Mechanisms of Hyperthermic Yoga for the Treatment of Depression

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Study Intervention Provided by: Bikram Yoga Boston and Bikram Yoga Cambridge

Tool Revision History
Version Number 1.9
Version Date: August 24th, 2020
Summary of Revisions to be Made:
We are proposing the following changes: 1) We are adding updated information regarding a Data Use Agreement (DUA) that we intend to submit.

Version Number 1.8
Version Date: July 13th, 2020
Summary of Revisions to be Made:
We are proposing the following changes: 1) We are adding information specifying the locations of where the analyses of biological samples (blood samples) collected will take place.

Version Number 1.7
Version Date: November 13th, 2019
Summary of Revisions to be Made:
We are proposing the following changes: 1) We are adding the ability to recontact patients for the sake of accurate reporting of yoga class attendance after they had finished participation in the study. Patient's will be contacted up to three times before they are considered "Lost to Re-Contact"

Version Number 1.6
Version Date: April 22, 2019
Summary of Revisions Made:
We are proposing the following changes: 1) We are increasing the language concerning enrollment from 80 to 160, as 80 is our target of patients to be randomized, while 160 is the number we plan to enroll to achieve this goal of randomization.

Version Number: 1.5
Version Date: January 11, 2019
Summary of Revisions Made:
We are proposing the following changes: 1) We are allowing clinician rated measures to be completed by phone for all visits when needed/indicated to reduce subject burden and protocol deviations. Subsequently, we have added a protocol for patients expressing active suicidal ideation over the phone. 2) We are updating the protocol to allow for the initial screening physical exam and EKG to be conducted at the baseline visit to accommodate clinician availability. 3) When blood cannot be obtained during the study visit by a member of the research staff, we are allowing the blood to be drawn at the phlebotomy lab at the MGH main hospital to accommodate the subject's schedule. 4) The psychoeducational session that takes place during the initial baseline (visit 2) and the repeat baseline (DYG, Visit 7) can now take place over the phone with Dr. Nyer to accommodate patient's schedules. 5) We are allowing participants to bring back their sleep diaries and saliva samples at the following visit if they forget them, as long as they were collected during the appropriate time. 6) We are adding the option to be re-contacted for future research studies to the informed consent. 7) The yoga studios have changed names and owners.

Version Number: 1.4
Version Date: July 10, 2017
Summary of Revisions Made:
We are proposing the following changes: 1) We are increasing the amount of blood collected throughout the study from a total of 13ml to 30ml per visit, resulting in a total amount of blood collected of 131mL for the immediate yoga group, and 221mL for the delayed yoga group. 2) We have added the University of Rhode Island Change Assessment Scale (URICA), which assess for readiness to change, to the baseline and end-of-study visits. 3) We are modifying our inclusion/exclusion criteria to specify that the consumption of coffee or other caffeinated beverages is allowable, and to alter the length required for a participant to be on a stable psychiatric medication prior to enrolling to the study from <3 months to <2 months, with a stable dose for <4 weeks. 4) We would like to ask participants to complete two paper-copy surveys (the EIFI and the STAI-State) 30 minutes pre and post each yoga class they attend. The questionnaires will be handed to participants after they are randomized at the end of their Baseline visit. 5) We included a column on the C-SSRS conducted at screen to assess for symptoms in the “past year”, in addition to “lifetime”. 6) We created an “informational form” to hand out to participants at baseline, which contains information about the studio locations and class scheduled, as well as reminders of how many classes to attend, which classes to attend, and that participants can attend classes at both studios for free for the duration of the study. 7) We updated the study protocol to specify how our randomization procedures are conducted (i.e., that participants are randomized at the end of their baseline visits by a research coordinator, and only informed of their group assignment once they have completed all study-measures). 8) We have changed the frequency of Dr. Nyer’s check-in calls with participants to once every two weeks, on weeks when participants do not come in to the program for an in-person assessment visit.

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1. STUDY OBJECTIVES

1.1 Primary Objective

Primary Aim I: To recruit and retain adults with depressive symptoms into a controlled trial of hyperthermic yoga, randomizing 40 participants to each the immediate yoga group (IYG) and delayed yoga group (DYG; control group) with seventy-five percent retention in both groups.

Hypothesis Ia: Seventy-five percent of the each group will complete a week-8 assessment.

Hypothesis Ib: Acceptability of hyperthermic yoga will be high on the Acceptability Interview.

Primary Aim II: To collect the Inventory of Depressive Symptomatology – Clinician Rated (IDS-CR) scores at screening; baseline; and weeks-1, -3, -5 and -8 to evaluate the efficacy of hyperthermic yoga for depressive symptoms.

Hypothesis II: The IYG will have greater decreases in Inventory of Depressive Symptomatology – Clinician Rated (IDS-CR) scores after 8-weeks compared to the DYG.

Primary Aim III: To collect (a) physiological and (b) psychological mediators of hyperthermic yoga in the IYG and DYG group at screening; baseline; weeks-1, -3, -5 and -8 to evaluate treatment response in the IYG compared to the DYG.

Hypothesis IIIa: Salivary cortisol, and inflammatory markers (i.e., Tumor Necrosis Factor [TNF-α], Interleukin-6 [IL-6], and C-Reactive Protein [CRP]) will significantly decrease following 5- and 8-weeks of hyperthermic yoga in the IYG compared to the DYG.

Hypothesis IIIb: Perceived stress, mindfulness, and rumination will improve by 5- and 8-weeks in the IYG compared to the DYG.

1.2 Secondary Objectives

Secondary Aim I: To collect measures of *quality of life, general health, physical functioning, decreased perceived stress, anxiety, and sleep* in the IYG and DYG at screening; baseline; and weeks-1, -3, -5 and -8 to evaluate hyperthermic yoga for secondary outcome measures.

Secondary Hypothesis I: The IYG will have significant improvements in quality of life, general health, physical functioning, perceived stress, anxiety, and sleep compared to the DYG.

Secondary Aim II (exploratory): To evaluate the long-term effects of the yoga intervention (1-month post intervention), while controlling for continued yoga participation.

