documented, but the effects of this voluntary exposure to particulate matter while exercising are not well known. The purpose of this study was to evaluate the hemostatic response to a color run.

Methods

Subjects

Ten recreationally active adults (3 males, 7 females) participated in the current study. Descriptive characteristics for the participants are shown in Table 1. Subjects met the following criteria for inclusion in the study: no history of chronic hypertension, cardiovascular disease, pulmonary disease, or allergies; no history of respiratory illness within the past six weeks; nonsmoker for at least the previous five years; and not on medications prescribed by a physician that could alter blood pressure. Additionally, subjects were instructed to abstain from caffeine ingestion and vigorous physical activity for at least 24 hours prior to testing. Prior to participation, subjects were informed both verbally and in writing of the study procedures as well as any potential risks and benefits associated with participation after which subjects were requested to sign a statement of informed consent if they elected to proceed. Study procedures were approved by the James Madison University Institutional Review Board prior to the onset of the study.

Experimental Design

Subjects performed a graded exercise test on a treadmill to assess VO$_2$\text{max} and completed familiarization trials for pulmonary function testing and recording of dietary intake. Following this preliminary session, each subject completed two exercise trials separated by a minimum of 7 days.
The trials consisted of subjects completing a 5k run at a self-selected pace around an outdoor loop of 400 meters with and without exposure to particulate matter in the form of a colored cornstarch powder (CP) every kilometer. Trials were randomly counterbalanced and split times were used to insure consistent pacing between trials for each subject. Ambient environmental conditions were monitored for consistency between trials with acceptable ambient temperatures during exercise of 7-25°C, average wind speed less than 10 mph, and relative humidity below 70%. In addition, repeated trials occurred in consistent temperatures for each subject (within ± 5 °C).

*Particulate Exposure*

Particulate matter was administered by 8-10 volunteers placed on both sides of the track covering approximately 20 feet of the course. CP was released into the air as subjects completed each kilometer with a double dose released at finish line of the course, in order to simulate conditions during color runs.

*Blood Sampling*

Prior to sampling, subjects assumed a consistent, semi-recumbent position. Venous blood was sampled from an antecubital vein 6 hours prior to exercise and immediately following the exercise trial. The pre blood draws occurred in the morning, following a 12-hour overnight fast. Baseline venipuncture occurred in a laboratory setting following 15 minutes of supine rest. Initial post-exercise venipuncture occurred in the field. Following sterile preparation with alcohol, approximately 10mL of whole blood was obtained in sodium citrate collection tubes. Samples were centrifuged at 10,000 rpm for 20 minutes at 4°C to obtain plasma, which was stored at -80°C for later analysis.
Factor VIII antigen (FVIII:ag), tPA activity, and PAI-1 activity were measured by an enzyme-linked immunosorbent activity assay (ELISA) (Affinity Biological Ancaster, ON, CA, and Eagle Biosciences, Nashua, NH, respectively).

Dietary and Exercise Controls

Subjects were instructed to avoid heavy physical activity for at least 24 hours prior to each exercise trial as well as over the seven-day period between trials. Subjects were also instructed to abstain from ingestion of any tobacco or caffeine products for at least 24 hours prior to both trials.

Statistical Analysis:

Repeated Measures Analysis of Variance was used to assess main effects of exercise (pre vs. post) and condition (color vs. no color), as well as the interaction between these two effects. Post-hoc tests were performed using t-tests to detect changes in Factor VIII antigen, tPA activity, and PAI-1 activity. A priori significance was set at P <0.05.

Results

Figure 1 displays the data for the PAI-1 activity. There was no main effect for exercise or condition, or an interaction effect; although a trend (P = 0.082) was observed for increased PAI-1 activity during exercise in the color condition (CoPre = 1.79 ± 1.50 U/mL, CoPost = 3.01 ± 2.96 U/mL) as compared with the no-color condition (NcPre = 1.43 ± 1.20 U/mL, NcPost = 1.40 ± 1.19 U/mL) (Fig. 1). No significant main effects or interaction effects (P<0.05) were observed among any of the variables in figures 2 and 3 which display the data for FVIII:ag (NcPre = 1.75 ± 0.23 IU/mL, NcPost = 1.65 ± 0.50 IU/mL, CoPre = 1.73 ± 0.30 IU/mL, CoPost = 1.70 ± 0.26 IU/mL) and tPA
activity (NcPre = 0.17 ± 0.20 IU/mL, NcPost = 0.21 ± 0.22 IU/mL, CoPre = 0.20 ± 0.24 IU/mL, CoPost = 0.19 ± 0.27 IU/mL), respectively.

