Study Title: Comparing nose & mouth breathing during exercise

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PROTOCOL TITLE:
Comparing nose & mouth breathing during exercise

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REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?

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1.0 Study Summary

Study Title	Comparing nose & mouth breathing during exercise
Study Design	Acute intervention (brief periods of breathing through nose or mouth only in the laboratory), observational
Primary Objective	To compare physiological responses in cardiovascular variables between nose and mouth breathing at rest and during exercise
Secondary Objective(s)	n/a
Research Intervention(s)/ Investigational Agent(s)	n/a
IND/IDE #	n/a
Study Population	Adults aged 18-30
Sample Size	30
Study Duration for individual participants	One visit will last up to approximately 2.5 hours
Study Specific Abbreviations/ Definitions	

2.0 Objectives*

2.1 Objective: To compare physiological responses between nose and mouth breathing at rest and during exercise.

2.2 Hypotheses: Breathing through the nose will elicit an improved autonomic cardiovascular profile (lower heart rate, higher heart rate variability, etc.) at rest and during exercise.

3.0 Background*

3.1 Breathing patterns can affect the cardiovascular system. Little is known about how nose versus mouth breathing affects cardiovascular variables (heart rate, heart rate variability, etc.) at rest and during exercise. It has been suggested that breathing through the nose can cause calmness and lower blood pressure. However, research is needed to examine the extent to which breathing through the nose affects cardiovascular variables at rest and during exercise.

4.0 Study Endpoints*

4.1 The primary outcome of this project is the difference in heart rate between nose and mouth breathing during cycling exercise. While this is a pilot trial (i.e., no aprior power analysis was conducted), we expect that having 20 individuals complete testing will inform us as to whether additional research is needed (e.g., a new, larger study or a request to increase the enrollment for the present IRB).

4.2 We expect very low attrition because there is only one visit. However, to be conservative, we will employ an attrition estimate of 33% due to potential screen failures, loss of interest, the decision to discontinue, etc. Thus, we will enroll up to 30 individuals in this project until we have collected data from 20 individuals.

4.3 There are no safety endpoints. Safety will be monitored throughout the study per IRB guidelines.

5.0 Study Intervention/Investigational Agent

5.1 Description: We will determine the extent to which cardiovascular variables (e.g., heart rate) change between nose and mouth breathing conditions at rest and during exercise.

5.2 Drug/Device Handling: N/A

5.3 N/A

6.0 Procedures Involved*

6.1 We will assess outcome measures following the consent and screening.

6.2 <u>A) Informed Consent and Assent</u>

A qualified member of the research team (e.g., Joseph Watso, Ph.D.) will perform the initial participant contact either via the telephone, email, or in person. This contact will include an explanation of study procedures. Interested individuals will be invited to the laboratory where a member of the research team will explain the study (purpose, procedures, risks, benefits, alternatives to participation, etc.) in lay language and will ensure the inclusion/exclusion criteria are satisfied. The potential participant will be provided with adequate time to read the consent, ask additional questions, and consider his/her options. Care will be taken that the participant is not pressured, and that staff is available to address any questions. If the potential participant indicates that they would like to participate, open-ended questions will be asked to assess comprehension. Once the researcher has ensured that the potential participant understands the protocol, s/he will be asked to sign the consent form. Staff phone numbers will be provided to the participants for any questions related to the study protocol.

Persons from vulnerable populations will not be invited to participate. Potential participants will not be coerced to participate, nor will coercive language be used in any recruitment materials. When presenting the study to participants, all risks will be outlined, the benefits will not be overly exaggerated, and it will be explained as completely voluntary.

B) Study Procedures, Materials, and Potential Risks

All devices used for research procedures are used routinely in clinical practice unless noted as ‘research only’ devices/procedures in the following section.

Individuals included as personnel in this IRB study will conduct the research procedures. The training and qualifications of researchers are available and include, but aren’t limited to, education in human physiology, clinical research experience, and CITI human subjects research certifications. All data processing would be completed while researchers are blind to the participant's condition (nose vs mouth breathing).