Secondary Hypothesis II: Subjects who choose to continue the yoga practice, either in the IYG or DYG, will have increased effects at 1-month post intervention, per the primary and secondary outcome measures compared to the week-8 assessment.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Depression is associated with significant mortality, morbidity, and disability, and is the fourth leading cause of global disease burden[1]. By 2020, Major Depressive Disorder (MDD) is projected to be the second leading cause of global disability in developed nations[2]. Even though more people are receiving treatments for depression, there is no evidence that the associated morbidity and mortality have significantly decreased. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study suggested relatively "weak effects" of antidepressants[3]. In addition, antidepressants also have common side effects, such as weight gain, sexual dysfunction, fatigue, and others[4]. Electroconvulsive therapy, and more recently ketamine infusion, provide more immediate relief to those with severe depression[3], though these benefits are often short-lived. Cognitive Behavioral Therapy (CBT), although effective, demonstrates remission rates no better than antidepressants[5,6]. The modest effects of current treatments may be due to their inability to fully address the physiological dysregulation of the stress response system[7].

2.2 Study Rationale

Proposed Intervention: Bikram Yoga (BY) is a standardized type of hyperthermic yoga, which synergistically combines Hatha Yoga postures, intensive exercise, mindfulness, and thermal therapy. It consists of 26 sequenced postures and two breathing exercises, practiced for 90 minutes in a room heated to 105-110˚F with 40–60% relative humidity. Table 1 summarizes the hypothesized active elements of BY.

Table 1. Mechanisms of Bikram Yoga.

Mechanism and Evidence Base	Inclusion in Bikram Yoga Protocol
Yoga: Yoga demonstrates antidepressant effects[8-12].	Includes postures indicated for depression, i.e., backbends and inversions[8].
Intensive Exercise: Exercise demonstrates antidepressant effects[13], and more intensive exercise produces higher remission rates as an augmentation for resistant MDD[14].	BY presents a more intensive form of exercise than most yoga modalities, and produces high metabolic energy output[15].
Mindfulness: Mindfulness-based interventions reduce depressive symptoms and prevent relapse[16].	The practice discharges agitation and facilitates mindfulness. Mastery of uncomfortable sensations may generalize to maintaining a calm mind during times of high stress.
Thermal Therapy: Heat[17-19], thermal therapy[20-23] and fever[24] demonstrate antidepressant potential.	The heat (105-110˚F) may enhance the benefits of the standard Hatha Yoga postures and allow for greater stretching of muscles[25], in addition to providing anxiolytic effects[26].

Characteristics of Bikram Yoga for Clinical Trials: Table 2 outlines the features that differentiate Bikram Yoga from other forms of yoga, and highlights the suitability of its use in clinical trials for depression.

Table 2. Features of Bikram Yoga.

Bikram Yoga Features	Clinical Trial Applicability
Classes are standardized in time, dialogue, posture sequence/duration, and room conditions. The unified consistent protocol allows for a rigorous clinical trial design because all patients assigned to yoga get the same intervention.	The lack of standardized yoga protocols[27] hinders methodological rigor in the yoga for depression literature base[9].
The standardized Bikram Yoga dialogue was designed to allow inexperienced students to safely complete the yoga postures[25].	The practice was developed so that participants of all levels would be able to take the classes[25].
Bikram Yoga focuses almost exclusively on the physicality of the practice, rather than the spiritual components of yoga.	Yoga modalities range in degree of physical and/or spiritual emphasis[27]. The physical focus may have broader, secular-based appeal to US public.
Widely Available: 285 Bikram Yoga studios in the US and 217 Bikram Yoga studios abroad, covering 42 countries and 464 cities; 18 Bikram Yoga studios in Massachusetts.	The intervention has broad potential for quick dissemination.

Yoga as a Treatment for Depression: Evidence is accumulating to support yoga as a treatment for depression. A 2005 literature review found 5 randomized controlled trials (RCTs)[11], whereas a 2013 meta-analysis found 12 RCTs[12]. Reviews suggest that yoga is a promising treatment for depression, but consistently note that a lack of rigorous study methodology prevents the firm conclusion that yoga be considered a treatment for depression[9, 12, 28]. Methodological limitations include: heterogeneity of yoga interventions and patient samples; inadequate sample size and randomization procedures; lack of intention-to-treat analysis and blinding of outcome assessors; inadequate control groups; and unreported dropout, relapse, and remission rates[9, 12]. Despite these flaws, the 2013 meta-analysis[12] did find moderate evidence for the short-term effects of yoga compared to usual care. A 2013 systematic review of yoga for depression[10] affirmed “Grade B” evidence for yoga as an acute treatment for depression. One controlled study used an Iyengar yoga treatment, a form of hatha yoga, that included back bends, inversions and vigorous standing postures thought to be particularly effective for alleviating depression[8]. These postures are also used in Bikram Yoga, which is also a form of hatha yoga. The Beck Depression Inventory (BDI) and Spielberger State-Trait Anxiety Index (STAI) measured mood and anxiety. Subjects had baseline BDI scores of 10 to 15 (mild-to-moderate depressive symptoms), similar to the proposed study. The 5-week yoga intervention decreased depression and anxiety, using similar measures to the proposed study. The evidence has been promising enough to warrant two current rigorous NIH R01 funded RCTs for non-heated yoga (NIH RePORTER: 1) PI: Streeter [NCCAM], and 2) PI: Uebelacker [NINR]). The proposed study has been constructed to address the concerns and apply rigorous methodology to an unexplored form of yoga.