**Discussion**

While no statistical significance was found among the tests, the major finding of this study is that there was a trend for increased PAI-1 activity during exercise in the color condition (Fig. 1). This response is atypical, as various studies have shown decreases in PAI-1 activity following exercise (35, 37). The increase in PAI-1 activity could be attributed to an increase in inflammation (4). One study conducted by Kupchak et al. found that PAI-1 levels were elevated in resistance trained men after a body muscle-damaging workout that resulted in acute inflammation (21). Since PAI-1 is a marker of inflammation, it could be assumed that the PM thrown during a color run could lead to acute inflammation following the bout of exercise. Since statistical significance was not met in this study, further exploration is required.

Factor VIII antigen levels are expected to increase during exercise as coagulation potential increases. This study did not find increased levels of FVIII:ag despite the possibility of increased inflammation markers (Fig. 2). This could be due to inadequate exercise intensity levels, as the intensity of the exercise must be above a specific threshold in order to enact the inflammation response (11). In a study by Davis et al., ten healthy males were exercised to exhaustion on a bicycle ergometer. The results showed that FVIII activity showed little increase until the subjects reached lactate threshold, with major changes being observed at 95-100% of maximum heart rate. Our study was likely conducted below this threshold, as the 5k was performed at a relatively low self-selected pace.

Likewise, tPA activity levels generally rise during exercise to combat the increase in coagulation potential with an increase in fibrinolysis. No signs of increased tPA levels were seen in this study (Fig. 3). Similar to FVIII:ag, tPA production is dependent on exercise intensity. In a study
conducted by Womack et al., equicaloric bouts of exercise above and below lactate threshold were conducted that varied in duration. The study found that intensity was the greater factor in promoting an acute fibrinolytic response (38). While our study may not have been conducted at a high enough intensity to elicit a pronounced fibrinolytic response in terms of tPA, an increase, even if slight, should have been observed. To our knowledge, this is the only study to not show increases in FVIII:ag and tPA post-exercise.

The findings of this study warrant further exploration. Color runs are an up-and-coming form of exercise and could pose potentially harmful implications if the increased inflammatory response present in this study is present. If color runs continue on their upward trajectory in terms of participation, then participants could be at increased risk for these health complications.

One significant limitation of this study was the number of subjects. This raises the question that the trend seen for increased PAI-1 levels could be statistically significant if more subjects were present in the study. Another limitation of this study is the fact that the findings can only be attributed to a 5 kilometer run at the assigned intensity, with these races being typically run at a higher relative intensity than the subjects employed in this study. The results of this study are not generalizable to other distances, intensities, or modes of exercise.

**Conclusion**

In summary, our findings suggest that there is a trend towards increased PAI-1 activity levels during exercise during color runs that could be due to increased inflammation. Furthermore, the lack of increased FVIII:ag and tPA suggests that the intensity of the exercise may not have been adequate and further research should be conducted in this area.
Manuscript References


Table 1. Mean (± SD) height (cm), weight (kg), age (years), and VO$_{2\text{max}}$ (mL/kg/min) of 10 recreationally trained subjects (3 male and 7 female).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>167.3</td>
<td>6.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.5</td>
<td>6.0</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (mL/kg/min)</td>
<td>51.3</td>
<td>9.6</td>
</tr>
</tbody>
</table>
**Figure Legend:**

Figure 1. Mean (± SE) PAI-1 activity pre-exercise (white) and post-exercise (black) for the no-color and color run conditions.

Figure 2. Mean (± SE) Factor VIII antigen pre-exercise (white) and post-exercise (black) for the no-color and color run conditions.

