MARY P. MILES
STEPHANIE M.G. WILSON
MORGAN L. CHAMBERLIN

SPORTS NUTRITION LABORATORY MANUAL

MARY P. MILES · STEPHANIE M.G. WILSON · MORGAN L. CHAMBERLIN

SPORTS NUTRITION **LABORATORY MANUAL**

1st edition. Updated February 2022.

This manual was made possible through the Montana State University Library.

PREFACE

Knowledge without practice is useless. Practice without knowledge is dangerous.

Confucius

Our hope is that this lab manual provides a foundation for sports nutrition application in the laboratory setting. Our desire to create this resource stemmed from a paucity of resources when designing a sport nutrition laboratory at Montana State University. We believe the concepts covered in this laboratory manual will support, reinforce, and bring to life the fundamental concepts taught in upper division undergraduate sport nutrition courses. Providing this hands-on experience will help strengthen student's knowledge and application of the material. Laboratories are designed to provide students an opportunity to learn more about exercise intensity and substrate utilization, measure glycemic responses with or without exercise to selected food items, design their own sports drinks and test rehydration responses, and learn about their own metabolic health. These unique active learning experiences provide an enjoyable way for students to enhance learning.

This laboratory manual is designed to appeal to all students as they play an active role in generating data as research participants. As a result, the concepts covered in laboratory are highly relevant to students and may be applied to a wide range of exercise ability and interest levels. We believe this provides a greater understanding of nutrition concepts as sport nutrition texts tend to focus on the elite athlete. Additionally, this manual provides a step-by-step guide to strengthen student's ability to generate data and convey their results in a scientific manner. Appendix A provides detailed information needed to write a formal laboratory report. Free on-line resources are additionally available on GitHub to increase access to sports nutrition laboratory materials.

We hope that this manual solidifies concepts for students and garners enthusiasm on the topic and practice of sports nutrition.

Mary P. Miles
Stephanie M.G. Wilson
Morgan L. Chamberlin

ABOUT THE AUTHORS

Mary P. Miles received her Ph.D. from the University of Massachusetts Amherst and is a Professor of Exercise Science at Montana State University. Miles has taught sports nutrition courses at the undergraduate and graduate level for more than 20 years. She leads the Nutrition Research Laboratory at Montana State University. Miles, a longstanding member of the American College of Sports Medicine, has served as a past co-chair of the college's Nutrition Interest Group and has been an associate editor for Applied Physiology, Nutrition, and Metabolism since 2013.

Stephanie M.G. Wilson received her Masters Degree in Exercise and Nutrition Sciences at Montana State University, Bozeman. She is a PhD candidate with Dr. Mary P. Miles. Wilson joined the Nutrition Research Laboratory at Montana State University in 2015 and has been a member of the American College of Sports Medicine since 2016.

Morgan L. Chamberlin received her Masters Degree in Exercise and Nutrition Sciences at Montana State University, Bozeman. She is a PhD student with Dr. Mary P. Miles. Chamberlin joined the Nutrition Research Laboratory at Montana State University in 2017. Chamberlin is also a registered dietitian and is licensed in the state of MT.

Contributors: Miles, Wilson, Chamberlin
Illustration, Design, Video: Wilson

TABLE OF CONTENTS

LABORATORIES

APPENDICES

LIST OF FIGURES

LIST OF TABLES

LABORATORY SAFETY AND STANDARD OPERATING PROCEDURES

1.1 BACKGROUND

Safety protocols and standard operating procedures are fundamental aspects of laboratory work and are used while completing experimental procedures and using laboratory equipment. It is important that everyone in a laboratory is using the same procedures for cleaning, disinfecting, disposing of waste, and preventing exposures to biohazards. Further, all universities have policies and procedures for working in laboratories that each laboratory must adopt to ensure that federal, state, and local regulations are being followed and documented.

Laboratories are rated by the U.S. Department of Health and Human Services according to the level of containment that is needed for biorisk management [1]. Biosafety levels and biorisk management requirements correspond to the type of biohazards present in a laboratory. Blood, sweat, saliva and urine from individuals in the general population are biohazardous materials considered to be moderate-risk infectious agents that "pose a risk if accidentally inhaled, swallowed, or exposed to the skin" [1]. Laboratories in which potential exposure to blood (**Figure 1.1**), sweat, saliva, or urine can occur must follow biosafety level 2 (BSL-2) containment procedures. This laboratory class will involve activities in which individuals are likely to sweat, testing procedures in which saliva will accumulate on mouth pieces and in saliva traps, and sampling of blood via finger sticks for a variety of measures. Thus, it is important for students to understand and adhere to protocols for BSL-2 containment.

1.2 OBJECTIVE

The purpose of this laboratory is for students to learn universal precautions for BSL-2 handling and disposal of biohazardous materials and proper cleaning and disinfecting procedures to keep the laboratory safe.

Figure 1.1: **Blood is considered a biohazardous material and warrants BSL-2 containment procedures.** Vials of blood taken in the course of patient care at the National Institutes of Health Clinical Center in Bethesda, Maryland. Test tubes. Blood test. Creator: Daniel Sone, Unsplash.

1.3 OVERVIEW

Safety in a BSL-2 laboratory is achieved by following universal precautions, which means that you must:

- Treat all blood and other potentially infectious materials (OPIM) including saliva, sweat, and urine as potentially infectious.
- Use personal protective equipment (PPE) including gloves, masks, eye wear, and lab coats (**Figure 1.2**)
- Decontaminate surfaces that may have been exposed to blood and OPIM such as tables and counters and equipment.
- Wash hands and non-disposable items thoroughly.
- Dispose of contaminated materials appropriately.
    - Lined bins marked for biohazard disposal for non-sharps
    - Sharps containers marked for biohazard disposal - lancets (including retractable safety lancets), needles, pipette tips, and anything else that could puncture biohazard bags

Figure 1.2: **Gloves and laboratory coats are considered personal protective equipment (PPE).** "Lab group" by Trust Katsande, Unsplash.

1.4 PROCEDURES

1.4.1 Complete Bloodborne pathogens training

Bloodborne pathogens means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Complete your bloodborne pathogen training by going to the website in this link. Then, proceed to do the following:

1. Enter your personal information
2. Launch course, listen to and complete knowledge-check questions during the 30-minute presentation
3. Take and pass the quiz. You must retake until you achieve a score > 80%.
4. You will receive an email with a link to your completion certificate once you have passed the final quiz. Save a PDF of your certificate to submit.

1.4.2 Safety Procedures Demonstrations

Attend the following procedures:

1. Procedures for disinfecting and cleaning laboratory spaces and equipment:
    - Use simple green spray and paper towel to clean tabletops and items touched with hands before and after use.
    - Use a paper towel damp with isopropyl alcohol to wipe computer keyboards and mice before and after use.
    - Dispose of paper towels in regular trash bins (non-red).
2. Procedures for using and disposing of PPE:
    - **Students will be provided laboratory coats in cases where biohazards will be present.** Laboratory coats are only to be worn in the laboratory and removed before leaving the laboratory. For example: do not leave the laboratory to go to the restroom while wearing a laboratory coat.
    - Appropriately sized gloves are to be worn on both hands. Gloves are to be removed before touching computers, cell phones, and other items that could become contaminated. As with laboratory coats, gloves are to be removed before leaving the laboratory.
    - The removal and disposal of gloves (**Figure 1.3**):
        - Step 1: Roll glove off of one hand so that it is inside out.
        - Step 2: Place that glove in the palm of the remaining gloved hand
        - Step 3: Roll glove off second hand so that the second glove is inside out with the first glove inside it.
        - Step 4: Dispose of gloves in red-bag lined biohazard bin
3. Procedures for handling and disposing of biohazards:
    - **Gloves and laboratory coats are to be worn whenever biohazards are to be handled.**

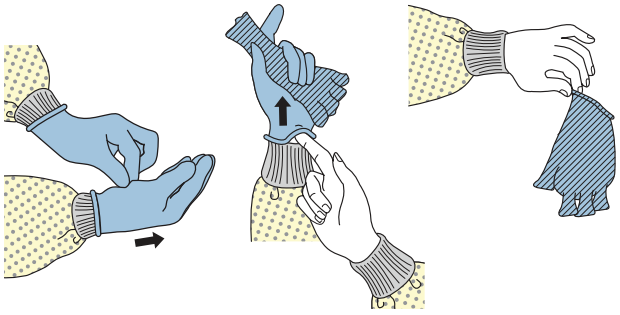


Figure 1.3: **Sequence for the safe removal of gloves.** Taken from the online PDF "Sequence for Putting on Personal Protective Equipment (PPE)". Centers for Disease Control and Prevention.

- Students may handle lancets used to stick their own fingers without gloves.
- Students will wear gloves to handle microcuvettes and other non-sharps for analysis of blood samples collected in the lab. Demonstrations of proper handling of biohazards will occur before laboratory activities take place.

  Note: Finger sticks for very small amounts of blood for analysis are the only method of blood collection that will be used by students in the laboratory course.
- Items that could potentially puncture a plastic bag must be disposed of in a hard-sided plastic biohazard sharps container. This includes **lancets for finger sticks** and other items as directed by the graduate teaching assistant (GTA).
  - Never reach into sharps containers.
  - Never fills the sharps container more than 75% full. If you notice that the sharps container has reached 75% capacity, notify your GTA for them to address the issue.
- Disposable non-sharps items that have been contaminated with blood should be disposed of a red-bag lined biohazard bin. This includes the absorbent laboratory mat, gauze, bandages, and other non-sharps.
- Towels that have been contaminated with saliva should be placed in a used towel bin so they can be laundered before reuse.

4. Hand washing and hand sanitizing requirements:
   - Use of hand sanitizer occurs:
     - Before you come into the lab
     - Before you leave the lab
   - Use of hand sanitizer or hand washing for 20 seconds occurs:
     - After you have taken off gloves
     - After you have touched anything that others may have touched
     - After you have used a facial tissue or other actions in which you may have contaminated your hands with your own bodily fluids
     - After you have used the restroom
5. Reporting an accidental exposure:
   - In the unlikely event of an exposure such as puncturing skin with a contaminated item or splashing blood or saliva into your mouth or eyes, it is important that you report the exposure to the instructor immediately.

- Instructors will notify the appropriate institutional authorities and the appropriate follow-up procedures will be taken to ensure your safety.

1.5 LABORATORY REPORT

There is no formal report for this laboratory. The following two documents should be submitted:

- OSHA Bloodborne Pathogen course completion report (PDF)
- Complete laboratory safety worksheet (PDF)

RECOMMENDED READINGS

1. US Department of Health and Human Services. *Science Safety Security: Finding the Balance Together*. https://www.phe.gov/s3/BioriskManagement/biosafety/Pages/Biosafety-Levels.aspx. 2020.

This chapter has online resources on GitHub.

NAME

LABORATORY 1 WORKSHEET

Universal Precautions for BSL-2

1. Our laboratories involve working with blood, saliva, sweat, and urine. Describe why these materials warrant BSL-2 containment procedures.

2. List at least three ways you can protect yourself from exposure to pathogenic microorganisms present in blood, saliva, sweat and urine.

Disposal of Biohazardous Materials

3. Specify what materials are allowed to go into the following receptacles:

General Waste	
Biohazardous Waste	
Sharps	

NAME

LABORATORY 1 WORKSHEET

Cleaning and Disinfection of Biohazardous Materials

4. You notice a small amount of blood on the table that your absorbent laboratory mat did not catch. How do you proceed in cleaning and disinfection of this area?

   Note: In your response, be sure to also include all materials you would need to do the job and how the materials are handled when you are done.

EXERCISE INTENSITY AND SUBSTRATE UTILIZATION

2.1 BACKGROUND

The relationship between exercise intensity and the substrates used to produce ATP underpins many nutritional strategies for sports and exercise. **One of the most important concepts that this relationship illustrates is the importance of carbohydrate availability to fuel moderate and high intensity physical activity** (1)**.** Additionally, application of the fundamental concepts linking substrate utilization and exercise intensity can be used to do the following things:

1. Estimate the amount of carbohydrate that needs to be consumed to prevent fatigue and or prevent use of protein as a fuel (2). This estimate can help individuals match carbohydrate intake to carbohydrate need before, during, and after exercise.
2. Target exercise intensity to maximize utilization of fat as a fuel. This can help endurance athletes prevent fatigue resulting from glycogen depletion (3).
3. Monitor training progress. For example, endurance athletes may train to increase their crossover point, which is the exercise intensity at which use of carbohydrate as a fuel begins to exceed that for fat (3). An increased crossover threshold would allow an endurance athlete to race at a higher intensity without depleting glycogen stores.

Energy to perform exercise is supplied by the chemical reaction of ATP → ADP + Pi + energy. There is a small amount of ATP stored in muscle cells that must immediately be resynthesized by the reverse chemical reaction of ADP + P_i + energy → ATP for exercise to continue.

The energy needed for the reaction to resynthesize ATP is produced through a combination of energy systems including:

- the **phosphagen system** (ATP and creatine phosphate),
- **glycolysis** (breakdown of glucose to pyruvate), and
- **oxidative phosphorylation** of substrates including carbohydrate, fat, and protein (4).

Substrate refers to the molecule that is acted upon by an enzyme or enzyme system. For example, creatine phosphate is a substrate for the phosphagen system, and glucose is the substrate for the glycolysis pathway.

The phosphagen system and glycolysis are **anaerobic** (not requiring oxygen) energy systems. Oxidative phosphorylation is the **aerobic** (requiring oxygen) energy system within mitochondria in which electrons are removed from energy substrates in the tricarboxylic acid (TCA) cycle (aka Krebs

or citric acid cycle) and used to create a proton gradient in the **electron transport system (ETS)** that generates energy to resynthesize ATP. The relationship between exercise intensity and use of aerobic and anaerobic energy systems for energy is illustrated in **Figure 2.1**.