Bikram Yoga Research: While there is no literature on Bikram Yoga for depression, several studies of Bikram Yoga demonstrate improvements in physiological and psychological processes implicated in depression. **Physiologically**: In a recent 8-week pilot trial, 3 weekly

Bikram Yoga classes improved glucose tolerance in 15 older obese adults compared to 14 young lean subjects. This supports Bikram Yoga as a therapeutic tool for metabolic dysfunction[29], and depression is associated with metabolic disorders[30]. Another study found that 8 weeks of 3 weekly Bikram Yoga classes improved arterial stiffness in 24 younger adults (30±1 years), and significantly reduced insulin resistance in 18 older adults (53±2 years)[31]. The relationship between depressive and anxiety disorders and early markers of arterial stiffness[32], indicates a common pathophysiology with depression. **Psychologically**: In an uncontrolled study of 54 healthy individuals, Bikram Yoga improved mindfulness and decreased stress[33], suggesting benefits in depression via effects on mindfulness and perceived stress. In an unpublished dissertation, DeBoer et al.[34] reported preliminary completer data of twice weekly Bikram Yoga classes (n=27) versus a waitlist control (n=25) for women (ages 25-45) with stress-induced eating and high perceived stress. This study demonstrated a dropout rate of 11.5%, lower than in most depression trials. The 4 subjects who discontinued yoga did so in the initial stages of the practice. This study provided preliminary evidence of the acceptability of recruiting and retaining a psychiatric population for this intervention. The effect size for the change in the Beck Depression Inventory (BDI-II)[35], based on Cohen's *d,* was medium (0.54), suggesting an advantage for Bikram Yoga over the waitlist. As this study did not require depressive symptoms for entry, the effect size for depression in the proposed study will likely be higher. The Beck Anxiety Inventory (BAI)[36] demonstrated a large effect for Bikram Yoga over control (*d*=1.19). There were also large effects for Bikram Yoga's ability to reduce scores on the Ruminative Responses Scale (RRS)[37] (.76), the Perceived Stress Scale (PSS)[38] (.83), and the Response to Positive Affect Scale[39] (.76). I will include the PSS and RRS in our study as secondary outcome measures, due to these results.

Thermal Therapy for Depression: Whole-body hyperthermia is receiving attention as an antidepressant. A large effect size (*d*=1.13) was found 5-days post a single total-body hyperthermia session (mean time 126.7 minutes) in 16 medically healthy adults with MDD on the Centers for Epidemiological Studies Depression Scale (CES-D). Only 3 of the 16 subjects were on antidepressants (SSRIs). When the 13 SSRI-free subjects were analyzed separately, the effect sizes increased (*d*=1.4). These findings are consistent with studies of mood induced hyperthermia in medically ill populations[23, 40]. In another study, fever induction in medication-free patients with melancholic MDD produced improvements in mood for all patients the day after induction, perhaps via inflammatory cytokines suppressing REM sleep, thus improving mood[24]. In another study, 28 medication-free patients with mild depression and somatic complaints randomized to 4 weeks of 5x/week (20 sessions) thermal therapy vs. non-thermal therapy (60°C for 15 minutes followed by 30 minutes of bed rest with blanket) demonstrated improvement in somatic and mental complaints, hunger, and relaxation[21]. Other preliminary data also support heat's beneficial effect on mood[17-19, 22]. Recent data suggest that Bikram Yoga similarly raises core body temperature[15]. There is evidence for a relationship between thermal regulation and serotonergic neural circuits controlling cognitive function and mood[41]. Depressed patients have disruption in thermoregulatory cooling, implicated in the maintenance of the disease[42]. Bikram Yoga offers both a mechanism for understanding this interaction and a means of treatment.

Yoga's Effects on the Stress Response Systems: Depression is linked to the body's physiological reaction to stress[43]. Yoga may restore homeostasis by increasing

parasympathetic nervous system (PNS) activity, restoring gamma-amino-butyric acid (GABA) activity, and reducing physiological effects of stress[7]. This may occur through a variety of mechanisms, including stimulation of the vagus nerve[7], related to vagus nerve stimulation for depression. **Hypothalamic-Pituitary-Adrenal Axis (HPA):** depression is commonly associated with increased cortisol levels[43]. Across populations yoga consistently reduces cortisol levels[47-55]. **Immune System Markers/Inflammatory Markers:** Inflammatory processes have been consistently implicated in the pathophysiology of depression[56]. In a meta-analysis of dozens of studies, medically-healthy MDD patients exhibit elevated serum interleukin (IL)-6, IL-1 (esp. IL-1-beta), and acute phase C reactive protein (CRP)[57]. Other studies report increased serum tumor necrosis factor alpha (TNF-α) in MDD patients[56, 58]. Expert hatha yoga practitioners, compared to novices, had lower levels of inflammatory markers at baseline, during, and after a protocol of stress induction and subsequent participation in hatha yoga[59]. In a non-randomized sample of patients with chronic diseases, inflammatory markers decreased after just ten days of yoga practice[60]. These data support our rationale for measuring HPA (cortisol), and immune system function (inflammatory markers) in our sample before, during, and after treatment.

Yoga and Functional Measures: Yoga may improve quality of life and functional measures associated with MDD. **Quality of Life**: Yoga has demonstrated improvements in quality of life in medically ill populations [61-66]. Since depression has a strong relationship with decreased quality of life[67], yoga may be of significant benefit. **Physical Functioning**: Depressed people have impairments in physical functioning similar to common chronic physical conditions[68-70]. Additionally, those with MDD and chronic illnesses have greater risk of subsequent illness, disability, and premature mortality[71]. In a meta-analysis of older adults, yoga may hold greater benefits than conventional exercise for health status, aerobic fitness, and strength[72]. In a recent literature review, yoga helped improve multiple markers of physical health[73].

Yoga and Secondary Outcomes: **Anxiety**: Symptoms of anxiety are often 40-50% comorbid with MDD[74-76]. Yoga may treat anxiety, though similar to the depression and yoga literature base, studies are methodologically flawed[77]. **Sleep**: Sleep disturbance and depression have been linked to fragmentation of sleep architecture and likely share common biological pathways[78]. Experienced Bikram Yoga practitioners using transition analysis had a faster return to sleep after nocturnal awakenings on nights when yoga was practiced that day compared to non-yoga days[79]. Bikram Yoga may directly and/or indirectly improve mood by simultaneously improving sleep. Improved sleep regulation, via improved sleep efficiency and quality, may be a mechanism for yoga's antidepressant effects[9].

3. STUDY DESIGN

The project is an acceptability, feasibility, and pilot efficacy trial of hyperthermic yoga vs. a waitlist control for 80 adults with at least mild depressive symptoms. The target enrollment for the study will be 160 to properly screen the number of needed randomized participants. The hyperthermic yoga intervention will take place in two community Bikram Yoga studios in the Boston area. Bikram Yoga Boston is owned by Jill Koontz and Bikram Yoga Cambridge is owned by Lucas Lambert. Participants will attend classes at either of these studios, free of charge – i.e., the intervention will be community-delivered. Participants will be asked to attend

at least two weekly 90-minute Bikram Yoga classes during the 8-week intervention phase of the study. All research related assessments will take place at the Depression Clinical and Research Program (DCRP) at the Massachusetts General Hospital (MGH). The enrollment period for participants will depend upon the arm to which they are randomized: 1) immediate yoga group (IYG) will complete the protocol in approximately 15weeks; and 2) the control or delayed yoga group (DYG) will complete the protocol in approximately 24weeks.

