PROTOCOL
Project Description for IRB
Title: Endocannabinoid and Psychological Responses to Yoga in Healthy Women

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Study Sites:
University of Wisconsin-Madison (lead site)
Medical College of Wisconsin (assays)

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Study Objectives

Yoga is an alternative form of exercise which has gained recent attention in the literature, ranging from children to older adults and healthy individuals to those with chronic illnesses. Physical benefits include improvements in anthropometric measures and blood pressure (Chu et al., 2016). Psychological benefits are demonstrated in a wide range of populations (e.g., Cramer et al., 2013; Hofmann et al., 2016), however these outcomes are less established. Further, there is relatively little known about the acute outcomes of participating in yoga, thus an understanding of the immediate impact of yoga is warranted. Further, it is important to understand the mechanisms behind these effects to develop protocols that are able to maximize the potential of yoga. One recent system proposed to be involved in the benefits of exercise is the activation of the endocannabinoid (eCB) system (Sparling et al., 2003). However, this has not been expressly tested using yoga. Thus, an exploratory pilot study is warranted to examine the acute responses of the eCB system to yoga as well as to understand the acute impact of yoga on mood.

> ***Primary Aim:*** *To determine the magnitude of change of eCBs (AEA, 2-AG) pre- to post-yoga and pre- to post- quiet rest (QR).*
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> ***Secondary Aim:*** *To determine the magnitude of change in mood (POMS- total mood disturbance; STAI- state anxiety; SF-MPQ- overall pain) pre- to post- yoga and pre- to post- QR.*

Study Significance

In the last 15 years, research determining the impact of yoga on physical and mental health has grown exponentially (Cramer et al., 2014). However, much of this research examines the benefits of long-term training, thus the acute effects of yoga are relatively unknown. Physical benefits of yoga training interventions include decreased body mass index (BMI), improved blood pressure, cholesterol, body weight, and triglycerides, as well as decreased heart rate (Chu et al., 2016). In addition, improvements in mental health such as anxiety in a healthy population (Hofmann et al., 2016), depression in individuals diagnosed with depression (Cramer et al., 2013), fatigue in women with breast cancer (Sadja et al., 2013), and pain in adults with low back pain (Goode et al., 2016) have been demonstrated. However, many of these studies report a need for further evidence before strong conclusions can be drawn.

Surprisingly few studies have examined the acute effects of yoga, thus the immediate experience of individuals practicing yoga are not well understood. Two recent studies demonstrated the acute benefits of yoga including decreased negative affect, increased positive affect (Gaskins et al., 2014; Szabo et al., 2017), and decreased state anxiety (Szabo et al., 2017). However, Gaskings and colleagues (2014) examined both acute and chronic effects of yoga and found no changes in perceived stress for either. Although these studies provide some insight, future research is required to replicate and expand this knowledge base.

There is even less known about the mechanisms behind the effects of participating in yoga. However, since yoga can be considered a type of exercise, we can draw from the mechanistic work in the exercise realm. One of the most recently proposed mechanisms is that of the endocannabinoid (eCB) system due to its role in several psychological constructs, such as depression and anxiety (Hill et al., 2009; Hill et al., 2008; Hillard et al., 2014; Mechoulam et al.,

2013; Riebe et al., 2011). The eCB system is a neuromodulatory system involved in numerous processes including inflammation, neurogenerative diseases, cognition, reward, memory, the stress response, and psychological disorders (Hillard, 2015; Mechoulam et al., 2013; Reibe et al., 2011). In brief, the eCB system consists of the cannabinoid receptors CB1 and CB2 as well, their effectors, N-arachidonylthanolamine (AEA, "anandamide") and 2-arachidonolyglycerol (2-AG), and the enzymes that degrade them (fatty acid amide hydrolase, FAAH; monoacylglycerol lipase, MAGL).

There is evidence that exercise impacts endogenous eCB signaling throughout the body (Tantimonaco et al., 2014). Sparling and associates (2003) were the first researchers to report increases in eCBs following 50 minutes of moderate cycling or running. Since then, strenuous hiking (Feurecker et al., 2012), intense cycling (Heyman et al., 2012), and treadmill running (Raichlen et al., 2012; Raichlen et al., 2013) have also been shown to influence the endocannabinoids. Further, recent work in our lab has demonstrated increases in eCBs following isometric exercise (i.e., static muscle contractions), and this was in conjunction with reductions in pain (Koltyn et al., 2014). However, no study, to our knowledge, has examined the eCB system in response to yoga.

Since it is currently unknown whether yoga influences the eCB system, an exploratory pilot study will be conducted to examine the eCB and psychological responses to yoga. Thus, we may determine the effect sizes for the proposed outcomes in order to sufficiently power subsequent studies.

Research Design and Procedures

The proposed study is an exploratory pilot study where participants will complete control and experimental conditions.

Subject Population

Twelve women from the University of Wisconsin-Madison will be recruited to participate in this study. Participants' ages will range from 18-45 years and will report being healthy.