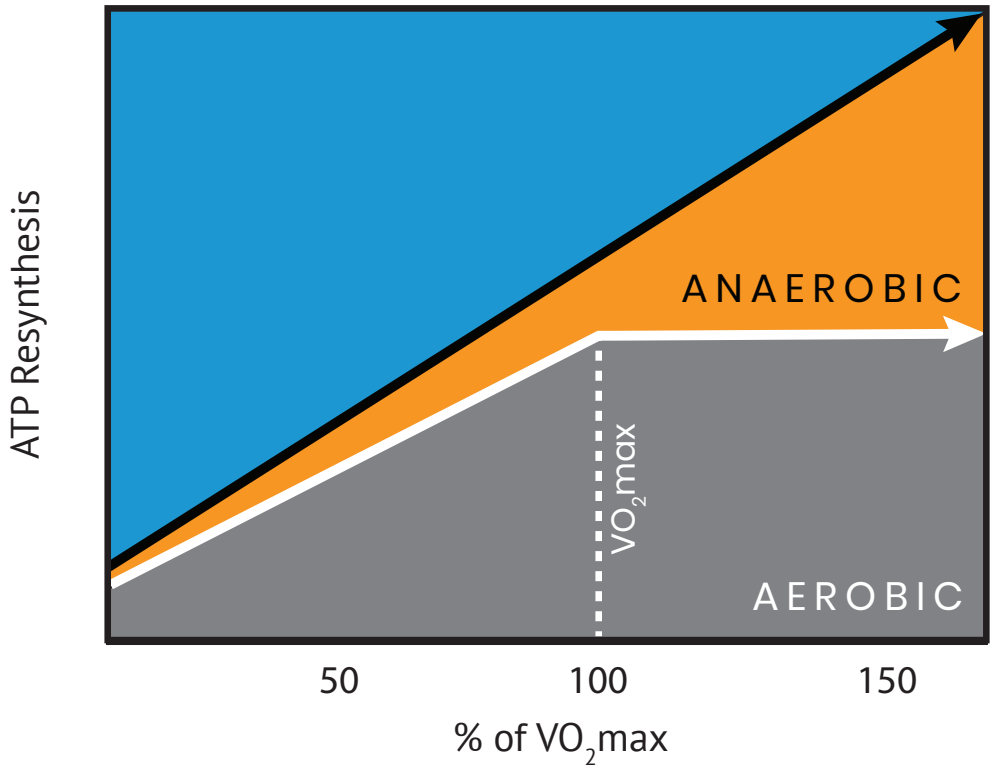


Figure 2.1: **Aerobic resynthesis of ATP increases linearly with increases in work rate and total energy expenditure as individuals go from resting to maximal aerobic capacity (VO_2max).** This relationship can be measured using a graded exercise test in which work rate is increased gradually in 2-3-minute stages (5). It is important to note that it takes time to match increases in work rate with aerobic ATP production, thus increases in work rate require anaerobic contributions to ATP resynthesis until oxidative phosphorylation catches up, when and if that occurs. As a result, it takes one to two minutes for increases in oxygen consumption (VO_2) to level off when increasing work rate during a graded exercise test. Additionally, the anaerobic contribution to ATP resynthesis increases as exercise intensity increases. The point at which work rate increases but VO_2 does not increase is the VO_2max, which typically coincides with maximal HR and volitional exhaustion. If an individual performs an anaerobic power test in which he or she goes directly to maximal work rate, that work rate may be approximately twice the work rate at his or her VO2max was measured. In other words, anaerobic power is greater than aerobic power.

Measurement of **oxygen consumption (VO_2)** and **carbon dioxide production (VCO_2)** during exercise can be used to determine the substrates utilized for aerobic ATP production. Common substrates that can be enzymatically modified to enter the TCA cycle include pyruvate, fatty acids, and amino acids. Thus, carbohydrates (source of glucose and other glycolytic intermediates from which pyruvate is produced), fatty acids (often referred to simply as fat) and protein (source of amino acids) are substrates for production of energy using the oxidative phosphorylation system.

Carbohydrate and fat are the primary substrates used for energy production during exercise. The proportions of their contribution to energy production are determined largely by exercise intensity and duration. As exercise intensity increases, there is a decrease in the proportion of energy derived from the oxidation of fat. The opposite is true of carbohydrate use during exercise. As exercise intensity increases, an increased proportion of energy is derived from the oxidation of carbohydrate. When exercising at intensities ideal for oxidation of fat as a fuel, maximal rates of fat oxidation typically occur after 30 or more minutes of exercise.

As illustrated in **Figure 2.1**., VO_2 max is the point at which work rate increases but VO_2 does not. Put another way, VO_2 max is the maximal amount of oxygen the body can consume during exercise. Because of this, it typically coincides with maximal heart rate as well as volitional exhaustion. As it pertains to energy production during exercise, a high VO_2max indicates an increased capacity for aerobic generation of ATP. For this reason, it may be used as a predictor of aerobic fitness. Relative exercise intensities refer to the proportion of VO_2 max at which exercise is taking place, which will be different absolute work rates from person to person.

For example, exercise at 50% of VO_2 max describes the exercise intensity at which oxygen consumption is 50% of that at VO_2 max for a specific individual. When comparing individuals with different VO_2 max levels, the absolute work rate at 50% of VO_2 max will be higher for a person with a higher VO_2 max. When describing substrate utilization during exercise, typical patterns of carbohydrate and fat utilization are described using relative exercise intensities. Perception of effort and time to fatigue are more comparable at relative intensities. Most people would consider exercise at 35% of VO2max to be low in intensity, 65% to be moderate in intensity, and 80% of VO2max to be somewhat high in intensity. Based on the relationship between exercise intensity and substrate utilization, how would you hypothesize that proportions of carbohydrate and fat will change from 35% to 65% to 80% of VO_2 max ?

The ratio of VCO_2/VO_2 is called the **respiratory exchange ratio (RER)**. If fat is the only substrate being used for oxidative phosphorylation, then the RER = 0.7. If carbohydrate is the only substrate being used for oxidative phosphorylation, then the RER = 1.0. The proportion of carbohydrate utilization increases proportionally as the RER increases from 0.7 to 1.0. This method is referred to as the 'non-protein RER' determination of substrate utilization because it assumes that amino acids contribute inconsequentially to the substrate mixture during exercise. It should be noted that while this assumption is generally acceptable in most circumstances, there are circumstances such as during fasting or when carbohydrate availability is limited, when amino acids are a substantial substrate for aerobic ATP production. Under these conditions, the non-protein RER estimation of substrate utilization will be less accurate.

2.2 OBJECTIVE

The purposes of this assignment are:

1. to compare carbohydrate and fat as fuels for oxidative phosphorylation (aerobic ATP resynthesis) during exercise
2. to quantify the relative and absolute contributions of carbohydrate and fat at 35, 65, and 80% of estimated VO_2max, and the percentage of VO_2max at which the crossover point occurs
3. to estimate the amount of carbohydrate that would need to be available to perform exercise or specified durations and intensities
4. to generate a hypothesis regarding the preferred substrate source at low, moderate, and high exercise intensities

2.3 OVERVIEW

The exercise will consist of a graded exercise test (increasing from low to high intensity in 2-minute stages). The graded exercise test will begin at a brisk walk and increase gradually until 85% of age-predicted HR maximum is reached (6). The metabolic cart will be used to measure VO_2 and VCO_2, an HR monitor will be used to measure HR, and ratings of perceived exertion will be reported by participants toward the end of each test stage. Oxygen consumption and HR data will be used to predict VO_2max. Oxygen consumption at 35, 65, and 80% of estimated VO_2max will be calculated. The amount of carbohydrate and fat oxidized at each of these intensities will be determined and used to calculate absolute and relative contributions of these fuels for ATP resynthesis at 35, 65, and 80% of VO_2max. The average of three tests within each lab team will be used for the laboratory report.

2.4 PROCEDURES

2.4.1 Data Collection: Graded Exercise Test

1. All students should fill out and electronically submit a 2021 PAR-Q+ (available online as a fillable form) prior to participating in any laboratory exercise activities.
2. Designate team members for the following tasks (rotate tasks across the three tests within your team):
    - Perform the exercise test (this will be "the participant")
    - Keep time and indicate when ratings of perceived exertion (RPE) should be taken and treadmill speed and grade should be changed.

- Record time, treadmill speed and grade, HR, and RPE on the data sheet
- Control treadmill speed and grade in addition to watching and communicating with the participant during the test
- Get mouthpiece, Rudolph valve, and nose clip rinsed and in hot soapy water after test

3. The participant will put on an HR monitor and warm up on a treadmill (or a cycle ergometer) at a self-selected low to moderate intensity for at least 5 minutes. Time this so that the warm-up is completed 2-3 minutes before beginning the exercise test for the lab. Heart rate should return to near resting levels before beginning the graded exercise test.
4. Use the data sheet to calculate age predicted HRmax (APHRmax) and HR at 85% of APHRmax.
5. The instructor will fit the mouthpiece, Rudolph valve, headgear, and nose clips for the participant and run the metabolic cart.
6. Graded exercise test protocol (**Figure 2.2**): Start with the treadmill level at a speed that elicits a low exercise intensity. This is likely to be a moderate to brisk walk but may be a slow jog for participants that are more endurance trained. If the test starts at a speed that is moderate to vigorous, then it will fail to demonstrate the intended relationship between substrate utilization and exercise intensity. **Table 2.1** features the recommended protocol, but the instructor may modify as needed during the test.

   The goal of this protocol is to start at a low exercise intensity and gradually (very important) increase exercise intensity over a period of 12-18 minutes until the participant reaches 85% of APHRmax. If possible, have the participant complete the 2-minute stage at or around 85%

Figure 2.2: **Student performing a graded exercise test on a treadmill.** A headpiece connects to a mouthpiece with a set of one-way valves which allows for transfer of expired gases via a collection tube to a metabolic cart for analysis of carbon dioxide and oxygen concentrations. A nose clip ensures that expired air is only leaving through the mouth.

Table 2.1: **Recommended protocol for graded exercise test.**

Stage	Time (min)	Grade (%)
1	0 - 2:00	0
2	2:00 - 4:00	2
3	4:00 - 6:00	4
4	6:00 - 8:00	6
5	8:00- 10:00	8
6	10:00 - 12:00	10
7*	12:00 - 14:00	12

* Continue increasing treadmill grade by 2% every 2 minutes until stage at which 85% APHRmax is reached or participant terminates the test, whichever comes first. If maximal treadmill incline is reached before hitting 85% APHRmax, then treadmill speed can be increased.

APHRmax so that you can get metabolic data at this final stage.

7. When the test is completed, the treadmill grade is returned to 0% and the treadmill is slowed down to allow the participant to cool down. The instructor will have the participant straddle the treadmill briefly to remove the mouthpiece, headgear, and nose clip and then allow the participant to continue the cool down.

2.4.2 Data Analysis: Estimate of VO_2max, Crossover Point, and Substrate Utilization

Data from the treadmill protocol will be exported by laboratory teaching assistants and be made available online for the class. Download the Excel file for each of the submaximal tests.

Required Graphs:

- Scatterplots to estimate participant VO_2 max: Use the VO_2 (ml/kg/min) data as the x-axis and heart rate (HR, bpm) as the y-axis to build a scatterplot for each participant. Graphs may be made separately. For formatting of your graphs, please follow the guidelines outlined in **Appendix A.2.2**.

  Add a trendline to each scatterplot. Your previously calculated age-predicted heart rate max serves as the y in the trendline equation, y = mx + b. The data uses a simple linear regression to determine m and b. Solve for x, your estimated VO_2 max.

  Note: If heart rate data is inconsistent and does not make sense (Ex. Is climbing within 120-130 bpm and suddenly falls to 60 bpm), you may have to remove datapoint(s) in order to improve the fit of your trendline. The R^2 value that accompanies the trendline is an indicator of fit, with a value of 1 indicating a perfect fit of the trendline to the data.

- Scatterplots showing substrate utilization over time: A scatterplot of time (x-axis) versus kcal of fat and carbohydrate expended per minute (y-axis) for the entire exercise period. The **crossover point** refers to the point at which the number of kilocalories of carbohydrate expended per minute exceeds the number of kilocalories of fat expended per minute. Find and record the VO_2 where this crossover point occurs. Determine what percentage this value is of the estimated VO_2 max.

Using your estimated VO_2 max, calculate 35, 65, and 80% of the estimated VO_2 max instructions. For each of these VO_2 values, refer back to the original data file and record the a) percentage of carbohydrate and fat expended and b) kcal of carbohydrate and fat expended per minute.

Average the percentage (relative) and kcal per minute (absolute) of carbohydrate and fat expended from your three tests at 35, 65, and 80% of VO_2 max. With these two sets of data, create **two** separate stacked bar charts, again following graph guidelines outlined in **Appendix A.2.2**.

For each participant, show your calculations for how much carbohydrate and how much fat would be burned during 60 minutes of exercise at 35% VO_2 max and at 65% VO_2 max. Do the same for 30 minutes of exercise at 80% of VO_2 max.

2.5 LABORATORY REPORT

Use the procedures, data, your calculations, and figures from this laboratory to complete a standard format laboratory report, as described in Appendix A.

- Deadline for Submission _____

RECOMMENDED READINGS

1. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol. 1993;265(3 Pt 1):E380-91. Epub 1993/09/01. doi: 10.1152/ajpendo.1993.265.3.E380. PubMed PMID: 8214047.

2. Jeukendrup A. A step towards personalized sports nutrition: carbohydrate intake during exercise. Sports Med. 2014;44 Suppl 1(Suppl 1):S25-33. Epub 2014/05/06. doi: 10.1007/s40279-014-0148-z. PubMed PMID: 24791914; PubMed Central PMCID: PMCPMC4008807.

3. Purdom T, Kravitz L, Dokladny K, Mermier C. Understanding the factors that effect maximal fat oxidation. J Int Soc Sports Nutr. 2018;15:3. Epub 2018/01/19. doi: 10.1186/s12970-018-0207-1. PubMed PMID: 29344008; PubMed Central PMCID: PMCPMC5766985.

4. Hargreaves M, Spriet LL. Exercise Metabolism: Fuels for the Fire. Cold Spring Harbor perspectives in medicine. 2018;8(8). Epub 2017/05/24. doi: 10.1101/cshperspect.a029744. PubMed PMID: 28533314; PubMed Central PMCID: PMCPMC6071548.

5. Beltz NM, Gibson AL, Janot JM, Kravitz L, Mermier CM, Dalleck LC. Graded Exercise Testing Protocols for the Determination of VO(2)max: Historical Perspectives, Progress, and Future Considerations. J Sports Med (Hindawi Publ Corp). 2016;2016:3968393. Epub 2017/01/25. doi: 10.1155/2016/3968393. PubMed PMID: 28116349; PubMed Central PMCID: PMCPMC5221270.

6. Graves S, Whitehurst M, Findley BW. Physiologic Effects of Aging and Deconditioning. In: ACSM, editor. ACSM's Resource Manual for Guidelines for Exercise Testing and Prescription. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 79-92.

This chapter has online resources on GitHub.

NAME

LABORATORY 2 DATA SHEET

DATE _____ Participant ID (no names) _____

Age-predicted heart rate max (APHRmax) Calculation

1. Age = _____

2. APHRmax = 208 bpm - (0.7 x _____) = _____bpm
   age

3. 85% of APHRmax = 0.85 x _____ = _____bpm
   APHRmax

Treadmill Protocol						
Stage	**Time***	**Grade %**	**Speed**	**HR**	**RPE**	**Notes**
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

*Time should be synchronized with the start of data collection on the computer so that the data sheet lines up with the metabolic measurements.

NAME

LABORATORY 2 DATA SHEET

DATE _____ Participant ID (no names) _____

Age-predicted heart rate max (APHRmax) Calculation

1. Age = _____

2. APHRmax = 208 bpm - (0.7 x _____) = _____bpm
   age

3. 85% of APHRmax = 0.85 x _____ = _____bpm
   APHRmax

Treadmill Protocol						
Stage	**Time***	**Grade %**	**Speed**	**HR**	**RPE**	**Notes**
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

*Time should be synchronized with the start of data collection on the computer so that the data sheet lines up with the metabolic measurements.

NAME

LABORATORY 2 DATA SHEET

DATE _____ Participant ID (no names) _____

Age-predicted heart rate max (APHRmax) Calculation

1. Age = _____

2. APHRmax = 208 bpm - (0.7 x _____) = _____bpm
   age

3. 85% of APHRmax = 0.85 x _____ = _____bpm
   APHRmax

Treadmill Protocol						
Stage	**Time***	**Grade %**	**Speed**	**HR**	**RPE**	**Notes**
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

*Time should be synchronized with the start of data collection on the computer so that the data sheet lines up with the metabolic measurements.