Retention Strategies: Patients with depressive symptoms frequently have impaired motivation. In the only known RCT of hyperthermic yoga for a psychiatric population, subjects who dropped out tended to do so after 1-2 sessions. Most who got through this critical period completed the study[34]. To address this: 1) study staff, the PI or designee, will administer a 50-minute pre-yoga baseline psychoeducation session with a preparatory handout, which they take home with them. 2) Participants will receive a bi-weekly telephone call from the PI, and when not available, another study staff member.

Subject Remuneration: Subjects in the DYG will be compensated for their time during the waitlist period ($25 for baseline; week 1, 3, 5 and 8; total=$125) to encourage participation. Subjects in IYG will only be paid for the 1-month follow up visit ($25), as they will receive free yoga classes at the outset of the study. All subjects will receive $30 per cortisol assessment.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

Inclusion Criteria

1. Adults ages 18-60
2. English language proficiency
3. Ability to provide informed consent
4. Score of >23 on the Inventory of Depressive Symptomatology – Clinician Rated (IDS-CR)[80]
5. Able and willing to attend two 90-minute hyperthermic yoga sessions per week
6. Willingness to keep existing exercise regimen (even if that is sedentary) stable over study course
7. Women of childbearing potential must use an acceptable form of birth control
8. Membership to the Bikram Yoga Boston and Bikram Yoga Cambridge studios via studio websites
9. Willingness to adhere to hydration requirements (i.e., an additional four 8 oz servings of water pre- and post-yoga classes)

4.2 Exclusion Criteria

1. Pregnancy or planned pregnancy during study
2. History of psychiatric hospitalization within the past year
3. Active suicidal intent within the past year ("yes" on item 4 or 5 on the Columbia-Suicide Severity Rating Scale[81])
4. History of neurologic disorders that could interfere in study participation
5. History of bipolar disorder, psychotic disorders, eating disorders, and/or substance abuse or dependence (within the last year), as per the Mini-International Neuropsychiatric Interview (MINI)[82]
6. Psychotropic medication use that has been stable for <2 months, and at a stable dose for <4 weeks
7. Use of stimulant medications or diet pills during the study, or any pre-workout powders or liquids designed to provide excessive energy (excluding coffee or caffeinated beverages)
8. Positive urine toxicology screen due to illicit drug use or other exclusionary medications. (Potential false positives will be addressed on a case-by-case basis at the discretion of the investigator)
9. Active conditions which may also cause depressive symptoms (e.g. epilepsy, hypothyroidism)
10. Medical conditions that may make participation unsafe (e.g., diabetes [I & II], cardiovascular disease, hypertension [>140 systolic and/or >90 diastolic], hypotension [<90 systolic and/or <60 diastolic, during screening], orthostatic hypotension [systolic drop of 20 points or 10 point diastolic or heart rate increase by 10], autoimmune disorders, malignancy, or autonomic dysfunction)
11. > 6 one hour classes of meditation or other mind-body disciplines (e.g., Tai chi or yoga) within the last 6 months
12. Current individual or group psychotherapy established for <3 months
13. A subject who in the opinion of the Principal Investigator would not be able to safely complete the study or would jeopardize study integrity

14. History of heat intolerance or heat stroke
15. Unsafe cardiac status as defined by abnormal ECG reading at screening visit as determined by medical monitor, study doctor, or subject's PCP or cardiologist

4.3 Study Enrollment Procedures

Recruitment: Clinician referral and advertisement for our program in local newspapers, internet, radio, and television; advertisements through internal research listings distributed via e-mail and posted online by MGH, RSVP for Health (a registry of MGH and Brigham and Women's Hospital where individuals receive information about clinical trials); a listing on ClinicalTrials.gov; flyers posted on hospital-approved bulletin boards or in local health clinics; study-specific postings on Craigslist; meetings with community organizations to recruit non-MGH patients; and study flyers posted at the Bikram Yoga Boston and Bikram Yoga Cambridge studios and on their websites.

Procedures: A Study Coordinator will determine possible subject eligibility by phone, utilizing the IRB approved Depression Clinical and Research Program (DCRP) General Recruitment Protocol (Protocol #: 2007P002312) - once this protocol is added by an approved amendment. Eligible subjects will be invited for a screen visit at the DCRP, where they will complete the consent process with MD or PhD-level staff. Prior to coming in for the screening visit, subjects will be asked to sign up for an online account with both yoga studios," through the Bikram Yoga Boston and the Bikram Yoga Cambridge websites. A screening log will be kept to document reasons for ineligibility and for non-participation of eligible subjects. Consented subjects who continue to meet inclusion criteria will be randomized to hyperthermic yoga or the waitlist control by their baseline visit.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

The study intervention is hyperthermic yoga, and in this case, Bikram Yoga, a form of hyperthermic yoga. The intervention will be community-delivered (free of charge) by two local Bikram Yoga studios, Bikram Yoga Boston and Bikram Yoga Cambridge. Participants will be asked to attend at least two weekly 90-minute classes over the course of an 8-week intervention period at either studio. The intervention period will be followed by a 1-month post-intervention visit; however, during the 1-month period between the active intervention and follow-up visit, yoga will not be provided free to participants. Participants may continue the intervention at their own cost, but this is entirely of their own volition.

Potential Adverse Effects: Bikram Yoga is performed in a heated room (105-110˚F), which may be uncomfortable. Discomfort may include cramping or feeling hot, lightheaded, dizzy, fatigued, or nauseous. More extreme and less frequent heat related risks include fainting, heat stroke, or cardiovascular events. Hydration is the best way to avoid these reactions to the heat, as such hydration recommendations will be provided to participants during their baseline visit prior to their first yoga class (i.e., an additional four 8 oz servings of water pre- and post-yoga classes). Bikram Yoga instructors may encourage participants to stay in the heated room

despite feeling overwhelmed, though they are free to leave the room – this point is emphasized during their baseline visit. Participation in yoga classes could also potentially aggravate old injuries and/or cause new injuries. Women experiencing hot flashes related to perimenopause or menopause may experience increased discomfort in the heated yoga room; however, there is no reason to believe that participation is unsafe. Stimulant and recreational drug use during the study may increase subject risk for heat related side effects during yoga classes (e.g., fatigue, nausea, dizziness). It is possible that participants may experience feelings of frustration and/or self-criticism due to the intensity of the exercise.