We will ask participants to avoid caffeine, alcohol, and vigorous exercise for 24 hours and food for 2 hours before each trial. We will assess body composition using waist and hip circumferences. We will measure blood pressure and autonomic cardiovascular function via ECG and beat-to-beat blood pressure. Participants will complete submaximal and maximal exercise testing, during which time we will assess heart rate, blood pressure, blood oxygenation, and perceptual ratings (e.g., exertion and breathlessness).

Data will be digitized upon acquisition and stored in password-protected computer folders. Only approved personnel will have access to participant files that will otherwise be locked. Members and staff of the Institutional Review Board (IRB) at Florida State University may review participant records. A representative of these IRBs may contact the participant for information about their experience with this research project. The participant may refuse to answer any questions that the representative of the IRB may ask. Participants’ information will be kept confidential unless the participant permits the researchers to share that information with others or if the investigators are required by law to release it. Hard-copy documents will be locked and only personnel who are involved in this research project will have access to the files. Information contained in reports or publications issued as a result of this

research project will be presented in such a way that the participant's identity will not be revealed. The signed consent form will be kept on file in the participant's laboratory folder. Disclosure of information to third parties will be prohibited.

PROCEDURES

You will be asked to visit the Cardiovascular and Applied Physiology (CAP) Laboratory for one visit lasting up to approximately 2.5 hours. We ask that you avoid caffeine, alcohol, and strenuous exercise for 24 hours and food for 2 hours before your laboratory visit. Please wear, or bring, exercise clothes (e.g., athletic shorts and a t-shirt).

Order of procedures:

- Consent
- Screening (e.g., blood pressure) & questionnaires
- Instrumentation (e.g., ECG for heart rate)
- Quiet seated rest baseline (~10 minutes)
- Submaximal exercise (~21 minutes)
- Maximal exercise (OPTIONAL; ~10-15 minutes)

B1. Body mass and waist-to-hip circumference. We will ask participants to step on a scale and use a tape measure to determine their waist and hip circumference.

B2. Heart rate and rhythm (electrocardiogram). We will place sticky pad electrodes on the skin of the torso. Participants might feel mild temporary discomfort when the sticky pads are removed.

B3. Arm blood pressure. We will measure brachial blood pressure in the laboratory. Participants might feel mild temporary discomfort when the cuff is inflated.

B4. Finger blood pressure and blood oxygenation. Blood pressure will be monitored using a cuff placed on the finger. Participants might feel mild temporary discomfort when the finger cuff is inflated - if needed, we can pause this device intermittently. We will place a pulse oximeter on a different finger.

B5. Fabric belt to measure respiration. We will place a soft, fabric belt around the ribcage on top of any clothing. This belt will measure ribcage expansion/contraction to record the rate of breathing.

B6. Submaximal cycling exercise. After a ~10-minute resting period, we will ask participants to pedal on a cycle at 75 watts (5.5 METS, or moderate intensity[1]) of resistance for approximately 21 minutes. For context, this exercise causes similar metabolic demand as a walk from the FSU football stadium to the Call Street parking garage (22.5 minutes @ 3.2 mph walking velocity; or 5.3 METS[1]). The 21 minutes of cycling will be broken up into three 7-minute segments: one with 'free' breathing, one with nose-only breathing (we'll remind individuals to only breathe through the nose by keeping their mouth closed), and one with mouth-only breathing (we'll provide participants with a soft nose clip to wear). The order of nose and mouth breathing will be randomly assigned. We will periodically show participants two printed visual scales (e.g., breathlessness from 1 to 10 with 'mild', 'severe' etc. as anchors) and ask them to rate their overall exertion and breathlessness.

**During the rest period and last 14 minutes of submaximal exercise testing, we will provide audio cues via a metronome to help pace breathing based on habitual breathing rate (e.g., if they breathe at 15 breaths/minute at rest, we will set the metronome to this pace for the ~10 minutes of resting data collection).*

[1]https://sites.google.com/site/compendiumofphysicalactivities

B7. Maximal cycling exercise (OPTIONAL). Participants will be asked to perform a maximal exercise test (i.e., strenuous exercise) while we progressively increase the cycling workload until participants can no longer sustain pedaling. This will take approximately 10-15 minutes. We will periodically show participants two printed visual scales (e.g., breathlessness from 1 to 10 with 'mild', 'severe' etc. as anchors) and ask them to rate their overall exertion and breathlessness.