NAME

LABORATORY 2 NOTES

CARBOHYDRATE SUPPLEMENTATION AND GLYCEMIC RESPONSES

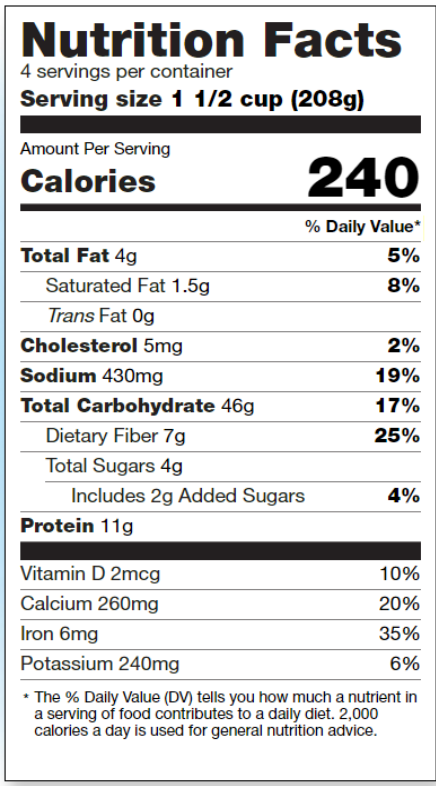
3.1 BACKGROUND

The need for carbohydrate supplementation during exercise is influenced by several factors that are important to understand if one is to utilize carbohydrates optimally for exercise and health. While carbohydrate availability during exercise is crucial to performance and influences the stress of exercise, carbohydrates can have undesirable consequences in other circumstances. Sports nutritionists, coaches, athletic trainers, and athletes are more likely to be concerned with making sure enough carbohydrate is consumed at the right time, in an optimal form, and with desired ratios of glucose and fructose (1). Individuals exercising to improve or maintain their fitness, lose weight, or improve their metabolic health potentially may be concerned with making sure too much carbohydrate is not consumed and that those consumed are in the healthiest forms (2). To navigate these differences, to make choices that match the person and situation, and to carry out dosing strategies, it is important to be able to:

1. Quantify carbohydrate intake
2. Identify glucose and fructose sources of carbohydrates
3. Differentiate lower and higher glycemic index foods

Quantifying carbohydrates in foods can be done by analyzing the nutrition data from food labels or from a variety of Internet sources, e.g. the United States Department of Agriculture's (USDA) nutrient data base FoodData Central or a variety of apps such as MyFitnessPal. Information about how to understand and use the Nutrition Facts Label on packaged foods can be found at this interactive Food and Drug Administration (FDA) website. As seen is **Figure 3.1**, nutrition facts labels include the following information for carbohydrates in each serving of the food:

- Total Carbohydrate (grams, g)
- Dietary fiber (g)
- Total sugars (g)
- Added sugars (g)



Nutrition Facts

4 servings per container

Serving size 1 1/2 cup (208g)

Amount Per Serving

Calories **240**

	% Daily Value*
Total Fat 4g	**5%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 430mg	**19%**
Total Carbohydrate 46g	**17%**
Dietary Fiber 7g	**25%**
Total Sugars 4g	
Includes 2g Added Sugars	**4%**
Protein 11g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 6mg	35%
Potassium 240mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Figure 3.1: **Nutrition Labels provide information about macronutrients and micronutrients for a given food serving.** Photo from USFDA.

Available carbohydrate is the total carbohydrate minus the dietary fiber. As an example:

- If total carbohydrate is 34 g per serving and
- Dietary fiber is 6 g per serving, then
- Available carbohydrate is 28 g per serving (34 g - 6 g = 28 g)

By definition, dietary fibers are plant polysaccharides that escape enzymatic digestion (3). While energy can be harvested from dietary fibers, this primarily occurs in the large intestine (colon) where intestinal bacteria capable of metabolizing and extracting energy from the fiber are most abundant (4). Thus, this portion of the total carbohydrate in food is not enzymatically digested to an absorbable monosaccharide unit and does not contribute to the glycemic response following ingestion.

Another important consideration in the calculation of carbohydrate intake is the serving size, which differs from food to food. For example, serving sizes of sports drinks, sodas, and fruit juices may range from 4 to 12 ounces. As a result, calculation of carbohydrate doses is achieved by determining a measurable quantity of food that contains the desired amount of carbohydrate, typically in grams.

In addition to quantifying the amount of food that contains a desired amount of carbohydrate, it is also important to know the type of available carbohydrate and the **glycemic index** of the food.

Types of carbohydrate available in food sources include fructose and glucose. Sources of glucose in the diet include dextrins and starches. Common sources of fructose include fruits and vegetables. Sucrose, commonly known as table sugar, is composed of one molecule of glucose combined with one molecule of fructose. It is important to be able to identify the type of available carbohydrate in foods and drinks as they are digested and absorbed differently.

In situations where exercise performance may be limited by the rate of glucose delivery from ingested carbohydrates during exercise, it is helpful to have a mixture of glucose and fructose in foods or beverages. Glucose is primarily absorbed in the small intestine via the transporter **sodium-glucose transporter 1 (SGLT1**). Fructose is primarily absorbed via GLUT5. Consumption of foods or beverages with both glucose and fructose in a ratio of approximately 2:1 (glucose:fructose) has been shown to maximize oxidation of exogenous (from food/beverage consumed during exercise) carbohydrate during exercise (1). Increasing oxidation of exogenous carbohydrate allows for higher intensity exercise to be performed when endogenous (glycogen and glucose made by the liver) carbohydrate is not sufficiently available. Consumption of multiple transportable carbohydrate (glucose and fructose) sources becomes increasingly important as exercise duration increases and is considered essential when exercising for greater than 2.5 hours (1).

Consumption of different foods that contain the same desired dose of carbohydrate may result in different rates of glucose digestion, absorption, and appearance in the blood. Glycemic index is a measure of glucose appearance in the blood during the first two hours after consumption of a quantity of food containing 50 g of available carbohydrate (5). The glycemic index is determined by comparing foods to standards that produce high glycemic responses, including glucose and white bread. The standards are given a glycemic index of 100. Foods that elicit smaller changes in blood

glucose have lower glycemic indexes and vice versa. Glycemic index ranges for foods compared to the glucose standard are given in **Table 3.1**. Glycemic index reflects one characteristic of foods and needs to be considered in the context of how much carbohydrate is consumed, other foods that may be consumed simultaneously, and many other factors that may influence glycemic responses. However, it can inform choices made to achieve different goals. For example, an exercising athlete with low muscle glycogen levels would get more benefit from a high glycemic index carbohydrate source than a low glycemic index carbohydrate source. However, foods with lower glycemic indexes are better for health and disease prevention and a wiser choice in most circumstances.

Table 3.1: **Ranges for foods categorized as low, medium, and high for glycemic index (GI).** Glycemic index is a ranking of carbohydrate-containing foods, based on the food's effect on blood sugar compared with a standard reference food.

Level	GI Range
Low	1 - 55
Medium	56 - 69
High	≥ 70

As demonstrated in *Laboratory 2: Exercise Intensity and Substrate Utilization*, carbohydrate is an important fuel for exercise at moderate and higher exercise intensities. The glucose used as fuel in muscles during exercise may come from glycogen inside the muscle cells or from glucose that is transported from the blood to the inside of the muscle cell, via **glucose transporter 4 (GLUT4).** GLUT4 can be induced to transport glucose into skeletal muscle via one of the following two mechanisms:

1. An increase in circulating concentrations of the hormone **insulin**. This will stimulate glucose uptake by all skeletal muscle and adipose tissue throughout the body.
2. Muscle contraction. This is known as the 'insulin-like effect of exercise' and will stimulate glucose uptake only in the skeletal muscles that are active.

These two mechanisms are independent of each other and either one or both may be responsible for GLUT4 transport of glucose from blood into muscle cells. If both are active simultaneously, then they have an additive effect that results in more glucose uptake than either would induce by itself.

3.2 OBJECTIVE

The purpose of this laboratory is:

1. to calculate the quantity of foods that contains 30 or 60 g of available carbohydrate
2. to compare glycemic responses to the same food with and without exercising after consumption.
3. to generate and test a hypothesis regarding the magnitude of glycemic response to:

   i) the same food with and without exercise after consumption

   ii) foods with lower versus higher glycemic indexes

3.3 OVERVIEW

At least two individuals from each lab team will participate in this experiment. Teams will first decide which food items and which carbohydrate dose they would like to use for the experiment. Teams should select a minimum of two foods for the experiment, one with a lower and one with a higher glycemic index. Then calculate the amount of each experimental food that contains either 30 or 60 g of available carbohydrate to use as the experimental dose. These amounts are based on recommended intakes for individuals exercising 1-2 hours and 2-3 hours, respectively (1).

For each of the foods tested in this experiment, blood glucose concentrations will be measured and compared in the same team member under the following two conditions:

1. food consumption followed by minimal activity (mostly sitting).
2. food consumption followed by exercise.

These conditions will be counter balanced over the course of two lab sessions for data collection, i.e. condition 1 then 2 for one team member and condition 2 then 1 for another team member. Participants in the experiment must come to the laboratory under the **same conditions** between the two data collection labs having refrained from consuming any calories and exercising at least three hours before the lab.

3.4 PROCEDURES

3.4.1 Week 1: Experimental Design and Collection

1. For the experiments to be performed in the next two lab sessions:
    - Select a minimum of 2 team members to perform the experiment ("the participants"). To participate in this experiment, team members must not have diabetes or any other health condition that would be negatively impacted by consumption of 30 or 60 g of carbohydrate. Team members who are not participating as participants will be "researchers".
    - Select a different food/drink for each participant **and** select at least one lower and one higher glycemic index food/drink.
    - Select a dose of either 30 or 60 g of carbohydrate. **All participants should consume the same dose of carbohydrate.**
    - Pick a mode of exercise available in the lab, either treadmill or cycle ergometer.
    - Set up a schedule for when each team member in the experiment must stop eating and should not exercise before the laboratory experiment. Think this through and plan details so that the conditions for each of the two lab sessions will be as similar as possible. For example, what will be eaten and when prior to the lab to fit the schedule and be feasible? A snack may be needed ~3-5 h before the lab to avoid feeling hungry. Control as much as possible between the two conditions so that the within and between experimental participant variation is minimized. Things that can be controlled to minimize impact on variability from one test day to the next include physical activity the day before and the day of the experiment, sleep, consumption of caffeine or alcohol in the 24 h prior to the

experiment, and recent diet.

- Perform and double check calculations for test foods that will be consumed in the experiment. Lab instructors will need this information before you leave the lab so that they can make sure the food is available for the experiments in weeks 2 and 3 of the lab.

2. Calculations of the amount of food that contains 30 or 60 g of available carbohydrate can be done in a variety of ways. It is important to make sure that you pay attention to the units and keep them consistent throughout the calculation for each food. Here are some formulas and sample calculations:

   - Select the units for measurement and calculation, e.g. grams, ounces, milliliters.
   - How many grams of available carbohydrate per unit? This information may be obtained from a nutrition facts label or from an online source such as the FoodData Central from the USDA. Available carbohydrate is equal to total carbohydrate minus total fiber.
   - Quantity (units) to be consumed to get desired g of carbohydrate = (desired g of carbohydrate)/(g of carbohydrate/unit)

Example Calculation:

Orange juice = 25.3 g of available carbohydrate (CHO) per 8 ounces of juice. How much orange juice needed to consume exactly 30 g of available carbohydrate?

Unit = ounce

$$\frac{25.3\ g\ CHO}{8\ oz\ juice} = 3.163\ CHO\ per\ oz\ of\ juice$$

To do the final calculation, you must invert the denominator fraction and multiply:

$$\frac{Desired\ quanity\ of\ CHO}{Quantity\ of\ CHO/unit}$$

Becomes the following:

$$30\ g\ CHO\ x\ \frac{1\ oz\ of\ juice}{3.163\ g\ CHO}$$

Units should cancel out to leave desired amount of carbohydrate:

$$30 \cancel{g\ CHO}\ x\ \frac{1\ oz\ of\ juice}{\cancel{3.163\ g\ CHO}} = 9.485 \text{ oz of juice}$$

9.5 ounces is the amount of orange juice that contains 30 g of carbohydrate.

3.4.2 Weeks 2 and 3: Data Collection

Participants should come to lab having followed the protocol set forward by team members during Week 1 of the lab. Participants will perform their own finger sticks, consume the foods, exercise or rest according to the protocol, and dispose of their biohazardous waste appropriately.

Researchers will measure out food quantities, keep track of time, oversee and document that the protocol is being followed, measure blood glucose, and record all data including preparation for the protocol, food quantities, timing of all experimental events, and blood glucose measurements.

1. Prepare the foods you have selected by measuring out the quantity of each that will contain the amount of carbohydrate you have chosen for your experiment (30 or 60 grams).
2. Prepare your testing area with the following testing materials for each person to be tested: 1 labmat (absorbent paper), 3 lancets, 3 glucose microcuvettes, 3 alcohol swabs, several pieces of gauze, 3 bandages, and a glucose meter.
3. Blood will be collected via finger stick at the following times:
   - Before consuming carbohydrate (T0)
   - 20 minutes after starting to consume carbohydrate (T20)
   - 40 minutes after starting to consume carbohydrate (T40)
4. Conditions: timelines of both conditions are presented in **Figure 3.2.**

   Exercise Condition: Pick a mode of exercise available in the lab, either treadmill or cycle ergometer. Measure T0 glucose, consume carbohydrate, begin exercising 10 minutes after consuming carbohydrate (T10) and continue exercising at a comfortable intensity for you, e.g. whatever you feel is moderate, for 30 minutes. After 10 minutes of exercise, pause briefly to measure blood glucose (T20), then continue for 20 more minutes (total exercise time = 30 minutes minus a few seconds to measure glucose at T20).

   No Exercise Condition: Measure T0 glucose, consume carbohydrate, and start the clock. Sit and perform minimal for activity for 40 minutes. Measure blood glucose at T20 and T40.

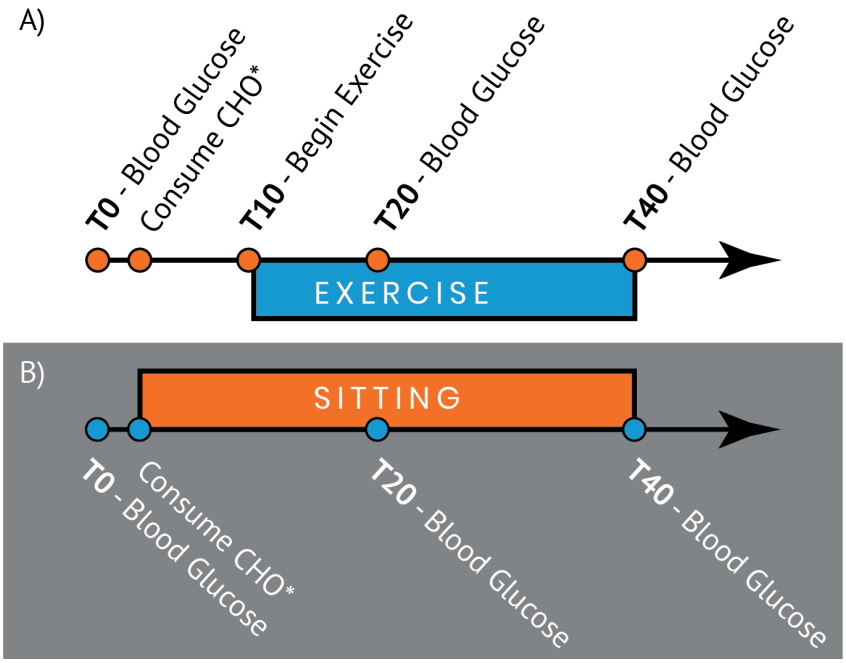


Figure 3.2: **Timeline for the A) Exercise and B) No Exercise Condition.** *The first sip or bite of carbohydrate-containing beverage or food is when you start your clock for either condition. CHO, carbohydrate.