5.2 Handling of Study Interventions

The infrastructure of the MGH DCRP will permit blinded MD and PhD-level clinician assessors or psychology interns (under supervision by a licensed clinical psychologist) at no additional cost to the study. Any unblinded assessors (MD and PhD- level staff or psychology interns designated prior to the start of the study) will not have access to data nor involvement in data monitoring or analyses. All psychology interns are closely supervised by MD and PhD-level staff, and are trained in the administration and rating of depression diagnostic scales.

Blinded DCRP assessors will perform primary and secondary endpoint collection. The research assistant welcoming the subject will remind the subject not to unblind the blinded assessors at each assessment visit. The research assistant will not be blinded, as they will be collecting attendance data from the yoga studios. If assessment visits are conducted by phone (on an as needed basis to reduce subject burden), blinded assessors will remind participants not to unblind them at the beginning of the call.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

Psychotropic medication use, other than stimulants, and ongoing stable psychotherapy (individual or group) is allowable as long as medications/psychotherapy have been stable for at least two months and at a stable dose for at least 4 weeks prior to screening visit.

If during the study subjects require regular care (i.e., worsening depression or safety issues) and do not have a provider, they will be allowed to begin 3-month free follow-up psychiatric care at DCRP and will be discontinued from the intervention at study clinician's discretion.

5.3.2 Required Interventions

N/A

5.3.3 Prohibited Interventions

Use of stimulant medications or diet pills, or any pre-workout powders or liquids designed to provide excessive energy are exclusionary. The consumption of coffee or other caffeinated beverages is acceptable.

5.4 Adherence Assessment

Participants are asked to attend at least two weekly hyperthermic yoga classes over an 8-week intervention, a minimum total of 16 yoga classes. Adherence will be defined at 75% of the minimum prescribed dose of the study intervention – i.e., adherence means that participants attended at least 12 out of 16 yoga classes over the 8-week study intervention period. The two yoga studios track attendance via computer systems. Attendance data will be collected by study staff (usually a research assistant) who will either email via Send Secure email, or utilize Dropbox to communicate with the studio owners, Jill Koontz (Bikram Yoga Boston) and Lucas Lambert (Bikram Yoga Cambridge), to obtain attendance data. All yoga students, whether study participants or not, are required to sign in at the front desk of the studios prior to class.

Yoga instructors act as front desk attendants for their own classes and sign participants into the computer system at this time.

Fidelity Assessment**:** The adherence of each of the two Bikram Yoga studios to the Bikram Yoga protocol will be established, prior to starting the proposed study and every six months thereafter with an adherence measure designed by Dr. Streeter.

Temperature Measurement: We will measure the temperature and humidity in the three heated yoga rooms with HOBO Bluethooth Low Energy Temperature/Relative Humidity Data. HOBO MX1101 data logger measures and transmits temperature and relative humidity data wirelessly to mobile devices via Bluetooth Low Energy (BLE) technology. These devices will be mounted in each of the three rooms. Study staff will collect data prior to study initiation and quarterly thereafter to monitor temperature and humidity- and to store the data for later analyses. These devices provide wireless and time stamped temperature and humidity data to smart phone device. The self-contained wireless data logger works with Onset's free HOBOmobile app for logger setup and data management. Data is accessible anytime from a mobile device for iOS or Android over a 100-foor range.

6. STUDY PROCEDURES

6.1.1 Immediate Yoga Group (IYG) Schedule of Evaluations

Assessment	V1	V2	V3	V4	V5	V6***	V7	V8	V9	V10	V11	V12
Informed Consent Form	X											
Demographics/Contact Information	X											
Clinician-Administered Psychiatric History Form	X											
Inclusion/Exclusion Criteria	X											
Safety Labs (collected by research coordinator) 1. Blood 2. ECG 3. Urine drug screen 4. Urine pregnancy test (Female)	X											
Medical History	X											
Physical Examination by DCRP psychiatrist	X					X	X					
Habits Questionnaire	X					X	X					
MINI	X											
C-SSRS (lifetime and past year V1; since last visit all other visits)	X				X	X	X					
Reproductive Status Questionnaire (Female)	X											
IDS-CR	X	X	X	X	X	X	X					
HAM-D-28		X				X	X					
Self-Rated Questionnaires: STAI; SF-36; Q-LES-Q; EIFI; PSS; PSQI; FFMQ; RRS, URICA	X	X	X	X	X	X	X					
URICA		X				X						
Concomitant Medications	X	X	X	X	X	X	X					
Vital Signs collected by research coordinator	X	X	X^{+}	X^{+}	X	X	X					
Randomization		After baseline visit										

Psychoeducational Session		X										
Waist-to-Hip Ratio measured by research coordinator		X			X	X	X					
Physiological Markers 1. Inflammatory markers (blood) 2. Cortisol (salivary)*		X			X	X	X					
Sleep Diary		X			X	X	X					
Adverse Events		X	X	X	X	X	X					
Hot Yoga Beliefs Scale		X				X	X					
Acceptability Interview						X						
MINI Mood Module						X	X					
QIDS-C and Weekly Phone Monitoring Form	----------> On weeks in between V2-V3, V3-V4, V4-V5, V5-V6 and V6-V7											
Pre- and Post-Yoga Scales (STAI State, EIFI)	30 minutes prior to and 30 minutes post each yoga class											

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6.1.2 Delayed Yoga Group (DYG) Schedule of Evaluations