C) Protection Against Risk

As with all studies involving human participants, there are risks associated with experimentation. To reduce these risks, all participants must meet the inclusion/exclusion criteria to participate in this research study to maximize the risk-to-benefit ratio. We will continually monitor the well-being of each participant. The risks and benefits will be explained to the participants before each signs an institutionally approved consent form. If any adverse events occur, participants will be notified and instructed to notify their primary care physician with any pertinent information/documentation.

The following list describes the precautions and safeguards that are in place to minimize risk and treat participants should an adverse event occur:

Exercise Testing: Participants will complete lower-body cycling on a constant-load semi-recumbent ergometer.

Risks and their mitigation: The major experimental procedure risk is the potential for abnormal cardiovascular responses (excessive increases in blood pressure, chest pain, etc) during exercise testing, particularly during maximal exercise. We will continue to follow ACSM guidelines for safely conducting exercise tests in clinical populations. Appropriate precautions are in place to prevent an adverse event and to treat the participant should an adverse event occur. If an adverse event should occur (e.g., chest pain following cessation of exercise), the participant will remain in the laboratory until they have fully recovered. Together, these precautions will minimize any risk to the participants such that the benefits derived from the proposed studies will far outweigh any risks.

**While unlikely, if anyone is unable to keep their breathing rhythm with the metronome or become uncomfortable with nose or mouth only breathing, you can breathe as you'd like at any time (e.g., remove the nose clip and breathe slower or faster). If this happens, the participant can choose to continue with the next procedure (e.g., maximal testing without breathing prompts) or discontinue in the study. Either way, if there are incomplete data from an individual, we will enroll (up to 30 participants) an additional individual to complete this study. Based on the low workload required, we don't expect that this will occur very often, if at all.*

Arterial blood pressure and heart rate: Arterial blood pressure will be monitored via automated oscillometric measurement, manual auscultation, and/or photoplethysmography (Finometer; beat-to-beat). Heart rate will be measured from the ECG.
Risks and their mitigation: There are no known risks involved with this procedure.

If an adverse event does arise, the study personnel will be responsible for immediate medical treatment as needed. Every investigator involved will be knowledgeable of emergency service contact information and the plan of action in place for adverse events. Information regarding unexpected adverse events that occur will be relayed to other participants in the study within 24 hours of the occurrence via telephone, email, or in person if necessary.

All unexpected serious adverse events will be immediately (within 24 hours) addressed and reported to the IRB. In accordance with the FSU Investigator Manual, the PI will complete the Report New Information SmartForm within 5 business days for any of the following information items: information that indicates a new or increased risk, or a new safety issue; harm experienced by a subject or other individual, which in the opinion of the investigator are unexpected and probably related to the research procedures (will be reported within 24 hours if an unexpected serious adverse event); non-compliance with the federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance; audit, inspection, or inquiry by a federal agency and any resulting reports; written reports of study monitors; failure to follow the protocol due to the action or inaction of the investigator or research staff; breach of confidentiality; change to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a subject; incarceration of a subject in a study not approved by the IRB to involve prisoners; complaint of a subject that cannot be resolved by the research team; premature suspension or termination of the protocol by the sponsor, investigator, or institution; or unanticipated adverse device effect (any unanticipated serious adverse device effect will be reported within 24 hours). The PI, co-investigators, and research staff will protect the rights, safety, and welfare of subjects involved in the research.

Overall Risk-Benefits Ratio: In total, the overall risk associated with participating in these studies is relatively low. The risks of this study are mitigated by carefully screening and consenting individuals. The benefits are that the data and mechanistic insight gained will help inform us about the effects of nasal versus mouth breathing on cardiovascular responses during exercise. It is our opinion that the benefits derived from the proposed studies far outweigh the risks.