Figure 3. Mean (± SE) tPA activity pre-exercise (white) and post-exercise (black) for the no-color and color run conditions.
Figure 1.
Figure 2.
Figure 3.
Attachment 1

Subject Prescreening Information

Subject #: ____________

Age: _____    Sex: ________

Height: ________    Weight: __________

Average Exercise Habits over the Past 2 Months:

Avg. # days of exercise per week: ______________

Avg. # of days of aerobic exercise per week: ______________

Avg. # of days of running per week: ______________

Allergies: ________________________________

Medications used: ________________________________

Recent respiratory illnesses: ________________________________

Smoking history: ________________________________
Attachment 2

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire

Assess your health status by marking all true statements

History
You have had:

- a heart attack
- heart surgery
- cardiac catheterization
- coronary angioplasty (PTCA)
- pacemaker/implantable cardiac
- defibrillator/rhythm disturbance
- heart valve disease
- heart failure
- heart transplantation
- congenital heart disease

Symptoms
You experience:

- chest discomfort with exertion
- unreasonable breathlessness
- dizziness, fainting, or blackouts
- take heart medications

Other Health Issues
You have:

- diabetes
- asthma or other lung disease
- burning or cramping sensation in your lower legs when walking short distances
- musculoskeletal problems that limit your physical activity
- concerns about the safety of exercise
- take prescription medication(s)

Cardiovascular risk factors
You are:

- a man older than 45 years
- a woman older than 55 years, have had a hysterectomy, or are postmenopausal
- smoke, or quit smoking within the previous 6 months
- your blood pressure is > 140/90 mmHg
- do not know your blood pressure
- take blood pressure medication
- your blood cholesterol level is > 200 mg/dl
- do not know your cholesterol level
- have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)
- are physically inactive (i.e., you get < 30 minutes of physical activity on at least 3 days of the week)
- are > 20 pounds overweight

If the marked statements indicate a history of illness or a condition that might be affected by exercise, you may need to use a facility with a medically qualified staff.

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a professionally qualified exercise staff to guide your exercise program.

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.
## 24-HOUR DIET RECORD

**Subject number**__________  **Date**____________  **Day of Week**__________

<table>
<thead>
<tr>
<th>Time</th>
<th>Food and/or Drink</th>
<th>Method of Preparation</th>
<th>Quantity Consumed</th>
<th>Brand Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment*. 2nd ed. United States of America: Mosby; 1996
INSTRUCTIONS FOR KEEPING YOUR 24-HOUR FOOD RECORD

Keep your record for three days per trial. You will include the day before, the day of, and the day after each trial. Include all meals, snacks, nibbling, and beverages including water and cocktails.

1. Fill out the date and day of the week at the top of food record sheet

2. Record the time you consumed your food and/or drink. To be most accurate, fill out the food record as soon as you finish eating.

3. List the first food and/or drink you consumed when you began your day and continue to record until you consume your last food and/or drink of your day (usually before bedtime)

4. List each food and/or drink on a separate line
   Example: cereal with milk, cereal and milk should each be on separate lines
   spaghetti, noodles and sauce should each be on separate lines

Combination foods:
List parts of food on separate lines
Include preparation method, quantity, and brand name of each food
Example: Sandwich (4 oz healthy choice turkey, 2 slices Sara Lee wheat bread, 1 tbsp Hellman’s light mayo, 2 oz Kraft American cheese, 1 slice of red fresh tomato)

5. Record the method of preparation
   Example: fried, baked, grilled
   - salt, oil (olive, canola, corn, other) butter or margarine, spices, etc.

6. Record quantity consumed
   Do not record any food not eaten
   Example: made 2 cups of vegetables but ate half so you would record 1 cup

   Quantity of food and/or drink
   Example: cups, ounces, liters, grams, each, or other unit of measure
   Example: 1 cup of vegetables, 4 ounces of meat, one medium apple

7. Record brand name
   Example: fast food chain name and/or package name
   Example: Wendy’s, Betty Crocker, Lean Cuisine, Gatorade, Thomas Bagel