5. Procedures for measuring blood glucose:

IMPORTANT: Keep all biohazardous materials contained on your labmat until all three glucose measurements are completed.

- Finger sticks will be done by the participants themselves.
- Wipe a fingertip with an alcohol wipe and allow to dry (can wipe with clean gauze to lessen the sting).
- Activate the lancet. Create pressure in the fingertip with the thumb and press the lancet to middle to outer side of the clean fingertip until stick occurs. Wipe the first drop of blood away with gauze.
- Use the thumb to create pressure and a nice 'bead' or 'drop' of blood on the fingertip. A lab partner wearing gloves will collect a drop of blood into the glucose cuvette and perform the glucose measurement.
- Wipe the finger stick area clean and put a bandage on.
- Keep all alcohol, gauze, bandages, and other material with blood on the labmat until you have collected all three samples.

- The person whose glucose was measured will dispose of biohazardous waste upon completion of all 3 glucose measurements.
    - Lancets and glucose microcuvettes should be disposed of into *sharps containers*.
    - Bandages, alcohol wipes, gauze, gloves, and lab mat should be disposed of in *non-sharps biohazardous waste* bins.
- All members of the lab team should clean the area where you collected blood and all other items touched during the lab with antimicrobial wipes or 10% bleach solution from spray bottle.

3.5 LABORATORY REPORT

Use data from this laboratory to complete a standard format laboratory report, as described in Appendix A.

- Deadline for Submission _____

RECOMMENDED READINGS

1. Jeukendrup A. A step towards personalized sports nutrition: carbohydrate intake during exercise. *Sports Med*. 2014;44 Suppl 1(Suppl 1):S25-33. Epub 2014/05/06. doi: 10.1007/s40279-014-0148-z. PubMed PMID: 24791914; PubMed Central PMCID: PMCPMC4008807.
2. Miles, M. P. (2012, 2012/03/01). Carbohydrates for Physical Activity: A Strategy to Avoid Undesirable Health Consequences. *American Journal of Lifestyle Medicine,* 6(2), 121-132. https://doi.org/10.1177/1559827611431053
3. Anderson, J. W., Baird, P., Davis, R. H., Jr, Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C. L. (2009). Health benefits of dietary fiber. *Nutrition Reviews*, 67(4), 188-205. https://doi.org/10.1111/j.1753-4887.2009.00189.x
4. Martinez-Guryn, K., Leone, V., & Chang, E. B. (2019, 2019/09/11/). Regional Diversity of the Gastrointestinal Microbiome. *Cell Host & Microbe*, 26(3), 314-324. https://doi.org/https://doi.org/10.1016/j.chom.2019.08.011
5. Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. S. (2005, 06/01). Glycemic index methodology. *Nutrition Research Reviews*, 18, 145-171. https://doi.org/10.1079/NRR2005100

This chapter has online resources on GitHub.

NAME

LABORATORY 3 DATA SHEET – WEEK 1

DATE _____ Participant ID (no names) _____

Food/Drink Selection and Calculations			
	Food/Drink Type	**Dose (30 or 60 g CHO)**	**Calculated Food Amount with units**
Low Glycemic Index			
High Glycemic Index			

Food Calculations

Scheduling and Controls for Weeks 2 and 3

NAME

LABORATORY 3 DATA SHEET - WEEK 2

Participant ID (no names)

Food/Drink Type and Amount for Ingestion:

Exercise Mode and Intensity:

Exercise Condition			
Time	**Blood Glucose**	**RPE**	**Notes**
T0			
T10			
T20			
T40			

Participant ID (no names)

Food/Drink Type and Amount for Ingestion:

No Exercise Condition		
Time	**Blood Glucose**	**Notes**
T0		
T20		
T40		

NAME

LABORATORY 3 DATA SHEET - WEEK 3

Participant ID (no names)

Food/Drink Type and Amount for Ingestion:

Exercise Mode and Intensity:

Exercise Condition			
Time	**Blood Glucose**	**RPE**	**Notes**
T0			
T10			
T20			
T40			

Participant ID (no names)

Food/Drink Type and Amount for Ingestion:

No Exercise Condition		
Time	**Blood Glucose**	**Notes**
T0		
T20		
T40		

NAME

LABORATORY 3 DATA SHEET - NOTES

HYDRATION

4.1 BACKGROUND

Water makes up 45-60% of the human body weight. Lean tissue has a higher proportion of water than adipose tissue, so body water percentage is also dependent on amount of body fat present. Most body water is found inside cells (intracellular) compared to outside of cells (extracellular).

During exercise, metabolic pathways are used to perform work. These systems are not 100% efficient, meaning that a portion of the energy derived from carbohydrate, fat, or protein is released as heat. Transcutaneous water loss, also known as sweating, allows for the majority of heat dissipation during exercise in many circumstances. The degree of fluid loss during exercise is dependent on exercise intensity and duration, environmental conditions, renal reabsorption and excretion, pre-exercise hydration status, and fitness level. Fluid loss through sweat must be replaced in circulation to allow for adequate cardiac output, optimal function of all physiological systems, and especially for temperature regulation.

Fluid retainment is essential for sport performance and rapid recovery, particularly in sports where there are multiple exercise bouts throughout the day (1). Without hydration strategies in place, **dehydration** can occur and negatively impact performance during endurance and high-intensity exercise. Increases in the **osmolality** (solutes in 1 kilogram of solvent) of body fluids are common indicators of dehydration and influence movement of water between body water compartments. Osmolality increases with dehydration and decreases as rehydration occurs. During dehydration, water moves from the intracellular space to the extracellular space, in part, due to an increased perfusion pressure and capillary area (2). Rehydration of compartments occurs sequentially going from the lumen of the gut, through the absorptive cells of the gut, into the blood (extracellular), to the interstitial space (extracellular), and finally into tissues (intracellular), e.g. muscle cells. Thus, ingested water moves sequentially from the gut, to the extracellular compartment, and finally to the intracellular compartment.

An **oral rehydration solution** (ORS) is a solution that can be used to prevent or correct dehydration. Common components of ORS can include

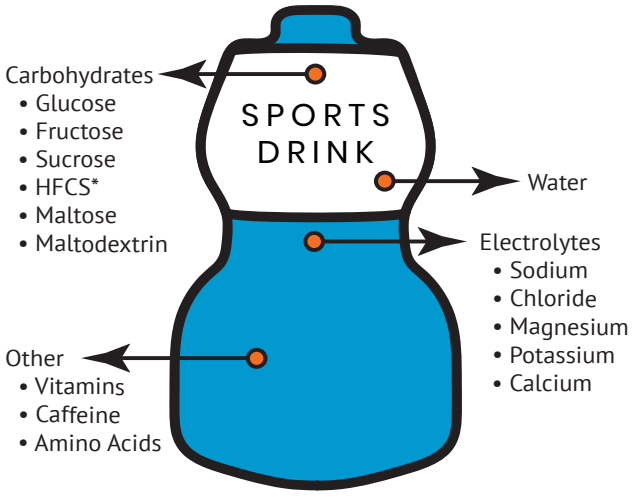


Figure 4.1 **Common components of an oral rehydration solution.** *HFCS, high fructose corn syrup.

electrolytes, macronutrients, caffeine, and other supplements (**Figure 4.1**). ORS can be consumed before, during, and after exercise. The goals of ORS are to:

1. Replace fluid volume
2. Supply macronutrients (typically carbohydrates) to working cells

Solutions that contain electrolytes and carbohydrates have been shown to restore plasma volume faster than water alone which is crucial for individuals performing multiple bouts of exercise per day (3). This difference in rehydration time is due to **the hemodilution effect of water,** which leads to large amounts of ingested fluid being lost to urine production (4). The addition of solutes like electrolytes and carbohydrates in ORS reduces water loss to urine by increasing intestinal water absorption via **osmosis**. Osmosis is the passive movement of solvent (water) in the direction that equalizes solute concentrations. In this way, changes in solute concentration result in net water flow between compartments and across membranes with water moving to the compartment with higher osmolarity. This is the underlying mechanism of hydration responsible for the sequential movement of water from the small intestine, to the blood, the interstitial space, and into cells.

Table 4.1: **Composition of common beverages including key carbohydrate sources.** Content redrawn from a table in a publication by Shirreffs (5) and includes osmolality data presented by Maricle and Pfeifer (6).

Beverage	Carbohydrate (g/100 mL)	Sodium (mmol/L)	Osmolality (mOsmol/kg)	Key Carbohydrate Source
Gatorade	8	20	280	sucrose, glucose, fructose
Powerade	6	23	285	maltodextrin, HFCS
Cola	11	3	700	HFCS
Orange Juice	10	4	660	fructose, sucrose, glucose
Apple Juice	13	1	330 - 696*	fructose, sucrose, glucose
Bottled Water	0	0	9	NA
Milk	5	26	288	lactose

*Osmolality values for apple juice are from a 2009 study by Maricle and Pfeifer (6).
Abbreviations: HFCS, high fructose corn syrup; wt/vol, weight by volume; mmol, millimole; mOsmol, milliosmoles.

The addition of carbohydrate (ie, glucose, table sugar), protein, or salt in ORS increases solution osmolality in a dose-dependent manner with total osmolality increasing with increasing amounts of added ingredients. The composition of common drinks are provided in Table 4.1.

Normal serum osmolality is typically **290 mOsm/L.** An ORS solution with a lower osmolality than serum osmolarity is considered **hypotonic**, while a solution with higher osmolality. is considered **hypertonic**. A solution with the same osmolality to serum is considered **isotonic**. ORS solutions that are close to isotonic are most effective for rehydration.

To estimate the effect common ORS components have on **osmolarity** (solutes in 1 L of solution), we first need to know the molecular weight of the component. In one mole of sodium chloride (NaCl),

we have one mole of sodium and one mole of chloride which in total weighs approximately 58 g. Similarly, one mole of glucose ($C_6H_{12}0_6$) has six moles of carbon, 12 moles of hydrogen, and six moles of oxygen and in total weighs approximately 180 grams. One mole of sucrose (a disaccharide containing one glucose and one fructose) has a weight of 342 g. The calculation for 1 mole of NaCl is provided below to show how molecular weight is calculated.

$$22.99\,\frac{g}{mol}\,Na + 35.45\,\frac{g}{mol}\,Cl = 58.44\,\frac{g}{mol}\,NaCl$$

Next, we need to know if the component has particles that dissociate in solution. As an example, NaCl dissociates into two molecules but glucose remains intact. We can use this information (in red) in conjunction with the component weight and component molecular weight (blue) to get osmolarity:

For 1 *g NaCl in* 1 *L water*:

$$1\ g\ NaCl\ x\ \frac{1\ mol}{58.44\ g\ NaCl}\ x\ \mathbf{2}\ x\ 1000 = 34.22\ mOsmol/L$$

For 1 *g glucose in* 1 *L water*:

$$1\ g\ glucose\ x\ \frac{1\ mol}{180.16\ g\ glucose}\ x\ \mathbf{1}\ x\ 1000 = 5.55\ mOsmol/L$$

Sucrose, also known as table sugar, dissolves in water but does not dissociate into ions. Thus, we would still use 1 as the number of particles that dissociate in solution.

For 1 *g sucrose in* 1 *L water*:

$$1\ g\ sucrose\ x\ \frac{1\ mol}{342.30\ g\ sucrose}\ x\ \mathbf{1}\ x\ 1000 = 2.92\ mOsmol/L$$

The overall osmolarity of a drink with multiple ingredients would be the sum of all component osmolarities.

4.2 OBJECTIVE

The purpose of this laboratory is to:

1. Assess daily fluid intake.
2. Compare body mass, intracellular and extracellular water volumes, and urine osmolality between euhydrated (optimal hydration) and hypohydrated (some amount of dehydration) conditions.
3. Create your own ORS and understand how solution components contribute to overall solution osmolarity.
4. Assess the hemodilution effects of water versus your ORS in the hypohydrated state.

4.3 OVERVIEW

Students should make an effort to arrive in lab for week 1 in a euhydrated (fully hydrated) state by drinking 500-1000 mL of extra fluids over the course of the morning of lab. In week 1, each team will design and create one ORS drink, calculate an approximate osmolarity of their ORS and perform baseline assessments including fluid intake, body water (via bioelectrical impedance analysis or BIA), urine osmolality, and thirst. Groups will also decide upon preparation procedures for weeks 2 and 3.

For weeks 2 and 3, two individuals from each lab team will be participants in the experiment involving the ORS drink and water with the other individuals facilitating data collection for the team (the researchers). The efficacy of the ORS drink in promoting hydration will be made in comparison to water.

Similar to laboratory 3, the majority of data collection will occur in weeks 2 and 3. Participants will come into laboratory for weeks 2 and 3 having performed fluid restriction or exercise to induce dehydration. The ORS or water will be provided to two hypohydrated participants in each group.

4.4 PROCEDURES

4.4.1 Week 1: Oral Rehydration Solutions and Baseline Assessments

To prepare for this laboratory, all individuals should:

- Arrive to the laboratory in a well hydrated state (500 - 1000 mL of extra fluid over the course of the morning before lab)
- Collect a urine sample at the start of the laboratory

1. Oral Rehydration Solutions

For this portion of the laboratory, groups will 1) determine **how much ORS and water** should be consumed by participants who have completed a hypohydration protocol, 2) **design and create one ORS** to be consumed by participants, and 3) **calculate estimated osmolarity** of the ORS. Groups will test the ability of their ORS to hydrate in comparison to water by comparing the hemodilution effect of equal volumes of each drink.

Determine ORS volume: The ORS and water volume should be the same for each participant. *Example*: If it is decided that 500 mL of fluid is adequate, the total volume that should be created during the week 1 laboratory is 1000 mL (1 L) to cover fluid intake for both data collection labs.

Note: While fluid replacement in laboratory 4 occurs while seated, it should be noted that fluid volume determination *during exercise* should take into consideration the length and intensity of exercise, training status, National Athletic Trainers' Position Statement on fluid replacement (7), and environmental conditions. Sweat rate is also another important factor to consider as sweat rate and sweat sodium can vary greatly between individuals.

Table 4.2: **Laboratory ORS ingredients and their general purpose in an ORS.**

Ingredient	Purpose
Table sugar, Glucose powder	Macronutrient, Sweetness
Aspartame	Sweetness
Citric Acid (or lemon juice)	Palatability, pH
Table salt	Electrolyte, Palatability
Kool-Aid powder	Palatability
Food coloring	Appearance

Design and Create one ORS: Discuss what components make a good ORS drink and what quantity of the component is optimal for correcting dehydration. Use available ingredients listed in **Table 4.2** and information provided in **Table 4.1** to help guide your drink creation.

Gather the following general items to create one custom ORS drink for your group:

- liquid measuring cup, scale, funnel, plastic weigh boats, plastic spoon, half gallon plastic jug. Once the group has determined what components and how much of each are going into the ORS, follow these instructions to create the ORS drink:
- Tare the scale with the weigh boat on it, and use the spoon to help weigh out correct amounts of the dry ingredients. Carefully add dry ingredients to the plastic jug using the funnel to transfer. Once all dry ingredients are added, the jug can be capped and shaken to thoroughly mix the dry ingredients.

- Use a liquid measuring cup for measuring out the appropriate volume of water (and lemon juice, if applicable). Combine the dry ingredients with liquid ingredients and shake until no dry ingredient clumps remain.
- Make sure the jugs are labeled with ORS and a form of group ID

Calculate estimated osmolarity of ORS: Use the example osmolarity calculations in the laboratory background section to calculate the estimated contribution of each component to ORS osmolarity. Once you have the calculated osmolarity for each component, add them together to find your ORS osmolarity. The main drivers of osmolarity in **Table 4.2** are the carbohydrates and salt; the effects of other components we will assume to be negligible.