Sample Size Justification

Based on our previous research showing a large effect of exercise on eCBs, a statistical power analysis was performed for sample size estimation based on a large effect size. Using Cohen's f effect size (ES) of .4, alpha = .05 and power = 0.80, the total sample size needed for this study is N = 10 (GPower 3.1) for a within subject repeated measures design. To account for participant attrition, up to 12 participants will be recruited.

Inclusion Criteria

Inclusion criteria will be as follows:

- $\geq$ 18 years old and $\leq$ 45 years old and
- report being healthy.

Exclusion Criteria
Exclusion criteria will include:

- Being pregnant or planning to become pregnant,
- currently smoking,
- having a history of light headedness or fainting during blood draws or physical activity,
- having a history of chest pain during physical activity,
- having a bone, joint, cardiac, or other medical condition that a doctor has said may be worsened by physical activity,
- taking medications for any chronic diseases such as high blood pressure or diabetes,
- responding 'Yes' to any of the seven questions on the Par-Q.

Identification and Recruitment of Subjects
Volunteers will be recruited from students, faculty, and staff at the University of Wisconsin-Madison to participate in this study. They will be between the ages of 18 and 45 and report being healthy. Participants will be recruited through flyers and direct recruitment. Flyers will be posted in approved locations across the UW campus. In addition, a study team member will directly recruit participants from lecture halls by making an announcement and handing out flyers. The instructor's consent will be sought before the team member attends the lecture. Volunteers will complete a phone screen prior to Study Visit #1 to determine eligibility. These recruitment methods were effective in previous studies conducted in the Exercise Psychology Laboratory.

Instruments
Demographic Form. The demographic form will consist of multiple choice and short answer questions (e.g., age, sex, ethnicity, etc.) and will be used to describe the sample.

Heart Rate. Heart rate will be collected to quantify the intensity of the study tasks using a heart rate monitor.

Profile of Mood States. The POMS (McNair, Lorr, & Droppleman, 1971) is a 65-item questionnaire using a 5-point Likert-type scale ranging from 0 "not at all" to 4 "extremely." It will be used to determine the acute mood state of participants which include: tension, depression, confusion, anger, fatigue, vigor, and total mood disturbance. Internal consistencies for the subscales on the POMS range from .84 to .95 (McNair et al., 1971).

Ratings of Perceived Exertion (RPE). A validated RPE scale (Borg et al., 1998) will be used to measure the perceived intensity of the exercise. The scale ranges from 6 "no exertion at all" to 20 "maximal exertion."

Short-Form McGill Questionnaire. The SF-MPQ (Melzack, 1987) is a 15-item survey with items rated on a 4-point scale ranging from 0 "none" to 3 "severe." Eleven items assess the intensity of sensory components of pain and four items assess the intensity of affective components of pain. In addition, a Present Pain Index (PPI) and a Visual Analogue Scale (VAS) are included in the questionnaire.

State-Trait Anxiety Inventory. The STAI Form Y-1 (Spielberger et al., 1983) is a 20-item questionnaire designed to measure state anxiety. Items ranged from 1 "almost never" to 4 "almost always" with higher scores indicating higher anxiety. The internal consistency for the STAI ranges from .86 to .95 (Spielberger et al., 1983).

Procedures

Prior to attending the study visits, volunteer eligibility will be determined through a preliminary phone screen. If the volunteer is eligible, they will be scheduled for Study Visit #1 and will be asked to refrain from exercise for 24 hours and to refrain from drinking or eating (except for water) for 2 hours prior to their study visit. Participants will attend a total of two study visits, each 60-75 minutes long, to complete both the control and experimental sessions. There will be between 2 and 7 days between Study Visit #1 and Study Visit #2. All visits will be completed independently and participants will spend a total of 120-150 minutes completing this study. Participants will be randomly assigned to the sessions.

> ***Experimental Task:*** The experimental task will consist of 40 minutes of yoga in a sound-dampened chamber. Participants will follow an online yoga video through YouTube and will be instructed to participate to the best of her ability. Heart rate (HR) will be collected every five minutes and the session intensity will be recorded through an over-all rating of perceived exertion at the end of the session (RPE; Borg et al., 1998). A study team member will monitor the participant via a window into the sound-damped chamber.
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> ***Control Task:*** The control task will consist of 40 minutes of quiet rest in a sound-dampened chamber. Participants will not be allowed to use electronics during this session, however reading material will be made available. HR will be collected every five minutes and RPE will be recorded at the end of the session. A study team member will monitor the participant via a window into the sound-damped chamber.