6.3 N/A

6.4 Data will be collected via the procedures mentioned in section 6.2 above.

6.5 N/A

6.6 N/A

7.0 Data and Specimen Banking*

7.1 Data will be stored in the laboratory and/or a study team member's office via locked filing cabinets (hard copies) and/or password-protected folders (digital).

7.2 All data and medical information obtained will be considered privileged and held in confidence. Study participants will be assigned unique subject identification (ID) numbers that will not contain any personal identifiers such as name, address, date of birth, zip code, etc. This study's subject ID number and the date of collection will be included in all collected data. A master list linking the participants' names and ID numbers will be kept in a separate locked file in the PI's office or kept in a computer file with password-protected access restricted to the PI and study team members. Specimens will likewise be labeled with coded IDs (e.g., STUDY##VISIT#TIMEPOINT#)

7.3 Efforts will be made to limit the use and disclosure of personal information, including research and medical records, to people who need to review this information. Organizations that may inspect and copy study information include the IRB and other representatives of this organization. The IRB will be granted direct access to participants' medical records to conduct and oversee the research. Also, de-identified data collected during this research can be used for future research studies or distributed to another investigator for future research studies.

8.0 Sharing of Results with Subjects*

8.1 When results are published or discussed at conferences, no information will be included that will reveal the identity of the participants. Participants have the opportunity to receive the results from their screening. Participants may request to obtain study results to bring to their physician.

9.0 Study Timelines*

9.1 Participants will be in the study for up to 2.5 hours during one laboratory visit. The duration to enroll all study participants is anticipated to take approximately one year. It is anticipated to take up to another year to complete primary analyses for this study.

10.0 Inclusion and Exclusion Criteria*

10.1 Individuals will be screened for eligibility usually during in-person, phone, or e-mail conversations.

10.2 The criteria below will determine who will be included or excluded in the final study sample.

10.3 Inclusion

- Age: 18-30 years old
- Body mass index: 18 - 30 kg/m^2
- Blood pressure: ≤140/90 mmHg

Exclusion

- Overt cardiovascular (e.g., diagnosed hypertension), respiratory, neurological, renal, liver, and/or metabolic health condition
- Current or recent (regular use within the past 6 months) use of tobacco or nicotine products (e.g., cigarettes)

11.0 Vulnerable Populations*

11.1 N/A

12.0 Local Number of Subjects

12.1 Up to 30 total participants will be accrued locally.

12.2 See section 4.1 above

13.0 Recruitment Methods

13.1 Participants will be recruited on a rolling basis from the greater Tallahassee area. Study recruitment will rely on paper flyers posted in/around campus at non-TMH public settings (e.g., parks, libraries, movie theaters, apartment complexes, malls, grocery stores, gyms, hairdressers, restaurants, community centers, etc.), flyers posted on social media, word of mouth, and flyers sent out as emails in approved registries (e.g., CAP laboratory registry). Individuals interested in the study will be able to contact the research study staff by telephone number and/or email address listed on the advertisement materials.

13.2 Participants will be recruited from the greater Tallahassee, Florida area.

13.3 Pre-Screening Procedures: Individuals that inquire about the study will be provided a brief overview of the study and, if still interested, pre-screened to determine eligibility for screening before scheduling their in-person visit. Eligibility will be based on inclusion/exclusion criteria and volunteer responses to questions about medical history. Pre-screening documentation will include personally identifiable information including name, email, and telephone number, and, therefore, will be kept in a locked cabinet and/or password-protected folder.

13.4 Recruitment/participant-facing document appendices:
Appendix A: Flyer (for emails, social media, online, and print circulation)
Appendix B: Health History Form
Appendix C: IPAQ (physical activity habits form)
Appendix D: RPE scale (exertion)
Appendix E: RPB scale (breathlessness)
Appendix F: Devices used for this research (e.g., brochures, etc.)

13.5 The payment schedule will be as follows:
Completing all required testing: $20
Completing maximal exercise testing: $20 (or $40 in total)

13.6 N/A

14.0 Withdrawal of Subjects*

14.1 Participants can be withdrawn from the study if they are not following instructions, the investigator decides that continuation could be harmful to the participant, the participant needs treatment not allowed in this study, the study is canceled, or for other administrative reasons.