Assessment	V1	V2	V3	V4	V5	V6***	V7	V8	V9	V10	V11	V12
Informed Consent Form	X											
Demographics/Contact Information	X											
Clinician-Administered Psychiatric History Form	X											
Inclusion/Exclusion Criteria	X											
Safety Labs (collected by research coordinator) 1. Blood 2. ECG 3. Urine drug screen 4. Urine pregnancy test (Female)	X											
Medical History	X											
Physical Examination by DCRP psychiatrist	X					X					X	X
Habits Questionnaire	X					X					X	X
MINI	X											
C-SSRS (lifetime and past year V1; since last visit all other visits)	X				X	X				X	X	X
Reproductive Status Questionnaire (Female)	X											
IDS-CR	X	X	X	X	X	X	X	X	X	X	X	X
HAM-D-28		X				X	X				X	X
Self-Rated Questionnaires: STAI; SF-36; Q-LES-Q; EIFI; PSS; PSQI; FFMQ; RRS	X	X	X	X	X	X	X	X	X	X	X	X
URICA		X				X	X				X	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs by research coordinator	X	X	X^{+}	X^{+}	X	X	X	X^{+}	X^{+}	X	X	X
Randomization		After baseline visit										
Psychoeducational Session		X					X					

Waist-to-Hip Ratio measured by research coordinator		X			X	X	X			X	X	X
Physiological Markers 1. Inflammatory markers (blood) 2. Cortisol (salivary)*		X			X	X	X**			X	X	X
Sleep Diary		X			X	X	X			X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X
Hot Yoga Beliefs Scale		X				X	X				X	X
Acceptability Interview											X	
MINI Mood Module						X					X	X
QIDS-C and Weekly Phone Monitoring Form	----------> On weeks in between V2-V3, V3-V4, V4-V5, V5-V6, V6-V7, V7-V8, V8-V9, V9-V10, V10-V11, V11-V12											
Pre- and Post-Yoga Scales (STAI State, EIFI)	30 minutes prior to and 30 minutes post each yoga class											

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6.2 Description of Evaluations

Figures 6.1.1 and 6.1.2 above outline a detailed description of evaluation visits. Additional information not included in this figure is listed below.

6.2.1 Screening Evaluation

Consenting Procedure
Prior to engaging in any study related activities, informed consent will be obtained by an MD- or PhD-level clinician or supervised psychology intern at the DCRP. One informed consent will be obtained for the screening and study procedures. Patients will be informed during the consent process that their participation is voluntary and their relationship with their prescribing physician will be unaffected by any choice to discontinue study participation. All participants will be informed that they can decline to answer any questions that they find upsetting and may withdraw from study participation at any time. The consent form will include the study procedures, information about potential risks and benefits of participation, and information regarding who they can contact with further questions or concerns. Patients will be alerted to the nature of the hyperthermic yoga practice during the consent process. Participants will have 24-hour pager access to an internal medicine physician, Darshan Mehta, MD, whose specialty is in alternative medicine. This information will be given in the consent form. Documentation of informed consent will be kept in the Documentation of the Informed Consent Process for Onsite Subject File.

Visit 1: Screen – IYG and DYG
The screening visit will last approximately 3 hours. The purpose of this visit is to determine study eligibility. The two Figures above (6.1.1 and 6.1.2) outline the procedures for the screening visit, which are the same for the IYG and DYG. All of the screening items will be completed prior to the baseline visit (within 21 days of the screening visit).

- Informed Consent Form
- Demographics/Contact Information
- Clinician-Administered Psychiatric History Form
- Inclusion/Exclusion Criteria
- Safety Labs (i.e., blood, ECG, urine drug screen, urine pregnancy test [female] collected by a trained research coordinator)
    - Blood draw: A phlebotomy trained research coordinator at the DCRP will draw 11mL of blood (one 8mL tiger top tube and one 3mL lavender top tube).
- Medical History
- Physical Examination conducted by DCRP psychiatrist
- Habits Questionnaire
- MINI
- C-SSRS Lifetime and Past Year
- Reproductive Status Questionnaire (female)
- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications

- Vital signs
- Give salivettes and instructions to bring back salivary cortisol samples to their baseline visit
- Give Sleep Diary

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment
Enrollment is defined as the date all of the screening criteria are met– occurring between screening (visit 1) and baseline (visit 2).

Baseline Assessments (Visit 2 for IYG; Visits 2 and 7 for DYG)
At this visit subjects will meet with a study clinician for a 50-minute psychoeducational (see First Heated Yoga Class: What to Expect) and motivational session. Subjects will be asked to discuss reasons for wanting to try the hyperthermic yoga and also to discuss potential barriers to participation.

Visit 2: Baseline - IYG and DYG
- IDS-CR
- HAM-D-28
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS, URICA
- Concomitant Medications
- Vital Signs
- Psychoeducational Session
- Waist-to-Hip Ratio
- Physiological Markers (i.e., inflammatory markers [blood], cortisol [saliva])
    - Please see section 9.5.2 for details
- Collect Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Collect salivates (can bring to the following visit as long as it was collected at the right time period)
- Adverse Events
- Hot Yoga Beliefs Scale

Visit 7: Repeat Baseline - DYG
- IDS-CR
- HAM-D-28
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS, URICA
- Concomitant Medications
- Vital Signs
- Psychoeducational Session (refresher)
- Waist-to-Hip Ratio
- Physiological Markers^ (i.e., inflammatory markers [blood], cortisol [saliva])
- Collect Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Adverse Events

- Hot Yoga Beliefs Scale

^If this visit takes place within 14 days of Visit 6, Physiological Markers will not be repeated at this visit.

At the end of the Baseline Visit that occurs before they start the yoga (V2 or V7), participants will be handed a packet of self-rated questionnaires (i.e. STAI-State and EIFI) to complete within 30 minutes prior and 30 minutes post each yoga class they attend. We will ask participants to note time and date on the top of each form. These questionnaires will provide data on acute mood changes pre- and post-yoga. Dr. Nyer's co-mentor Dr. Streeter uses these questionnaires to gather the same data in her RO1 (5R01AT007483-05). Including this will allow us to compare data across studies.

Participants will also be handed a "Yoga Information" form at the end of the Baseline Visit that occurs before they start the yoga (V2 or V7), which will provide information about the studio locations and the class logistics.

Pre- and Post-Yoga Classes Scales – IYG and DYG
- Self-Rated Questionnaires (STAI-State only, EIFI)

Randomization
Patients will be randomized prior to visit 3, assessment visit 1. Research Coordinators will conduct the randomization and will inform Dr. Nyer and participants of which group they have been randomized too at the end of their Baseline Visit, once all measures have been completed.