2. Baseline Assessments

Daily Fluid Intake: All team members will complete the beverage intake questionnaire (BEVQ-15) designed by Hedrick and colleagues (8) in your data sheet. The BEVQ-15 allows you to estimate your habitual average daily intake of beverages.

- Score the BEVQ-15 by following this equation for each beverage category:

$$\textit{Average daily consumption} = \textit{Frequency} \times \textit{Amount Consumed}$$

  Where frequency ("How often") is converted to the unit of times per day and amount consumed ("How much each time") is in fluid ounces.

- The total average daily consumption can be obtained from summing average daily consumption of beverage categories.

Thirst Assessment: Researcher(s) will prompt participants to assess thirst using the visual analog scale on the laboratory data sheet.

Urine Collection: One urine sample per participant will be collected to assess baseline hydration status.

- Prepare urine collection supplies for each participant:
  - 1 urine cup and 1 cooler
  - Label urine cup with participant name
- Use the restrooms down the hall to collect a urine sample. If you can, fill the collection cup halfway. Void the rest of the urine into the toilet.
- Provide your capped and labeled cup to your instructor so that they can run the samples through the osmometer. Osmolality of a solution can be determined by testing a small sample of fluid on an osmometer, which utilizes a technique called freezing point depression.

Body Water via BIA: Each participant will perform the BIA test according to manufacturer standard operating procedure.

- Record what clothes were worn during the body composition test in the data sheet. For future body composition assessments during laboratory four, the same clothing should be worn.
- Record body weight, total body water, extracellular water, and intracellular water data in your laboratory data sheet. If system data provided in pounds, convert to kilograms.

3. Preparation Procedures for weeks 2 and 3.

Decide on hypohydration protocol: The goal is for participants to arrive to the start of week 2 and week 3 laboratory in a hypohydrated state. This can accomplished in a safe manner through either fluid restriction or exercise before the laboratory. Each group must decide the hypohydration protocol its participants will follow. The hypohydration protocol should be the same between participants and between conditions.

- *Fluid Restriction*: Refrain from drinking any beverages in the 18 hours prior to the start of laboratory time. A modest amount of fluids can be ingested with meals. Keep water or another beverage with you during the day. If you are excessively thirsty or feeling as if you are becoming dehydrated (lightheaded, generally fatigued, headache, nausea), then drink fluids as needed.
- *Exercise*: The group must determine a type and length of exercise that can be performed by participants before the start of the laboratory with the goal to have a workout that achieves fluid loss via sweating.

Set a schedule for participants: Outline a clear schedule for participants to follow to ensure instructions are followed. Consider other variables you may want to record and control for in your upcoming data collection. Adherence to preparation instructions will promote stronger data collection during weeks 2 and 3.

4.4.2 Week 2 and 3: Data Collection

For weeks 2 and 3 of this laboratory, participants should prepare by:

- Bringing the same fitness clothes as worn in week 1
- Following the predetermined hypohydration protocol and other preparation instructions as decided upon by the group.

During the experiment, participants will perform their own urine collection, complete BIA measurements, and remain sedentary. Researcher(s) will facilitate data collection during weeks 2

and 3 by ensuring adherence to the experimental protocol, keeping time, preparing data collection materials, and recording/sharing data.

Start Conditions: Participants on each team must decide which drink participants will consume on week 2. One participant should begin with the ORS while the other participant begins with water. In week 3, the participant will consume the drink they did not consume during week 2.

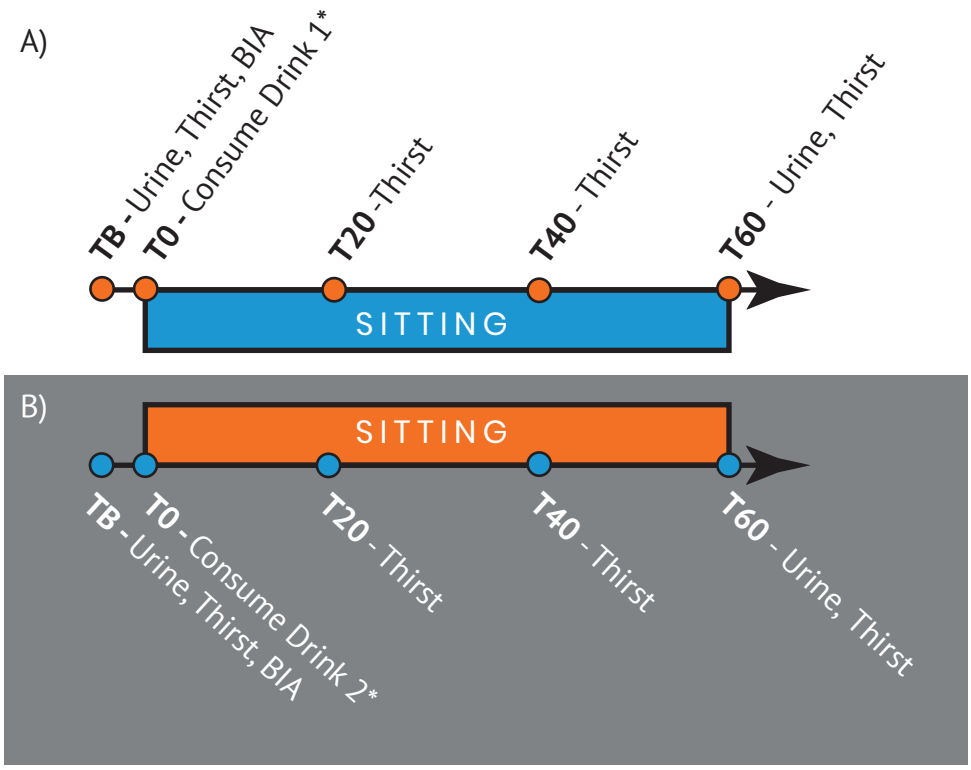


Figure 4.2: **Timeline for the A) Condition 1 and B) Condition 2.** *The first sip of the drink at T0 is when the clock starts for each condition. BIA, bioelectrical impedance analysis; TB, baseline; T0, T20, T40, and T60 refer to minute time points.

An overview of the design protocol is provided in **Figure 4.2**. For both hydration drinks, the following overall procedure is followed:

1. **Drink Consumption**: Obtain ORS or water from refrigerated storage. The volume of drink to be consumed was previously calculated during Week 1. Recall that drink volume should be the same between conditions for the same participant.
    - When prompted by the team researcher, the entire drink must be consumed in 5 minutes.
2. **Thirst assessment**: Thirst assessment questionnaire delivered by researcher(s) to participants at TB, T20, T40, and at T60 with both hydration drinks. Assess thirst using the visual analog scale on your laboratory data sheet.

3. **Urine Collection**: A total of two urine samples per participant will be collected during one lab period. Collection occurs at TB and T60 for both participants.

IMPORTANT: Keep urine specimen collection cup contained in biohazard cooler while transporting to the lab from the bathroom.

- Prepare urine collection supplies for each participant:
  - 1 urine cup and 1 cooler
  - Label urine cup with participant name
- Use the restrooms down the hall to collect a urine sample. If you can, fill the collection cup halfway. Void the rest of the urine into the toilet.
- Provide your capped and labeled cup to your instructor so that they can run the samples through the osmometer. Record the osmolality of urine samples in your lab data sheet.
- Once urine osmolality has been determined on all participant samples, proceed to disposal of biohazardous waste and disinfection of collection areas and equipment.

4. **Body Water via BIA**: Perform the BIA test according to manufacturer standard operating procedure. The BIA test will occur at TB in both conditions. Record body weight, total body water, extracellular water, and intracellular water in your laboratory data sheet. If system data provided in pounds, convert to kilograms.

4.5 LABORATORY REPORT

Use data from this laboratory to complete a standard format laboratory report, as described in Appendix A. Use information collected from the BEVQ-15 and thirst assessment to build your report. The report should address findings in context of the laboratory objectives.

Required Graphs

- **Changes in urine osmolality**: For each participant, create one bar graph comparing urine osmolality in a euhydrated state (week 1) to the urine osmolality of both water and ORS at TB and T60 (weeks 2 and 3). Urine osmolality (mOsm/kg) will be the y-axis and week 1 euhydrated, and TB and T60 timepoints for each drink will be your x-axis. There will be two graphs in this format (one for each participant).
- **Changes in body compartment water**: For each participant, use BIA data to create one graph comparing intracellular hydration from euhydrated state (week 1) to hypohydrated state prior to each drink condition (weeks 2 and 3). Create the same graph for extracellular hydration for each participant. Intracellular and extracellular hydration in kg will be your y-axis and the three different measurements will be your x-axis. There will be four graphs in this format (an intracellular and extracellular hydration graph for each participant).

For graph formatting, please follow the guidelines outlined in Appendix A.2.2.

Deadline for Submission _____

RECOMMENDED READINGS

1. Seifert, J., Harmon, J., & DeClercq, P. (2006). Protein added to a sports drink improves fluid retention. *Int J Sport Nutr Exerc Metab,* 16(4), 420-429. doi:10.1123/ijsnem.16.4.420
2. Institute of Medicine (US) Committee on Military Nutrition Research; Marriott BM, editor. Fluid Replacement and Heat Stress. Washington (DC): National Academies Press (US); 1994. 11, Shift in Body Fluid Compartments After Dehydration in Humans. Available from: https://www.ncbi.nlm.nih.gov/books/NBK231136/
3. Shirreffs, S. M., Aragon-Vargas, L. F., Keil, M., Love, T. D., & Phillips, S. (2007). Rehydration after exercise in the heat: A comparison of 4 commonly used drinks. *International Journal of Sport Nutrition and Exercise Metabolism*, 17(3), 244-258. doi: https://doi.org/10.1123/ijsnem.17.3.244
4. Shafiee, M. A., Charest, A. F., Cheema-Dhadli, S., Glick, D. N., Napolova, O., Roozbeh, J., . . . Halperin, M. L. (2005). Defining conditions that lead to the retention of water: The importance of the arterial sodium concentration. *Kidney international*, 67(2), 613-621. doi:https://doi.org/10.1111/j.1523-1755.2005.67117.x
5. Shirreffs, S. (2009, 11/17). Hydration in sport and exercise: Water, sports drinks and other drinks. Nutrition Bulletin, 34, 374-379. https://doi.org/10.1111/j.1467-3010.2009.01790.x
6. Maricle, B. R., & Pfeifer, A. M. (2009). Ascorbate concentrations and osmolalities in orange and apple juices. Transactions of the Kansas Academy of Science (1903-), 112(1/2), 77-86. http://www.jstor.org/stable/40588223
7. Casa, D. J., Armstrong, L. E., Hillman, S. K., Montain, S. J., Reiff, R. V., Rich, B. S., Roberts, W. O., & Stone, J. A. (2000). National athletic trainers' association position statement: fluid replacement for athletes. Journal of athletic training, 35(2), 212-224. https://pubmed.ncbi.nlm.nih.gov/16558633
8. Fausnacht, A. G., Myers, E. A., Hess, E. L., Davy, B. M., & Hedrick, V. E. Update of the BEVQ-15, a beverage intake questionnaire for habitual beverage intake for adults: determining comparative validity and reproducibility. *Journal of Human Nutrition and Dietetics*. https://doi.org/10.1111/jhn.12749

This chapter has online resources on GitHub.

NAME

LABORATORY 4 DATA SHEET - WEEK 1

DATE

Oral Rehydration Solution		
Component(s)	**Weight (g)**	**Osmolarity Calculation(s)**
		Total Osmolarity

Body Water				
Participant ID	**Body Weight (kg)**	**Total Body Water (kg)**	**Intracellular Water(kg)**	**Extracellular Water (kg)**

Urine Collection	
Participant ID	**Osmolality (mOsM/kg)**

Also complete:

- BEVQ-15 and BEVQ-15 Scoring
- Thirst Assessment

Beverage Questionnaire (BEVQ-15)

Instructions:

For the past month, please indicate your intake for each beverage type by marking an "X" in the bubble for "how often" and "how much each time".

1. Indicate how often you drank the following beverages, for example, if you drank 5 glasses of water per week, mark 4-6 times per week.
2. Indicate the approximate amount of beverage you drank each time, for example, if you drank 1 cup of water each time, mark 1 cup under "how much each time". If applicable, indicate the specific type of beverage by marking an "X" in the bubble by the one used (i.e., type of nut milk).
3. When trying to estimate your intake throughout the day, (i.e., water) think about the total amount you drink. For example, 3 times per day and 20 fl oz each time = 60 fl oz per day. **If you consume more 60 fl oz per day select "1 time per day" and write the TOTAL daily amount in the last column.**
4. Do not count beverages used in cooking or other preparations, such as milk in cereal.
5. Count milk/creamer added to tea and coffee in the tea or coffee with creamer beverage category, NOT in the milk categories; this includes non-dairy creamer. Please indicate the type of creamer (flavored, plain or sugar-free) and sweetener used by marking an "X" in the bubble by the one used, if applicable.

Type of Beverage	HOW OFTEN (MARK ONE)							HOW MUCH EACH TIME (MARK ONE)					
	Never or less than 1 time per week (go to next beverage)	1 time per week	2-3 times per week	4-6 times per week	1 time per day	2 times per day	3+ times per day	Less than 6 fl oz (¾ cup)	8 fl oz (1 cup)	12 fl oz (1½ cups)	16 fl oz (2 cups)	20 fl oz (2½ cups)	> 20 fl oz (specify TOTAL daily amount)
Water or unsweetened sparkling water	O	O	O	O	O	O	O	O	O	O	O	O	_____
100% Fruit Juice	O	O	O	O	O	O	O	O	O	O	O	O	_____
Sweetened Juice Beverage/Drink (fruit punch, juice cocktail, Sunny Delight, Capri Sun)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Whole Milk: red cap, Reduced Fat Milk 2%: purple cap, or Chocolate Milk	O	O	O	O	O	O	O	O	O	O	O	O	_____
Low Fat 1%: green cap, Fat Free/Skim Milk: light blue cap, Buttermilk or Soy Milk	O	O	O	O	O	O	O	O	O	O	O	O	_____
Nut Milk (almond, cashew, coconut) O Flavored, Original, or Plain O Unsweetened	O	O	O	O	O	O	O	O	O	O	O	O	_____
Soft Drinks, Regular	O	O	O	O	O	O	O	O	O	O	O	O	_____
Energy & Sports Drinks, Regular (Red Bull, Gatorade, Powerade)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Diet or Artificially Sweetened Soft Drinks, Energy & Sports Drinks (Diet Coke, Crystal Light, artificially sweetened sparkling water, Sugar-Free or Total Zero Red Bull, Powerade Zero)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Sweet Tea (with sugar)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Tea or Coffee, black (no creamer or milk) O Sugar, O Artificial Sweetener, O N/A	O	O	O	O	O	O	O	O	O	O	O	O	_____
Tea or Coffee (w/ milk &/or creamer) O Sugar O Artificial Sweetener O N/A Milk &/or Creamer: O Milk O Half & Half or Cream O N/A Creamer: O Flav. O Plain O Sugar-Free	O	O	O	O	O	O	O	O	O	O	O	O	_____
Wine (red or white)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Hard Liquor (vodka, rum, tequila, etc.)	O	O	O	O	O	O	O	O	O	O	O	O	_____
Beer, Ales, Wine Coolers, Non-alcoholic or Light Beer	O	O	O	O	O	O	O	O	O	O	O	O	_____
Other (list):_____	O	O	O	O	O	O	O	O	O	O	O	O	_____

Virginia Tech, 2016.