14.2 Participants will be contacted through their provided contact information and informed of their termination from the study. The reason(s) will be thoroughly explained to each participant, and any further questions will be answered.

14.3 Any data already collected from the withdrawn subject will be retained and used in the final analysis.

15.0 Risks to Subjects*

15.1 Risks

Exercise
The major experimental procedure risk is the potential for abnormal cardiovascular responses (excessive increases in blood pressure, chest pain, etc) during exercise testing, particularly during maximal exercise. We will continue to follow ACSM guidelines for safely conducting exercise tests in clinical populations. Appropriate precautions are in place to prevent an adverse event and to treat the participant should an adverse event occur. If an adverse event should occur (e.g., chest pain following cessation of exercise), the participant will remain in the laboratory until they have fully recovered. Together, these precautions will minimize any risk to the participants such that the benefits derived from the proposed studies will far outweigh any risks.

Loss of confidentiality:
There is a slight risk that information collected about participants could be revealed inappropriately or accidentally. Taking part in this research involves providing information that is considered confidential or private. Efforts such as coding research records, keeping research records secure, and allowing only authorized people to have access to research records, will be made to keep volunteer information safe. All data and specimen samples will be labeled with a unique series of numbers. If samples will be transmitted outside Florida State University, all identifying information will be removed from the samples before they are released to any other investigators. Researchers will take appropriate steps to protect any information collected.

Unknown Risks:
In addition to the risks listed above, participants may experience a previously unknown risk or side effect.

All unexpected serious adverse events will be immediately (within 24 hours) addressed and reported to the IRB. In accordance with the FSU Investigator Manual, the PI will complete the Report New Information SmartForm within 5 business days for any of the following information items: information that indicates a new or

increased risk, or a new safety issue; harm experienced by a subject or other individual, which in the opinion of the investigator are unexpected and probably related to the research procedures (will be reported within 24 hours if a unexpected serious adverse event); non-compliance with the federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance; audit, inspection, or inquiry by a federal agency and any resulting reports; written reports of study monitors; failure to follow the protocol due to the action or inaction of the investigator or research staff; breach of confidentiality; change to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a subject; incarceration of a subject in a study not approved by the IRB to involve prisoners; complaint of a subject that cannot be resolved by the research team; premature suspension or termination of the protocol by the sponsor, investigator, or institution; or unanticipated adverse device effect (any unanticipated serious adverse device effect will be reported within 24 hours). The PI, co-investigators, and research staff will protect the rights, safety, and welfare of subjects involved in the research.

15.1 N/A

15.2 N/A

15.3 N/A

15.4 N/A

16.0 Potential Benefits to Subjects*

16.1 There are no direct potential benefits to participants (aside from learning about their health status, upon request). The final results of the study will not be available until all analyses are completed. This may take up to two years.

16.2 N/A

17.0 Data Management* and Confidentiality

17.1 See section 4.1.

17.2 De-identified data collected will be securely stored in a filing cabinet and/or password-protected folder in our laboratory. The data will only be accessible to the PI and study team. The data will be linked to subjects via codes to ensure their protection and confidentiality; the codes will not include HIPPA identifiers, such as initials, etc. The encryption code for participants will be kept in a password-protected folder. Research staff will be trained on the importance of participant confidentiality.

17.3 Efforts will be made to limit the use and disclosure of personal information, including research studies and medical records, to people who need to review this information. If identifiers are removed from the identifiable private information that is collected during this research, that information could be used for future research studies or distributed to other investigators for future research studies without additional informed consent. The published results of this study will not report any identifying

information. A description of this clinical trial will also be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify the participant. At most, the website will include a summary of the results.

17.4 Data will be handled safely and will be coded, as indicated in previous sections of this protocol, by trained study team members. See section 7.0.