6.2.3 Blinding
The study clinician-assessors will be blinded. The research assistant welcoming the subject will remind the subject not to unblind the assessors at each assessment visit. Blinded assessors will complete all primary and secondary endpoint collection.

The randomization scheme will be executed by our biostatistician who will be blinded to treatment assignment and will also have no influence on outcome assessments. Our biostatistician will establish the blind and deliver it to the designated research assistant for this study. The Principal Investigator (PI) will not be blinded, since the PI will be delivering the psychoeducation preparatory visit (pre-yoga start) and also will be performing the bi-weekly phone monitoring. The PI will not have access to the data until the study database is locked. The DSMB identified in the submission will have access to unblinded data and will monitor adverse and serious adverse events.

Breaking the Blind: the PI, Maren Nyer, PhD, and Medical Monitor, Darshan Mehta, MD, are both unblinded to the intervention arm of each subject. In the event of a medical AE/SAE, Dr. Mehta or his designee will be responsible for assuring safety of the patient. In the event of a psychiatric AE/SAE, Dr. Nyer or her designee will be responsible for assuring to the safety of the patient.

6.2.4 Follow-up Visits

Phone visits will occur bi-weekly from baseline through the end of the study intervention period. During the 1 month follow-up period, there will be a phone call during the midpoint (2-weeks into the follow-up period).

Assessment visits will be completed within a reasonable time frame (+/- 3 workday window), but may occur outside this window if deemed appropriate by study staff.

Visit 3 (Week-1 Assessment)* – IYG and DYG

- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs[+]
- Adverse events

Visit 4 (Week-3 Assessment)* – IYG and DYG

- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs[+]
- Give salivettes
- Give Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Adverse Events

Visit 5 (Week-5 Assessment) – IYG and DYG

- C-SSRS since Last Visit
- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs
- Waist-to-Hip Ratio
- Physiological Markers (i.e., inflammatory markers [blood], cortisol [saliva])
- Give salivettes
- Collect and give Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Adverse Events

Visit 8 (Week-1 Yoga Assessment) – DYG

- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs[+]
- Adverse Events

Visit 9 (Week-3 Yoga Assessment) – DYG
- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs[+]
- Give salivettes
- Give Sleep Diary
- Adverse Events

Visit 10 (Week-5 Yoga Assessment) – DYG
- C-SSRS since Last Visit
- IDS-CR
- Self-Rated Questionnaires: STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS
- Concomitant Medications
- Vital Signs
- Waist-to-Hip Ratio
- Physiological Markers (i.e., inflammatory markers [blood], cortisol [saliva])
- Give salivettes
- Collect and give Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Adverse Events

[+]Note that assessment visits will in most cases take place in person, however may take place over the phone in extenuating circumstances to reduce subject burden or accommodate subject schedules. In these cases, visits may be split into two separate occasions within the appropriate window with clinician-rated measures taking place by phone if needed. If assessment visits 3, 4, 8, and 9 take place by phone, no vital signs will be obtained. For all other visits blood is required for either safety procedures or outcome data.

6.2.5 Completion/Final Evaluation

Visit 6: Study Primary Endpoint (Week-8) for IYG and DYG (repeated for DYG in Visit 11 as 9-week Yoga Assessment)/Early Discontinuation Visit – IYG and DYG
- Physical Examination
- Habits Questionnaire
- C-SSRS since Last Visit
- IDS-CR
- HAM-D-28
- Self-Sated Questionnaires (STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS, URICA)
- Concomitant Medications
- Vital Signs
- Waist-to-Hip Ratio
- Physiological Markers (i.e., inflammatory markers [blood], cortisol [saliva])

- Collect Sleep Diary (can bring to the following visit as long as it was completed for the right week)
- Adverse Events
- Hot Yoga Beliefs Scale
- Acceptability Interview (completed at Visit 11 for DYG)
- MINI Mood Module

Final Evaluation: 1-Month Follow-Up Post-Yoga Visit – IYG (Visit 7) and DYG (Visit 12)
- Physical Examination
- Habits Questionnaire
- C-SSRS since Last Visit
- IDS-CR
- HAM-D-28
- Self-Rated Questionnaires (STAI, SF-36, Q-LES-Q, EIFI, PSS, PSQI, FFMQ, RRS)
- Concomitant Medications
- Vital Signs
- Waist-to-Hip Ratio
- Physiological Markers (i.e., inflammatory markers [blood], cortisol [saliva])
- Collect Sleep Diary
- Adverse Events
- Hot Yoga Beliefs Scale
- MINI Mood Module

Potential Reasons for Early Termination
Subjects can be potentially dropped from the intervention due to: 1) adherence to yoga or assessment visits, 2) being deemed unsafe to continue the yoga intervention, 3) worsening of depressive symptoms, or 4) suicide risk.

Please see section 8 (Intervention Discontinuation) for operational definitions of the above.

7. SAFETY ASSESSMENTS

Patients will be called on a bi-weekly basis to assess for AE/SAEs and worsening symptoms of depression and suicidality throughout the active intervention and once during the 1-month follow-up period.

Suicide risk will be assessed through clinical interview and clinical assessment items on the MINI, BDI, PHQ-9 and CSSR-S. Homicidality, a less common occurrence, will be assessed as per all our clinical trials at the DCRP, through MD or PhD level or supervised psychology intern assessors trained to assess for homicidality on clinical interview. All necessary legal standards, including duty to protect, will be followed in the event of a serious homicidal threat.

Expected AE/SAEs

Heat related side effects could occur, including: cardiac events, cramping, dehydration, fainting, fatigue, heatstroke, light headedness, muscle soreness, nausea, or worsening of hot flashes (for peri – or menopausal women).

7.1 Specification of Safety Parameters

Any abnormalities in psychiatric status, labs, vital signs, ECG, or physical exam on any assessment visit will be assessed for safety. Dr. Mehta (or his designee) will be responsible for any medical abnormalities, and Dr. Nyer (or her designee) will be responsible for any psychiatric abnormalities.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Please see section above. Any abnormalities will be addressed within a 48 hour workday window.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

Any abnormal (outside the normal range) liver function values or thyroid values will be followed up on and if necessary referred for appropriate clinical care.