Visit 1

Participants will be randomly assigned to completing the experimental or control task prior to coming in for Study Visit #1. All study visits will be completed in the Exercise Psychology Laboratory in the University of Wisconsin-Madison Natatorium building. Upon arrival, participants will enter a sound-damped chamber to ensure privacy. They will complete an informed consent form and a packet of questionnaires consisting of: the demographic questionnaire, Profile of Mood States (POMS; McNair et al., 1992), State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983), and the Short-Form McGill Pain Questionnaire (SF-MPQ; Melzack, 1987). Upon completion of questionnaires, participants will have their age, sex, weight, and height measurements assessed. Participants will then be informed of their task and will be outfitted with a heart rate monitor and a study team member will explain the RPE scale. Next, participants will have their blood drawn (5ml) immediately before completing the visit 1 task (experimental or control). Immediately upon task completion, the participants will be asked for their RPE, have their blood drawn (5 ml) and will complete the same questionnaires listed previously (POMS, STAI, SF-MPQ, and VAS-F). Blood samples will be prepared for analysis within 10 minutes of collection (see 'Endocannabinoid Assays' section below).

Visit 2
Participants will complete the task that was not assigned to them during study visit #1 (control or experimental task). Although they will not complete the informed consent or demographic questionnaire, all procedures will be the same. Participants will complete the following questionnaires pre- and post-task completion: POMS, STAI, and SF-MPQ and will be asked their RPE. Further, two separate blood samples (5ml) will be taken immediately pre- and post-task completion and will be processed within 10 minutes of collection. Participants will receive $30 gift card upon completion of both study visits.

Endocannabinoid Assays
Blood samples will be collected using EDTA containing tubes (BD Vacutainer, K3E EDTA K3) and centrifuged within 10 minutes of collection. Separated plasma will be frozen at -80 C until analysis. Analysis will be conducted at the Medical College of Wisconsin under the supervision of Dr. Cecilia Hillard. The analysis will quantify the plasma concentrations of eCBs (AEA and 2-AG) and their related biolipids (PEA, OEA, 2-OG) using an isotope-dilution, electrospray ionization liquid chromatography/mass spectrometry of the daughter ions (LC-ESI-MS-MS). This protocol has been previously described (Koltyn et al., 2014).

Data Analysis
The design of this study is a within subject, repeated measure design where participants complete pre-post measures during two study visits. Since this is an exploratory pilot study, our aims are to determine the magnitude of change of our outcomes, using Cohen's *d*. Specifically, effect sizes for the change in eCBs (AEA and 2-AG) pre- to post- condition will be determined for yoga and QR. In order to meet the secondary aim, Cohen's *d* will be conducted for each of the psychological outcomes (POMS-total mood disturbance; STAI-state anxiety; SFMPQ- pain) to determine the pre- to post-changes for each task (yoga, QR).

Data Collection

Data will be collected through self-report and biological samples. The only identifiable information will be collected through the informed consent document. The informed consent documents (hard copy), which include the participant's assigned identifier, will be stored in a locked cabinet in the PI's office located in the Kinesiology Department at the University of Wisconsin-Madison Natatorium building. These will be separate from the other study documents and only the PI will have access. All data will be coded using a personal identifier assigned to the participant after informed consent is signed, thus all collected data will be coded. All information collected on paper (i.e., age, HR, height, RPE, weight), besides the informed consent, will be stored in a locked cabinet in the Exercise Psychology Lab, which is part of the Kinesiology Department. Self-report data (questionnaires) will be collected electronically through Qualtrix and only the research team will have access to both electronic and hard-copy data. Raw data will be input into a computer for data analysis where it will be stored in a secured file on the Exercise Psychology Lab server which is located in a password protected area with firewall protection and backed up daily. All team members will have access to the coded information if located in the Exercise Psychology Laboratory. Biological samples (blood samples) will be stored in a freezer in the Biodynamics Laboratory located in the Natatorium at the University of Wisconsin-Madison after which they will be transferred to a container of dry ice and driven by the PI to Dr. Hillard's laboratory at the Medical College of Wisconsin. The

blood samples then will be stored in Dr. Hillard's lab until eCB assays are performed. They will be exhausted during the eCB extraction processes. The blood samples then will be stored in Dr. Hillard's lab after which they will be exhausted. The blood samples will contain only the participant ID number, time of collection, and session date; thus, participants will not be able to be identified. Moreover, the screening data for eligible participants will be kept and secured in a locked file cabinet in the Exercise Psychology Lab until the participant has either completed the study, removed themselves from participation, or is excluded from participation. All screening data for eligible participants will be shredded upon participant completion, dropout, or exclusion of the study. Phone screening data from any interested potential participants not meeting inclusion criteria will be shredded immediately. Biological samples will be exhausted upon completion of endocannabinoid assay. Raw data will be shredded after 7 years and coded data will be kept indefinitely.

Sample/Specimen Collection

Blood samples will be collected by a trained phlebotomist using EDTA containing tubes (BD Vacutainer, K3E EDTA K3) and centrifuged. Plasma will be frozen at -80 C in a freezer in a designated area on the UW-Madison campus until all samples are collected. All samples will be shipped to Dr. Hillard's lab at the Medical College of Wisconsin and will be stored in a freezer until analysis. No samples will include identifiable information and the results from the eCB assays will be shared between study team members using the participant ID numbers

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