8. Place any helpful food labels in manila envelope that is attached to folder
<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beverages</strong></td>
<td>Sugar or creamer?</td>
</tr>
<tr>
<td></td>
<td>Regular or sugar-free?</td>
</tr>
<tr>
<td></td>
<td>Alcohol content?</td>
</tr>
<tr>
<td></td>
<td>Name of drink and ingredients (if mixed drink)</td>
</tr>
<tr>
<td><strong>Breads</strong></td>
<td>Butter or margarine added?</td>
</tr>
<tr>
<td><strong>Cereal/Milk</strong></td>
<td>Milk, sugar, or fruit added?</td>
</tr>
<tr>
<td></td>
<td>The type of milk? (skim, 1%, 2%, whole)</td>
</tr>
<tr>
<td></td>
<td>Cereal: dry or cooked measure?</td>
</tr>
<tr>
<td><strong>Dairy</strong></td>
<td>Is yogurt fruited or plain?</td>
</tr>
<tr>
<td></td>
<td>% fat of milk or yogurt?</td>
</tr>
<tr>
<td></td>
<td>Indicate brand name of cheese substitute and/or nondairy creamer.</td>
</tr>
<tr>
<td><strong>Desserts</strong></td>
<td>Whipped topping added?</td>
</tr>
<tr>
<td></td>
<td>Frosting?</td>
</tr>
<tr>
<td></td>
<td>Fat modified (i.e., reduced)?</td>
</tr>
<tr>
<td></td>
<td>Sugar-free?</td>
</tr>
<tr>
<td><strong>Eggs</strong></td>
<td>Preparation method (scrambled, hard-boiled, etc)?</td>
</tr>
<tr>
<td></td>
<td>Fat used in cooking?</td>
</tr>
<tr>
<td><strong>Fast Food</strong></td>
<td>What restaurant?</td>
</tr>
<tr>
<td></td>
<td>If not a national fast food chain, describe food in detail</td>
</tr>
<tr>
<td></td>
<td>Size order of fries? Super-size?</td>
</tr>
<tr>
<td></td>
<td>Extra toppings on sandwich?</td>
</tr>
<tr>
<td><strong>Fats/Oils</strong></td>
<td>Regular or salt-free?</td>
</tr>
<tr>
<td></td>
<td>Stick, tub, or liquid margarine?</td>
</tr>
<tr>
<td></td>
<td>Reduced calorie or diet product?</td>
</tr>
<tr>
<td><strong>Fish</strong></td>
<td>Water or oil packed (fresh or canned)?</td>
</tr>
<tr>
<td></td>
<td>Baked or fried (With batter or without)?</td>
</tr>
<tr>
<td></td>
<td>Type of fat added?</td>
</tr>
<tr>
<td></td>
<td>Raw or cooked weight?</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td>Sweetened or unsweetened?</td>
</tr>
<tr>
<td></td>
<td>Fresh, canned, or frozen?</td>
</tr>
<tr>
<td></td>
<td>With or without skin?</td>
</tr>
<tr>
<td><strong>Meats</strong></td>
<td>Visible fat removed?</td>
</tr>
<tr>
<td></td>
<td>Light or dark meat? Raw or cooked?</td>
</tr>
<tr>
<td><strong>Sugars and Sweets</strong></td>
<td>Regular or reduced-calorie?</td>
</tr>
<tr>
<td></td>
<td>Don't forget hard candy as well as chocolate.</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td>Raw or cooked?</td>
</tr>
<tr>
<td></td>
<td>Fresh, frozen, or canned?</td>
</tr>
<tr>
<td></td>
<td>Low-sodium or regular?</td>
</tr>
<tr>
<td></td>
<td>Added fat or sauce?</td>
</tr>
</tbody>
</table>
Helpful Hints with Portion Sizes

- 1 teaspoon (5 ml)
  - about the size of the top half / tip of your thumb
- 1 oz (28 g)
  - approximately inch cube of cheese
  - volume of four stacked dice
  - slice of cheese is about the size of a 3 1/2 inch computer disk
  - chunk of cheese is about as thick as 2 dominoes
  - 1 handful (palm) of nuts
- 2 ounces (57 g)
  - 1 small chicken leg or thigh
  - 1/2 cup of cottage cheese or tuna
- 3 ounces (85 g)
  - serving of meat is about the size of a deck of playing cards (3 exchanges)
  - the size of the palm of your hand
  - 1/2 of whole chicken breast
  - 1 medium pork chop
  - 1 small hamburger
  - unbreaded fish fillet
- 1/2 cup (118 ml)
  - fruit or vegetables can fit in the palm of your hand
  - about the volume of a tennis ball
- 1 cup (236 ml)
  - about the size of a woman's fist
  - breakfast cereal goes halfway up the side of a standard cereal bowl
  - broccoli is about the size of a light bulb
- 1 medium apple = A tennis ball