NAME

BEVQ-15 SCORING

Type of Beverage	Average Daily Consumption (oz)
Water or unsweetened sparkling water	
100% Fruit Juice	
Sweetened Juice Beverage/Drink (fruit punch, juice cocktail, Sunny Delight, Capri Sun)	
Whole Milk: red cap, Reduced Fat Milk 2%: purple cap, or Chocolate Milk	
Low Fat 1%: green cap, Fat Free/Skim Milk: light blue cap, Buttermilk or Soy Milk	
Nut Milk (almond, cashew, coconut) O Flavored, O Unsweetened Original, or Plain	
Soft Drinks, Regular	
Energy & Sports Drinks, Regular (Red Bull, Gatorade, Powerade)	
Diet or Artificially Sweetened Soft Drinks, Energy & Sports Drinks	
Sweet Tea (with sugar)	
Tea or Coffee, black (no creamer or milk) O Sugar, O Artificial Sweetener, O N/A	
Tea or Coffee (with creamer) O Sugar O Artificial Sweetener O N/A O Milk O Half & Half or Cream O N/A Creamer: O Flavored O Reg. O Sugar-Free O N/A	
Wine (red or white)	
Hard Liquor (vodka, rum, tequila, etc.)	
Beer, Ales, Wine Coolers, Non-alcoholic or Light Beer	
Other (list):_____	
Other (list):_____	
Other (list):_____	

NAME

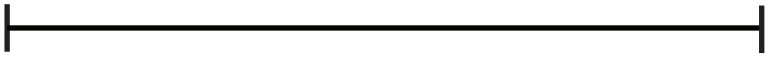
LABORATORY 4 DATA SHEET – THIRST

Participant ID (no names)

Researcher: Instruct participants to make a mark on the line on the visual analog scale (VAS) at the given time collection. Cover past responses with a folded sheet of paper.

Condition 1 - TB

How thirsty do you feel right now?



Not thirsty Very thirsty

Condition 1 - 20

How thirsty do you feel right now?

Not thirsty Very thirsty

Condition 1 - 40

How thirsty do you feel right now?

Not thirsty Very thirsty

Condition 1 - 60

How thirsty do you feel right now?

Not thirsty Very thirsty

NAME

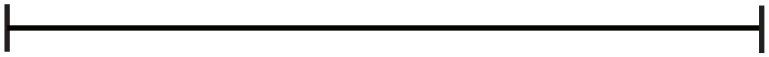
LABORATORY 4 DATA SHEET – THIRST

Participant ID (no names)

Researcher: Instruct participants to make a mark on the line on the visual analog scale (VAS) at the given time collection. Cover past responses with a folded sheet of paper.

Condition 2 - TB

How thirsty do you feel right now?



Not thirsty — Very thirsty

Condition 2 - 20

How thirsty do you feel right now?

Not thirsty — Very thirsty

Condition 2 - 40

How thirsty do you feel right now?

Not thirsty — Very thirsty

Condition 2 - 60

How thirsty do you feel right now?

Not thirsty — Very thirsty

NAME

LABORATORY 4 DATA SHEET - CONDITION 1

Participant ID (no names)

Drink:

Urine Collection			
Time	**Collection Start Time**	**Osmolality (mOsM/kg)**	**Notes**
TB			
T60			

Body Water				
Time	**Body Weight (kg)**	**Total Body Water (kg)**	**Intracellular Water(kg)**	**Extracellular Water (kg)**
TB				

NAME

LABORATORY 4 DATA SHEET - CONDITION 2

Participant ID (no names)

Drink:

Urine Collection			
Time	**Collection Start Time**	**Osmolality (mOsM/kg)**	**Notes**
TB			
T60			

Body Water				
Time	**Body Weight (kg)**	**Total Body Water (kg)**	**Intracellular Water(kg)**	**Extracellular Water (kg)**
TB				

NAME

LABORATORY 4 NOTES

METABOLIC HEALTH AND ENERGY AVAILABILITY

5.1 BACKGROUND

Metabolic health refers to the overall functional status of our biochemical, cellular, and physiological systems. This functional status affects how we process nutrients and has a wide array of interconnected downstream impacts on health. Metabolic health is heavily impacted by lifestyle factors including physical activity and diet. Adaptations that occur in response to physical activity and exercise training include increased physical fitness and exercise capacity. These adaptations improve health and lower disease risk. Examples of how metabolic and cellular adaptations to exercise simultaneously improve health are given in **Table 5.1**.

Table 5.1: **Examples of Exercise-Induced Adaptations that Prevent or Reverse Disease Processes.** (1,2).

Exercise-induced Adaptation	Impact on Exercise Performance/Recovery	Impact on Health	Diseases Potentially Prevented
↓ inflammation	↑ function ↑ recovery	↓ insulin resistance	type 2 diabetes cardiovascular diseases neurodegenerative diseases cancer
↓ oxidative stress	↓ cellular damage ↑ mitochondrial function	↓ inflammation ↓ cellular damage ↓ dysfunction	type 2 diabetes cardiovascular diseases neurodegenerative diseases cancer
↑ insulin signaling	↑ glycogen resynthesis	↓ lipolysis ↑ glycemic control ↓ visceral adipose tissue ↓ insulin	type 2 diabetes cardiovascular diseases neurodegenerative diseases cancer
↑ muscle mass	↑ force production	↑ glucose control via tissue to take up glucose with elevated insulin	type 2 diabetes cardiovascular diseases
↑ vascular endothelial function	↑ blood delivery ↑ VO_2 max	↓ blood pressure	hypertension
↑ neurogenesis	↑ cognitive function	↑ neural health	neurodegenerative diseases

Diet and physical activity are the first line of defense against development of many chronic diseases. Healthy foods can have disease preventing impacts like that of exercise (1, 3). For example, fruits and vegetables have anti-inflammatory phytochemicals, foods with lower glycemic indexes aid in maintaining glycemic control, and n-3 fatty acids have anti-inflammatory and other health benefits (3). The amount of fat that is stored in adipose tissue also influences health. As the size of adipose cells increases, the endoplasmic reticulum becomes stressed, and adipocytes secrete pro-inflammatory damage signals (4). These signals stimulate inflammation and make the adipose tissue dysfunctional. Loss of function in adipose tissue leads to increased release of free fatty acids and other disease promoting factors. This dysfunction is particularly disease promoting when it occurs in the adipose tissue within the abdominal compartment, known as **visceral adipose tissue** (VAT).

Physical activity and diet influence the total amount of fat stored in adipose tissue as well as VAT. Caloric restriction and weight loss may be helpful to improve health if approached in a long-term, sustainable manner. Increasing physical activity may be an effective way to promote a negative energy balance. However, nutrients and energy are both needed to support health and adaptations to exercise. For example, energy, amino acids, and several micronutrients (vitamins and minerals) are needed for protein synthesis to produce the adaptations listed in **Table 5.1**. Thus, it is important to know the minimum amount of energy that is needed to support healthful adaptations to exercise so that health is not diminished by caloric restriction.

One method to assess the adequacy of energy intake is to calculate **energy availability** per kilogram of **fat-free mass** (FFM). Adipose tissue has lower metabolic demands compared to FFM. Thus, estimates of energy need will be more accurate if calculated for FFM rather than total body mass. The following recommendations for energy availability are generally accepted by a variety of sports nutrition experts (5):

- **High** for growth and body mass gain, >45 kcal/kg FFM
- **Adequate** for weight maintenance, ~ 45 kcal/kg FFM,
- **Reduced** but adequate for healthy weight loss, 30-45 kcal/kg FFM or
- **Low** with health implications, <30 kcal/kg FFM

Energy availability is the amount of energy that is available for metabolic activities after subtracting the cost of physical activity from total energy intake. In addition to physical activity, the major metabolic needs are for growth, cellular maintenance, thermal regulation, and reproduction. A minimum of 30 kcal/kg FFM is needed to support non-physical activity metabolism. If this minimum amount of energy is not available, then one or more of these major metabolic needs will be negatively impacted. Adaptations to physical activity and exercise training are a form of growth, so low energy availability could impair adaptations to and recovery from exercise and increase risk for overtraining. Immune and reproductive system function is also vulnerable to low energy availability and insufficient resources to support these systems may have downstream health impacts, e.g. susceptibility to infection or decreased bone mass.

Example calculation of low energy availability:

- 60 kg female with 20% body fat = 12 kg fat mass (FM) and 48 kg fat-free mass (FFM)
- Her daily energy intake is restricted to 1800 kcal
- Energy cost of exercise = 500 kcal
- Energy availability = Energy intake – Energy cost of Exercise
    - 1800 kcal intake - 500 kcal energy cost during exercise = 1300 kcal of energy available for the remaining 4 major metabolic activities (growth, cellular maintenance, reproduction, thermoregulation)
- Energy availability by kg of FFM = $\frac{1300\ kcal}{48\ kg\ FFM} = \mathbf{27}\ \frac{\boldsymbol{kcal}}{\boldsymbol{kg\ FFM}}$

5.2 OBJECTIVE

The purpose of this laboratory is to:

1. Assess your own metabolic health and gain an understanding of the tools and measures used to assess risk of metabolic diseases.
2. Use body composition assessment of fat free mass to calculate the amount of energy availability recommended to maintain health during exercise training.

5.3 OVERVIEW

In week 1, students will learn how to do the assessments and interpret results related to metabolic health. Appointments will then be made for an individual time slot to perform assessments in the morning after an overnight fast. Everyone will assess their own metabolic health via analysis of finger stick blood samples and body composition.

Body composition will be assessed using bioelectrical impedance analysis (BIA) to measure VAT and FFM. The amount of VAT is important for assessment of disease risk, and FFM will be used to calculate the amount of energy that needs to be available to support both health and adaptations to physical activity. These measurements will be used to evaluate disease risk and potential to improve health.

5.4 PROCEDURES

The following assessments will be made during this laboratory:

5.4.1 Metabolic Syndrome Criteria

Metabolic syndrome is a cluster of interrelated variables known to increase risk for type 2 diabetes and cardiovascular disease. To be diagnosed with metabolic syndrome, individuals must meet a minimum of three of the following criteria (6):

- **Elevated waist circumference:** ⩾ 88 cm in females or ⩾ 102 cm in males
    - Waist circumference is highly correlated with and considered a proxy measure for VAT.
- **Elevated fasting blood glucose (FBG)**: ⩾ 100 mg/dL (5.56 mmol/L)
    - Impaired fasting glucose levels are an indication of hepatic insulin resistance. Insulin inhibits gluconeogenesis in the liver. Thus, if the liver is resistant to insulin, then it will overproduce glucose. This insulin resistance and overproduction of glucose is evident under fasting conditions.
    - With respect to diabetes, prediabetes is defined as having an FBG of 100-125 mg/dl (5.56 - 6.94 mmol/L) and diabetes is defined as an FBG ⩾ 126 mg/dl (7 mmol/L). However, a number of factors can influence FBG, and this measure alone or on a single occasion is not sufficient to indicate presence of diabetes.
- **Elevated fasting triglycerides (TG)**: ⩾ 150 mg/dl (1.69 mmol/L)
    - Dyslipidemia in metabolic syndrome is evident with an increase in circulating triglycerides. Under fasting conditions, most triglycerides in the blood are part of the very low-density lipoproteins (VLDL). Elevations in triglyceride may occur for a number of reasons, including high carbohydrate intake and insulin resistance.
- **Reduced high-density lipoprotein (HDL)**: < 40 mg/dl (1.03 mmol/L) in males and < 50 mg/dl (1.29 mmol/L) in females
    - This lipoprotein has many functions including reverse cholesterol transport, to remove excess cholesterol from tissues and plaques forming in blood vessels. Higher levels of HDL are protective against cardiovascular disease and vice versa.
- **Elevated systolic (SBP) or diastolic (DBP) blood pressure**: SBP ⩾ 130 mmHg or DBP ⩾ 85 mmHg
    - Potential mechanisms linking metabolic syndrome to elevated blood pressure are elevated sodium levels secondary to renal insulin resistance and increased blood volume resulting from altered metabolism and production of hormones that increase blood pressure.

5.4.2 Hemoglobin A1c (HBA1c)

Hemoglobin A1c (HbA1c) is a measure of glycosylated hemoglobin and reflects glycemic control over the preceding 90 days. Glycosylation of proteins occurs when the concentration of glucose is elevated. Red blood cells have a life span in the blood of approximately 90 days. Thus, the extent to which glucose binds to hemoglobin in red blood cells reflects the 'average' level of glucose over that 90-day period. As such, HbA1c is a diagnostic marker used to classify individuals as non-diabetic (<5.7%), prediabetic (5.7-6.4%), or diabetic (≥ 6.5%). (7)

5.4.3 Visceral Adipose Tissue (VAT) Volume

Algorithms to estimate VAT from BIA have been validated against the gold standard measure of magnetic resonance imaging. Elevated levels of VAT are undesirable because pro-inflammatory mediators and free fatty acids secreted by VAT are delivered through the blood.

5.4.4 Blood Lipids

In addition to assessing triglycerides and HDL within the context of metabolic syndrome criteria, a standard lipid panel can be used to determine how multiple components of the lipid panel may affect disease development. Current practice is to interpret lipid panels in the context of other risk factors. For example, the decision to treat elevated LDL is informed by age, family history, diabetes status (having diabetes would be an indication for more aggressive treatment), risk factors, and presence of atherosclerotic cardiovascular disease (8). Diet, physical activity, and not smoking are pillars of primary prevention for chronic diseases. Implementing primary prevention strategies at an early age is important for everyone and intensive primary prevention is recommended for young adults (20-39 years of age) with suboptimal lipid panel values (9). The references ranges for four key lipid panel components are outlined in **Table 5.2**.

Table 5.2: **Target levels for fasting lipid panel components** (6, 9).

Marker (Units)	Reference Range
Total Cholesterol (mg/dL)	Desirable <150; Borderline High 200-239; High ≥ 240
HDL (mg/dL)	Optimal > 40 for males and >50 for females
LDL (mg/dL)	Optimal <100; Near Optimal 100-129; Borderline High 130-159; High 160 - 189
Triglycerides, TG (mg/dL)	Normal <150; Borderline High 150 - 199; High 200-499; Very High ≥ 500

5.4.5 Framingham Risk Score Calculator

Calculations of relative risk and odds ratios from prospective cohort research studies have been used to develop tools that individuals may use to estimate their risk of coronary heart disease in the next 10 years. There are a number of calculators available to estimate risk based on the key variables that were most predictive of coronary heart disease risk. These variables typically include age, sex, smoking status, blood pressure, HDL level, and total cholesterol level.

The **Framingham Heart Study** is a seminal epidemiological study in which a variety of measures were made in a large population of people (the population of Framingham, Massachusetts at the onset) and then the population was followed over time and monitored for coronary heart disease (**Figure 5.1**). After many years, an analysis was performed to see which of the variables best predicted risk of developing disease. That analysis was then used to generate an algorithm and risk calculator (10). This is just one of many tools that can be used to understand disease risk. The tool does not take into account other lifestyle factors such as the type of diet you eat and your level of physical activity. While these may be indirectly reflected in the measures you enter into the calculator, the estimated risk is independent of any dietary improvements or changes in physical activity that might be undertaken. That is, a person can make lifestyle improvements to lower risk. The minimum age for this calculation is typically 30 years. However, there are calculators that will go as low as 20 years.