18.0 Provisions to Monitor the Data to Ensure the Safety of Subjects*

18.1 During ~weekly laboratory meetings, we will discuss any potential harms/benefits observed with pertinent data. Should any potential harms become apparent, we will follow IRB reporting guidance. Safety information will be recorded based on observations from the data and/or participant reports. The PI (Joseph Watso, Ph.D.), along with pertinent study team members will review safety information. Thus, Dr. Watso will lead the data safety and monitoring activities for this study.

19.0 Provisions to Protect the Privacy Interests of Subjects

19.1 Any personal information collected will be kept private and confidential. Participants will be reassured that all necessary precautions and procedures are being taken to protect their privacy and interactions.

19.2 Participants will be informed of the main objectives of the study and the rationale for each procedure being performed. Participants will also be informed of the strict confidentiality policies regarding data storage and protection. The PI or study team will inform the participants of the rationale for the health history questionnaire and that the questions are in place to ensure their safety.

19.3 All data collected in hardcopy will be stored and locked in a filing cabinet. Only the PI and study team will have a key to access the locked filing cabinet. All the data collected in softcopy will be stored in password-protected folders. The PI and study team will have access to electronic data that does not include any personally identifiable data, such as initials.

20.0 Compensation for Research-Related Injury

20.1 Florida State University has no program to pay for medical care for research-related injuries. In the case of unforeseeable risks occurring, medical care will be made available to the participant. This care is not covered by the university and will be billed to the participant, their insurance, or other third parties.

20.2 N/A

21.0 Economic Burden to Subjects

21.1 Participants are responsible for transportation to and from the FSU campus, if necessary. A parking pass will be provided to participants during their visits if

requested (e.g., a student living on/near campus and/or with a parking pass may not need a pass).

22.0 Consent Process

22.1 For this study, we will follow the SOP: Informed Consent Process for Research (HRP-090). Participants who have been briefed on study procedures will be provided the opportunity to consent to participate. The principal investigator and/or study team member(s) will brief potential participants on the purpose of the study, the procedures involved, risks, and expectations and requirements to participate in the study. Study consent briefings will be scheduled to occur in person and will not occur over the phone. Study briefings will occur in room 1101 William Johnston Building on the Florida State University campus or at a study team member's office. Prospective participants will be familiarized with the study procedures and informed verbally and in writing of their rights to withdraw from any part of the study without penalty or prejudice. The principal investigator or designee will answer all questions. No study procedures will occur before any volunteer has given their informed consent. After the volunteer has had the informed consent explained to them, they will have as much time as desired before signing. A copy of the informed consent document will be offered to the volunteer with the original kept for study documentation. Participants who have consented will be informed of any new information or changes to the protocol. Participants will be allowed to withdraw at any time without penalty or loss of benefits to which they would otherwise be entitled. An investigator may stop an individual’s participation in the study if the participant is unwilling or unable to complete study procedures or follow study instructions. An investigator may also withdraw a participant if the individual becomes ill or injured or if it would not be in the participant’s best interest to continue. If the participant is withdrawn by the investigator or decides to withdraw voluntarily, all further data collection will discontinue, but the data that was already collected will be included in the final analysis.

23.0 The Process of Document Consent in Writing

23.1 For the proposed research study, we will follow SOP: Written Documentation of Consent (HRP-091).

23.2 N/A

23.3 The consent document is attached to the smart form portal (e.g., RAMP).

24.0 Setting

24.1 We will conduct research in the Cardiovascular and Applied Physiology research laboratory. See section 13.1 for recruitment details.

25.0 Resources Available

25.1 Recruitment flyers will be posted in and around the Florida State University campus and throughout Tallahassee, thus, a large number of students, staff, faculty, and the

larger community will be able to see the posted flyers. Visits will be conducted in room 1101 of the William Johnston Building or a study team member's office. We do not anticipate that participants will need medical or psychological resources throughout the study. However, if medical or psychological resources are necessary, participants will be advised to see their primary care physician. All personnel assisting with this study will be thoroughly briefed regarding the study protocol and established safety precautions. The PI and study team will assist in all aspects of the study including scheduling, record keeping, maintenance of confidentiality, recruitment, data collection, data analysis, and writing the final reports.

26.0 Multi-Site Research*

N/A