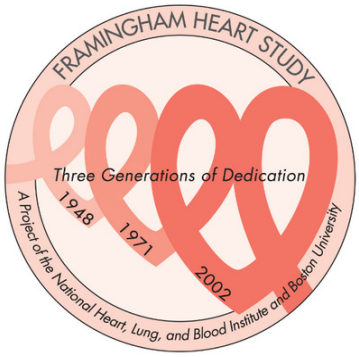


Figure 5.1: **The Framingham Heart Study.** Since 1948, the Framingham Heart Study has followed the population and enrolled additional cohorts of individuals. Logo from @FraminghamStudy on Twitter.

- Cardiovascular Disease Risk Online Calculator (Ages 30 - 74)
- Cardiovascular Disease Risk Online Calculator (Ages 20 - 74)

5.4.6 Schedule a Visit

1. Sign up for an appointment slot to come into the laboratory for a 20-minute appointment.
2. Preparation the day before your appointment can influence the measures. For best results, on the day before the appointment, you should do the following things:
    - Eat a diet that is balanced (roughly 40-60% carbohydrate, 20-30% fat, 10-20% protein) in macronutrient composition and not out of the ordinary for any one macronutrient. For

example, consuming a lot of sugar or a lot of fat can impact glucose and triglyceride measures.

- Avoid prolonged strenuous or unaccustomed exercise.
- Hydrate.
- Do not consume alcohol.
- Consume nothing other than water starting 12 hours before your appointment.
- Get a good amount of sleep the night before the test.

3. Show up to lab approximately 5 minutes before your appointed time.
4. The following measures will be made at the appointment:
    - BIA for determination of FFM and VAT
    - Waist circumference
    - Blood pressure
    - HbA1c via finger stick
    - Glucose, total cholesterol, triglycerides, and HDL via finger stick

5.5 LABORATORY REPORT

A slightly modified version of the standard laboratory report should be prepared for this laboratory. This report should have an introduction with a purpose statement (there will not be a hypothesis), a brief methods section (less than typical reports), an extended results section (details below), and a 1-2 paragraph discussion (less than typical reports) describing your metabolic health and potential dietary and lifestyle changes that you could make to improve your metabolic health and lower disease risk.

Use your data to calculate and or report the following information (your data compared to the optimal, recommended, or target for the measure) in the results section (include calculations and units where appropriate):

- What is the minimum amount of energy you should consume to ensure adequate energy availability if you perform 30, 60, or 90 minutes of physical activity per day?

You may use the Compendium of Physical Activity Energy Expenditure website to choose physical activities that are most typical for you (11). Use this equation:

$$\text{Energy expenditure (kcal)} = \text{METs} \times \text{mass} \times \text{duration}$$

Where kilocalories is the amount of energy expended during the activity, mass is your body mass in kilograms, duration is the amount of time in hours spent doing the activity, and MET is metabolic equivalents (1 MET = resting energy expenditure).

Example

Energy expenditure = 7.5 MET (kcal/kg/h) x 80 kg x 1.5 h = 900 kcal

Present your value and the metabolic syndrome criteria or each of the five metabolic syndrome measures.

- How many and which metabolic syndrome criteria do you have?
- Is the volume of VAT that you have ideal, or does it put you at elevated risk for metabolic diseases?
- How do your lipid panel measures compare to target values?
- What is your estimated risk for coronary heart disease in the next 10 years based on the Framingham risk calculation at the present time? Using your current values for lab measures and changing your age in the calculator. How does this risk change each decade until you are in your 90s?
- Are you non-diabetic, prediabetic, or diabetic according to your HbA1c?

Cautionary Note Regarding Measurement of Disease Risk Factors

Having increased risk for disease is not the same as having a disease and does not mean that the disease will occur. It is an indication of the likelihood or odds that the disease will occur. Most of us will develop chronic diseases if we live long enough. The value in assessing risk is that it provides information that can be used to prevent or delay the onset of disease with lifestyle changes or other therapeutic strategies. Healthy lifestyles help to increase health span by pushing the onset of disease to older age.

Please note that any concerning laboratory values and findings should be discussed and or confirmed with your personal physician. If you have immediate concerns, please do not hesitate to discuss those with the instructor.

REFERENCES CITED AND RECOMMENDED READINGS

1. Miles MP, Wilson S, Yeoman CJ. Physical Activity and Inflammation Phenotype Conversion. Journal of Clinical Exercise Physiology. 2019;8(2):64-73.

2. Pareja-Galeano H, Garatachea N, Lucia A. Exercise as a Polypill for Chronic Diseases. In: Bouchard C, editor. Molecular and Cellular Regulation of Adaptation to Exercise. 135. Waltham, MA: Academic Press; 2015. p. 498-526.

3. Salas-Salvado J, Martinez-Gonzalez MA, Bullo M, Ros E. The role of diet in the prevention of type 2 diabetes. Nutr Metab Cardiovasc Dis. 2011;21 Suppl 2:B32-48. PubMed PMID: 21745730.

4. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annual review of immunology. 2011;29:415-45. Epub 2011/01/12. doi: 10.1146/annurev-immunol-031210-101322. PubMed PMID: 21219177.

5. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance. Journal of the Academy of Nutrition and Dietetics. 2016;116(3):501-28. Epub 2016/02/28. doi: 10.1016/j.jand.2015.12.006. PubMed PMID: 26920240.

6. Grundy SM. Metabolic syndrome update. Trends in cardiovascular medicine. 2016;26(4):364-73. Epub 2015/12/15. doi: 10.1016/j.tcm.2015.10.004. PubMed PMID: 26654259.

7. Chamberlain JJ, Rhinehart AS, Shaefer CF, Jr., Neuman A. Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. Ann Intern Med. 2016;164(8):542-52. Epub 2016/03/02. doi: 10.7326/m15-3016. PubMed PMID: 26928912.

8. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 Suppl 2):S1-45. Epub 2013/11/14. doi: 10.1161/01.cir.0000437738.63853.7a. PubMed PMID: 24222016.

9. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139(25):e1082-e143. Epub 2018/12/28. doi: 10.1161/cir.0000000000000625. PubMed PMID: 30586774; PubMed Central PMCID: PMCPMC7403606.

10. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53. Epub 2008/01/24. doi: 10.1161/circulationaha.107.699579. PubMed PMID: 18212285.

11. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Jr., Tudor-Locke C, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011;43(8):1575-81. Epub 2011/06/18. doi: 10.1249/MSS.0b013e31821ece12. PubMed PMID: 21681120.

This chapter has online resources on GitHub.

NAME

LABORATORY 5 DATA SHEET

Metabolic Syndrome Criteria	
Marker	**Measurement**
Waist Circumference	
Fasting Blood Glucose	
High-density Lipoprotein	
Triglycerides	
Blood Pressure	

Bioelectrical Impedance Analysis	
Marker	**Measurement**
Fat-Free Mass	
Visceral Adipose Tissue	

Hemoglobin A1c
Measurement:

NAME

LABORATORY 5 NOTES

GUIDELINES, FORMAT, AND REQUIREMENTS FOR LABORATORY REPORTS

A.1 Guidelines

All laboratory reports must be generated using computer word processing and graphing programs. Laboratory reports should be single spaced with 1-inch margins. A clear sans serif font (e.g, Arial, Calibri, Helvetica) with 12-point sizing should be used. The report should include a title and the following sections: introduction, methods, results, discussion, and references.

A.1.1 Title

The title should be a brief declarative statement that focuses on results presented in the report.

A.1.2 Introduction

Describe what the purpose of the laboratory is and what practical or theoretical implications you can gather from the laboratory findings. State the research question and aim(s) of the laboratory in concise language. Leading up to this aim(s) statement, there should be text utilizing peer-reviewed literature to provide sufficient context for the reader to understand the rationale for the laboratory. The text of the introduction should be 2-3 paragraphs or approximately 0.5 pages in length.

A.1.3 Methods

The methods section describes how the study was performed. The purpose of the methods is provide the reader with information to assess the suitability of the experimental design and methods used. Sufficient information should be provided that would allow the reader to replicate the experiment. The text of the methods section should be approximately 0.5-1 pages in length.

Methods should have distinct paragraphs for:

1. a summary of the experimental design that identifies the main variables measured
2. short, concise paragraphs describing the experimental procedures and method for data reduction, if applicable
3. analysis of data

The methods section should not include study findings. The methods section should let the reader know who was involved in the study and what happened during the study. In describing who was involved, it generally begins with a statement of the number and type of subjects involved in the

experiment. The bulk of the methods section should describe the experimental protocol, the equipment used, and what measures were obtained during the protocol.

Data reduction is the process of calculating variables, e.g. the average of two trials was taken, or %VO_2max was calculated and the kcal of carbohydrate expenditure to the VO_2 at 35, 65, and 80% of VO_2max was determined by selecting the measured values nearest to the calculated VO_2 percentages. In other instances, direct measurements are used and a description of data reduction is not required.

In this laboratory course, analysis of data will consist primarily of descriptive statistics, e.g. calculating the mean of measures from 2+ members of the lab group. In some instances, there may be data from only one member of the lab group for a given condition. In this instance, descriptive statistics are not needed and do not need to be described.

A.1.4 Results

The results section is the most important part of your laboratory report as it indicates if your experiment did or did not accomplish what the experiment was set up for. Results should present findings clearly, in a logical order, and be relevant to the research question in the introduction. There should be a very concise narrative that describes the key findings and refers to the figures or tables in which the data described are presented. The results should be presented in this section but save the discussion of study implications or conclusions for the discussion section. Keeping figures to the approximate size of those shown in this chapter, the results section will be approximately 1 page in length.

Tables and figures can be used to provide organized or visual summaries of findings. The tables and figures should follow required guidelines including size, clarity, labeling, titles, and figure legends. The text should not repeat numbers that are provided in tables and figures but should refer to these tables and figures and highlight key features.

A.1.5 Discussion

The discussion section answers the research question presented in the introduction and draws reasonable conclusions and presents their implications. To accomplish this, you may refer to study results but do not waste space directly repeating them. Instead, explain your study results in a larger context. In addition, use this section to provide possible explanations if the results did not match your hypothesis. Text of the discussion should be 2-4 paragraphs or approximately 0.5 to 1.0 pages in length. The discussion should:

- Interpret major findings related to the research question
- Relate findings to previous peer-reviewed research (previous supporting evidence **and** contrary evidence)
- Present final conclusions and their theoretical and/or practical implications

A.1.6 References

Citations are important in scholarly writing to show readers that you have performed the appropriate research. By listing your sources, you acknowledge and give credit to other researchers for their work and ideas and avoid plagiarism.

A minimum of 2 peer-reviewed research articles must be cited in the text of each laboratory report. Research articles cited in text must be also presented in the references section of your report. At least one of these articles should be an original research study, i.e. summarizing a research experiment and containing introduction, methods, results, and discussion sections.

Follow the citation format outlined in the APA 7th edition for your in-text citations and your reference page. As an example, consider the article (**Figure A.1)** by Asker Jeukendrup (on PubMed Central here):

Sports Med (2017) 47 (Suppl 1):S101–S110
DOI 10.1007/s40279-017-0690-6

CrossMark

REVIEW ARTICLE

Training the Gut for Athletes

Asker E. Jeukendrup[1]

Figure A.1: **Peer-reviewed journal article on sports nutrition by Asker Jeukendrup.** The first page of a journal article typically contains the information you need to create a citation.

From the first page, we can obtain the elements required for the citation for your reference list:

- Author - Asker E. Jeukendrup
- Publication Date - 2017
- Publication Title - Training the Gut for Athletes
- Publication Source - Sports Med 47 (Suppl 1): 101-110. DOI 10.1007/s40279-017-0690-6

When citing this article in your text, it would read in parentheses as (Jeukendrup, 2017). Once you have utilized the citation in-text, it also goes into your reference list at the end of your report. In your reference list, it would read as:

> Jeukendrup, A. E. (2017). Training the gut for athletes. *Sports Med, 47(Suppl 1)*, 101-110. DOI 10.1007/s40279-017-0690-

It is important to note that citations will look different based on features like number of and type

of work. Programs called citation managers can help you keep track of articles and create article citations for you in the APA 7th edition. Major citation programs can work inside Microsoft Word (some can work inside Google Docs) to help you cite articles as you write your report. A good citation manager will work with your needs and budget. Common citation managers include:

- EndNote, Mendeley, RefWorks, Zotero

A.2 Format

A.2.1 Tables

Tables can be placed into Word documents from Excel. General points for creating tables include:

- Each table should have a clear and descriptive title.
- Table footer should indicate what values are represented (Ex. mean vs median, standard deviation vs standard error).
- Variables in the table should have units (Heart rate, beats per minute).
- Any abbreviations used should be described in the table footer.

A.2.2 Figures

Figures can be created using graphical and statistical programs. General points for creating figures include:

- Each figure should have a clear and descriptive title.
- Figure caption should indicate what values are represented.
- Dependent variable on the y-axis, Independent variable on the x-axis
- X and Y axes should have labels in a font size that can be read easily.
- Axis scaling should be clear and make sense.
- Variable groupings should be indicated by a clear figure legend.
- Symbols are distinguishable from other symbols.
- Any abbreviations should be described in the figure caption.
- Use error bars when applicable to indicate standard error or standard deviation.
- Data should portrayed in the clearest manner. Avoid over-plotting.

The figures in the example lab report were created using Excel and models these general points. An

Excel template (data included) for creating similar figures is available on this manual's GitHub repository under Appendix A. The repository also contains the same data used to create similar figures but was created using the program RStudio. The code to recreate this figure is also available on GiHhub under Appendix A. Readers are encouraged to build skills in RStudio as it is a free and powerful data analysis and visualization program.

A.3 Sample Lab Report

The following document provides an example of how to complete each component of a laboratory report with color highlights linked to specific report requirements. Students should use this sample report to guide the content, format, and structure of lab reports. Each section is broken down to illustrate how each component of the sample lab relates to the content and structure requirements for lab reports.

Impact of Pre-Exercise Feeding on Substrate Utilization and Perception of Effort

Component Legend

Part 1: Hook

Part 2: Research background

Part 3: Research need or importance

Part 4: Specific aim of study

Part 5: Hypothesis

Part 6: Reasoning behind hypothesis

Introduction

Carbohydrate (CHO) quantity before exercise influences performance. Adequate CHO consumption prior to exercise promotes muscle and liver glycogen storage which may then be used for energy production during exercise. The International Olympic Committee on Sports Nutrition recommends athletes consume 5-12 grams (g) CHO per kilogram (kg) of body weight each day according to duration and intensity of exercise training. A CHO rich meal containing 140-330 g, 3-5 hours prior to exercise improves aerobic performance. Carbohydrate availability during exercise influences substrate utilization and the perception of effort during a bout of exercise (Melzer, 2011). Understanding how pre-exercise feeding affects substrate utilization and ratings of perceived exertion (RPE) during exercise is important for guiding pre-exercise meal decisions.

The purpose of the study is to determine how substrate utilization and perceived effort during 30 minutes of vigorous exercise are impacted by pre-exercise feeding. It is hypothesized that a CHO rich pre-exercise meal will result in greater rates of CHO oxidation and lower reported RPE during exercise. During high intensity exercise, muscle glycogen is the main fuel source used for energy production (Romijn et al., 1993). For this reason, glycogen depletion during exercise is a limiting factor on performance and one cause of fatigue. Ingestion of CHO before exercise increases the availability of glucose for CHO metabolism during vigorous exercise and spares glycogen depletion. If pre-exercise feeding lowers

perception of effort during exercise, then training may be performed at higher exercise intensities and/or for a longer duration of time resulting in greater training benefits. Understanding the relationship between pre-exercise CHO feeding and the RPE is needed to guide athletes in nutrition decisions for training goals.

Part 7: Significance of research

Methods

Experimental design

This study was conducted for the Sports Nutrition Lab (SNL) at Montana State University (Bozeman, MT, USA). One 22-year-old female subject completed two testing sessions conducted over two weeks, one with and one without pre-exercise feeding. The subject was 56 kg, 1.57m tall and an experienced runner. Exercise testing was completed to determine substrate utilization and RPE in an unfed and fed state. The Physical Activity Readiness Questionnaire (PAR-Q) was completed prior to testing to determine eligibility for physical activity.

Part 1: Summarize experimental design and the main variables measured. Provide description of participants including age, sex, and anthropometric measurements.

Experimental procedures

The subject repeated the same exercise testing under two conditions, an unfed state and a CHO fed state. Both testing conditions were conducted at the same time of day. The subject was instructed to abstain from food and beverages (except water) for five hours before testing. A pre-exercise meal was consumed in the SNL 4 hours before the scheduled exercise test (140 g CHO's, 10 g fat, 6 g pro). A pre-exercise snack was provided to the subject to consume 45 min before returning for testing (45 g CHO, 3 g fat, 2 g pro). The meal CHO content was determined based on International Olympic Committee on Sports Nutrition daily CHO recommendation (5 g CHO/kg body weight) divided in half. The subject was fitted for a chest heart rate (HR) monitor (Polar Electro Oy, Polar H10 Heart Rate Sensor, Kempele, Finland) then performed a 10-minute warm-up on a Monark cycle ergometer. HR measurements were recorded every minute during testing. CHO and fat utilization was measured with a metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT, USA). The subject was fitted with a mouthpiece, headgear, and Rudolph valve that was connected to the metabolic card prior to testing. In order to test vigorous exercise, the participant was instructed to run at a minimum of 77% of age predicted HR max (APHR; 208bpm – (0.7 x age) = bpm) and maximum of 95% APHR. The American College of Sports Medicine defines vigorous exercise as 77-95% of HR max. Treadmill speed was adjusted according to subject's HR to main the target range. RPE was recorded every 2 min. The subject completed 10 min self-paced cool down after 30 min of vigorous

Part 2: Describe the experimental procedures. Include the manufacturer and data collection settings used for all equipment and instrumentation in the study.

exercise.

Part 3: Describe methods used for calculations and statistical analysis.

Data analysis

Substrate oxidation of CHO and fat were calculated from respiratory quotient (RQ), the ratio of carbon dioxide (CO_2) and oxygen (O_2) produced and consumed. Descriptive statistics were used to compare conditions. HR averages were reported as mean and standard deviation. Microsoft Excel was used for data entry and to calculate and visualize the data.

Results

Part 1: Summarize the purpose of the lab and what was done (1-2 sentences).

Part 2: Body of the results section. Fully describe data in an impartial way. Describe overall trends of the data and compare differences in data points. Reference tables and figures as needed.

Part 3: Conclusion of the results section. Connect study results to the study aim and hypothesis.

RQ and RPE were measured to determine the perceived effort and substrate utilization during unfed and fed conditions. Overall, the subject reported higher RPE during unfed exercise (Figure 3). Similar RPE was self-reported during the first 6 minutes of the exercise test under both conditions (Figure 3). There was greater CHO oxidation during the fed condition compared to the unfed condition (Figure 1). During the unfed condition, greater fat oxidation was observed when compared to the fed condition (Figure 2). The subject APHR was 193 bpm, 77%-95% of APHR was calculated to be 148-182 bpm. Average heart rate during the unfed condition was slightly higher than the fed condition (155±4.7bpm, 154±3.5bpm). Average treadmill speed was X in the unfed condition, and X in the fed condition. In summary, a CHO rich meal 4 hours and snack 45 min before exercise decreased the reported RPE during vigorous exercise, increased CHO oxidation and decreased fat oxidation.

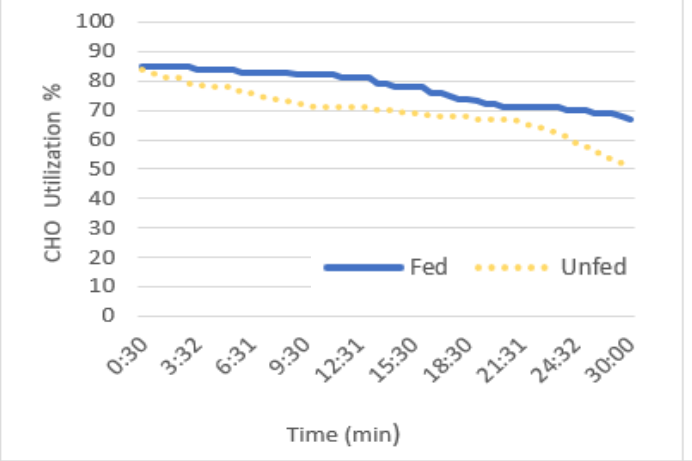


Figure 1. CHO Utilization During Vigorous Exercise
Carbohydrate (CHO) utilization measured by respiratory quotient greater than 1

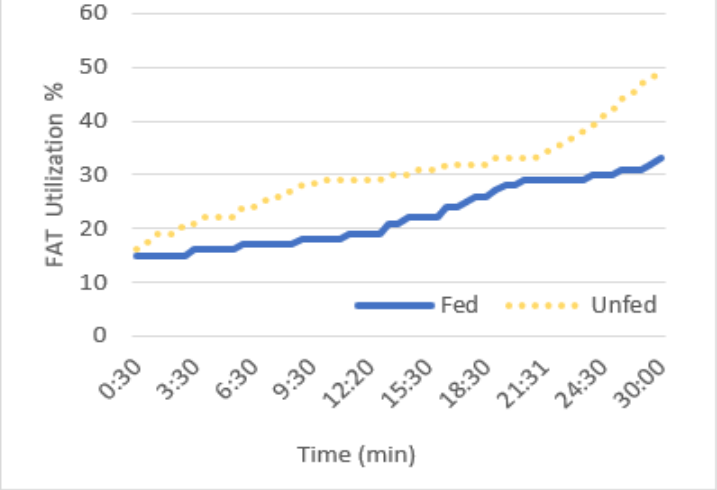


Figure 2. FAT Utilization During Vigorous Exercise
Fat utilization measured by respiratory quotient of 0.7

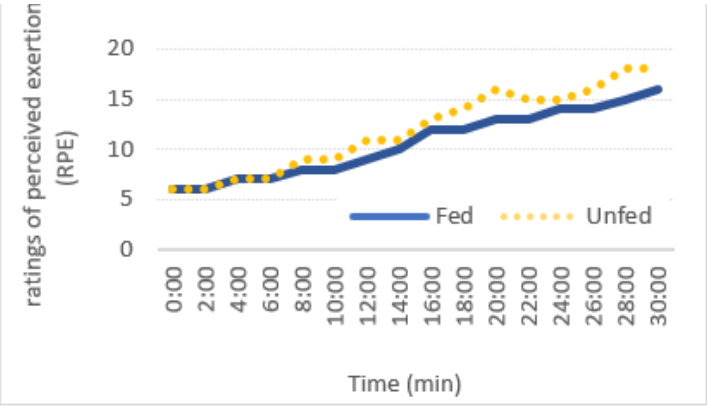


Figure 3. RPE During Vigorous Exercise

Discussion

The purpose of this study was to determine the impact of a CHO rich meal and snack of 140 g and 45 g CHOs on substrate utilization and perceived effort during a 30-minute bout of vigorous exercise. The study subject repeated a vigorous exercise test under both fed and unfed conditions. Lower ratings of perceived exertion and higher carbohydrate utilization were measured in the fed compared to unfed condition. Exercising in an unfed condition utilizes less muscle glycogen and plasma glucose for energy production resulting in reduced glycogenolysis and glycolysis rates. These findings support the hypothesis that pre-exercise

Part 1: Begin with a short, clear, accurate summary of the study (2-4 sentences).

Part 2: Elaborate on Part 3 of the results section by connecting study results to your research question and study aims.

Part 3: Compare your findings with other published data. Interpret findings in the context of 1-2 peer-reviewed research studies. Were your results similar, different, both?

Part 4: Apply study findings to a theoretical or practical context.

Part 5: Describe the relevance of study results. Do not generalize beyond the data and make conclusions that are not based directly on what you measured.

CHO consumption would increase use of CHO as a fuel during vigorous exercise.

The RPE measured between conditions in this study are similar to findings of previous research. In a population of middle-aged runners, Utter et al. demonstrated a lower RPE during an unfed 3 hour run at 70% V02max compared to consumption of a CHO beverage prior to exercise (Utter et al., 2004). The difference in substrate utilization and perception of effort between fed and unfed conditions highlights the importance of carbohydrate consumption for high intensity exercise. As the duration of vigorous exercise increased, RPE was 1-2 points lower after CHO consumption. This difference could help to increase the amount of time that a person could exercise at that intensity. Alternatively, lower RPE at a given work rate may be an indication that an individual could increase exercise intensity after carbohydrate and perceive the exercise to be the same effort as lower intensities in the unfed state, thereby supporting a greater training stimulus. Similarly, in our study, a CHO rich meal 4 hours prior to exercise followed by a CHO snack 45 minutes before exercise increased CHO utilization during exercise.

In summary, we found that consumption of a carbohydrate rich meal and snack before vigorous exercise increased use of carbohydrate as a fuel and decreased perceived effort compared to an identical exercise bout without the meal and snack. Based on these findings, we conclude that endurance athletes may benefit from consumption of a pre-race or exercise event meal rich in CHO to enhance exercise training or performance.

This chapter has online resources on GitHub.

BEST PRACTICES TO PREVENT LABORATORY ACQUIRED INFECTIONS

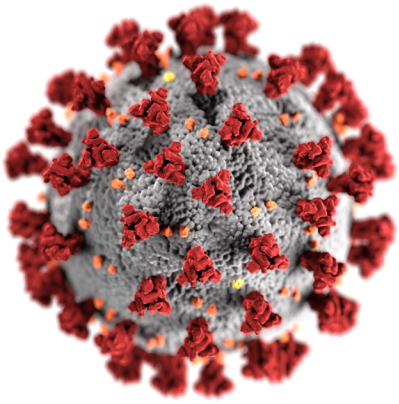


Figure B.1: **Structure of the novel coronavius.** Also known as the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-COV-2). "23312" by CDC/Alissa Eckert, MSMI; Dan Higgins, MAMS

B.1 INFORMATION

Health and human performance laboratory courses involve activities in which students will have direct contact with one another while exercising and collecting biohazardous samples. Best practices should be followed to ensure that laboratory activities are performed in a manner that reduces the risk of transmission of infectious agents including coronavirus. Students and instructors are required to adhere to the following procedures to minimize risk of transmission while in laboratory spaces.

B.2 HAND SANITATION

Wash hands for a minimum of 20 seconds:

- After you have taken off gloves
- After you have used the restroom
- After blowing your nose, coughing, or sneezing

B.3 USE OF PERSONAL PROTECTIVE EQUIPMENT (PPE)

During the course of this laboratory, please refer to and follow your institutional policy on transmissible diseases.

B.3.1 **Cloth Face Coverings and Face Shields**

Wear a face cloth mask or face shield:

- At all times while in your designated laboratory space.
- Students performing metabolic testing with a mouthpiece and a nose clip are exempt from wearing a mask while testing.
- For more information on face coverings, please visit the Centers for Disease Control and Prevention (CDC) website on Cloth Face Coverings.

If you do not have a mask or face shield, then one will be provided to you so that you can participate in the laboratory activities should you choose to do so.

B.3.2 **Disinfectant**

Spray bottles with disinfecting solutions and paper towels will be provided. Wear gloves when handling spray bottles. Spray and wipe laboratory surfaces:

- Before and after each lab for the tables and chairs where you will sit
- After touching equipment and surfaces during the laboratory, e.g. rails, bicycles, treadmill screens, etc.
- After touching anatomical models
- After using exam tables

Disinfection with spray bottle disinfecting solutions is not always the most appropriate method (ie, computer keyboard and other electronics). Alcohol-based wipes will be available for use in these instances.

B.3.3 **Gloves**

Wear gloves in the following circumstances:

- When any direct contact with another person will occur
- When handling anything that may be biohazardous, e.g. contaminated with sweat, blood, or saliva
- When using chemicals or washing/disinfecting laboratory equipment
- When using disinfectants, e.g. spray bottles, sanitary wipes

ADDITIONAL CAUTIONS:

- Do not touch your face unless you have clean hands.
    - Disinfectant is provided for you. Keep shared equipment/items clean to reduce the risk of infection
- Remind others to follow procedures if you see them making a mistake.
- Be very careful with your cell phone. If you touch things in the lab and then touch your phone, then you have potentially infected your phone.

REFERENCES

1. US Department of Health and Human Services. *Science Safety Security: Finding the Balance Together*. https://www.phe.gov/s3/BioriskManagement/biosafety/Pages/Biosafety-Levels.aspx. 2020.

SUGGESTED LABORATORY EQUIPMENT

C.1 LABORATORY 1 - LABORATORY SAFETY AND STANDARD OPERATING PROCEDURES

No equipment needed.

C.2 LABORATORY 2 - EXERCISE INTENSITY AND SUBSTRATE UTILIZATION

Bike: Cycle ergometer (Monkark, Ergomedic 828 E, Sweden)

Borg scale: Scale accessible through the following reference:

- Borg GA. Perceived exertion: a note on "history" and methods. Med Sci Sports 1973; 5:90–3.

Heart Rate Monitor: Polar Electro Oy, Polar H10 Heart Rate Sensor (Polar, Kempele, Finland).

Metabolic Cart: TrueOne 2400 (ParvoMedics, Sandy, UT, USA)

Treadmill: Treadmill (Woodway, Waukesha, USA)

C.3 LABORATORY 3 - CARBOHYDRATE SUPPLEMENTATION AND GLYCEMIC RESPONSES

BIA: Bioelectrical impedance analyzer MC-980u plus (Tanita, Arlington Heights, Illinois, USA)

Glucometer: Hemocue Glucose 201 System (HemoCue, Brea, CA, USA)

- Hemocue 201 Microcuvettes

C.4 LABORATORY 4 - HYDRATION

BIA: Bioelectrical impedance analyzer MC-980u plus (Tanita, Arlington Heights, Illinois, USA)

Osmometer: Model 5010 Osmette III Automatic 10 uL Osmometer (Precision Systems Inc., Natick, MA, USA)

- 10 uL Sample Tubes and Cleanettes
- CON-TROL 290 Reference Solution, Calibration Standards (100, 500, 1500, 2000 mOsm/kg)

C.5 LABORATORY 5 - METABOLIC HEALTH AND ENERGY AVAILABILITY

Anthropometric tape measurer

BIA: bioelectrical impedance analyzer MC-980u plus (Tanita, Arlington Heights, Illinois, USA)

Blood pressure cuff: Omron Digital Blood Pressure Monitor (Omron Healthcare Inc., Bannockburn, IL, USA)

Glycated hemoglobin: Afinion 2 Analyzer (Abbott, San Diego, CA, USA)

- Abbott Hba1c Test Cartridges
- Abbott Hba1c Controls

Lipid Testing: Alere Cholestech LDX Analyzer (Abbott, San Diego, CA, USA)

- Alere Cholestech LDX Test Cassettes
- Alere Level 1 and 2 Controls