AEs that will be solicited on bi-weekly phone call (Bi-Weekly Phone Monitoring Form) will be: depression, suicidality, muscle soreness, or AEs/SAEs.

Unsolicited events will be captured during bi-weekly phone calls (Bi-Weekly Phone Monitoring Form) or during assessment visits (Adverse Events Form).

Reporting procedures are described in section 7.4 below.

7.4 Reporting Procedures

The PI will be responsible for the reporting of any SAEs or AEs to the IRB, DSMB, and NCCIH as necessary. Study-related adverse events will be reported to the IRB and NCCIH as soon as they are discovered by any study staff member and discussed with PI or designee (within 24 hours). AE and SAE reports and annual summaries will not include subject- or group-identifiable material. Each report will only include the identification code. Other adverse events will be reported following the rules of the Partners IRB. Short-term elevations in participant distress are not considered adverse events.

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the mentorship team, Independent Monitors, IRB, and NCCIH in accordance with requirements.

Unexpected fatal or life-threatening AEs related to the intervention will be reported to the NCCIH Program Officer within 7 days. Other serious and unexpected AEs related to the intervention will be reported to the NCCIH Program Official within 15 days.

Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the IRB, NCCIH, and other oversight organizations in accordance with their requirements.

7.5 Follow-up for Adverse Events

Subjects will continue to be monitored for safety after an AE/SAE has occurred until the issue has resolved or is considered stable. Subjects will be called or asked to come in to assess AE/SAEs if determined necessary by study clinician.

7.6 Safety Monitoring

A data safety monitoring board (DSMB) has been assembled prior to the start of the study. The board consists of three staff-level investigators who are not key personnel involved with this grant. They are qualified to review the patient safety data generated by this study. One has expertise in mind-body interventions (John Denninger, MD, PhD); the second has expertise in clinical trial design (Andrew Nierenberg, MD); the third has expertise as a statistical consultant (Johannes Laferton, PhD). The DSMB will meet at least quarterly per year to review study progress, address any difficulties with recruitment, and address any safety related matters that may arise. Details of the logistics of the DSMB are as follows:

a. **Unblinded Reporting** – Safety information for this study will be reported to the DSMB in an unblinded manner. A statistical penalty will not be assessed for the ongoing unblinded review of safety by the DSMB. Unblinded data will not be released to the investigators unless necessary for safety reasons.

b. **Range of Safety Reporting to the DSMB** – It is considered necessary for the purpose of monitoring the safety of the study that the DSMB review not only AEs and SAEs, but other data that may reflect differences in safety between treatment groups. This

includes treatment retention rates, reasons for drop-out, and laboratory values reflecting potential danger to patient.

c. **Serious Adverse Events** – Expedited review will occur for all events meeting the FDA definition of SAEs. This also includes any event that a study investigator or the DSMB judges to impose a significant hazard, contraindication, side effect, or precaution.

d. **Non-Serious Adverse Events** – At periodic intervals (quarterly during the course of the study and then again at its completion), the DSMB will be provided with un-blinded summaries of the numbers and rates of adverse events by treatment group. These reports will include types of events, severity, and treatment phase. Data on individual non-serious adverse events is not expected to be needed for this review.

e. **Other Safety-Related Reports** – At twelve-month intervals throughout the course of the study, the DSMB will also receive unblinded summary reports of treatment retention and reasons for drop-out, by treatment arm and study phase.

f. **Study Stopping Rules** – If at any time during the course of the study, the DSMB judges that risk to subjects outweighs the potential benefits, the DSMB shall have the discretion and responsibility to recommend that the study be terminated.

8. INTERVENTION DISCONTINUATION

If a subject's overall depressive symptoms significantly increase, or if a subject develops suicidal intent, he/she will be evaluated by a study clinician to determine if referral for clinical intervention is needed. This will be determined per study clinician judgment.

- Subjects who demonstrate an increase in the IDS total score of 25% or greater over baseline or whose QIDS-C score is a >16 (indicating severe depression) will be assessed for referral to evidence-based clinical treatment.
- A "yes" on items 4 or 5 on the Columbia-Suicide Severity Rating Scale, a score of 3 on the suicidal ideation question of the QIDS-C, or a score of 3 on the IDS-CR suicide item will prompt assessment, and if necessary, appropriate referral.
- If a subject reports that worsening severity is due to situational factors and there appears to be no acute risk of self harm or other significant worsening, they will be given the option to be reassessed within 72 hours (most likely via phone) to determine the safety of continuation.
- Patients will also be dropped from the study if they are, in the judgment of the study clinician, deemed to be a safety risk, the intervention appears to making their condition worse, or if new information is uncovered re: an untreated condition (e.g., onset of substance abuse or physical condition) that would affect participation or threaten the integrity of the study.

Disconsolation due to Non-Adherence: if a subject does not attend a yoga session for two weeks, he or she will be discontinued from the intervention – though he or she will be asked to come in for a final assessment visit. If a subject misses more than two consecutive assessment visits, he or she will also be discontinued from the intervention, unless there are extenuating circumstances communicated to the study team. Subjects will be allowed to continue if they are only attending one yoga class during any specified week, though this will be controlled for in analyses – their data will still be useful. If subjects are discontinued from the intervention, they will be included in the modified ITT (MITT) analyses (see section 9 below) and invited for a Visit 6 (final exit visit).

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This study is a randomized controlled trial (RCT) with a waitlist control group with a crossover design. This is a relatively rigorous design for this stage of investigation. Double blinding is not possible in this study, as the participants will know whether or not they are attending the yoga classes. The waitlist intervention will control for time, attention during assessment visits, and the cyclical nature of depression for this early stage investigation.

Hypotheses are clearly outlined in section 9.6 below. Primary and secondary outcome measures are outlined in sections 9.5.1 and 9.5.2 below.

9.2 Sample Size and Randomization

Sample Size and Power Estimation: Although this study will test for statistical significance, the primary aim of this pilot RCT is to estimate the intervention's effect size to conduct a more definitive R01 study, as is outlined in NIH guidelines for developing behavioral interventions[83]. The study was powered conservatively based on an 80% (n=64) ITT sample. Effect sizes for the degree of change in IDS-CR (primary outcome) were estimated for the hyperthermic yoga versus the control using Cohen's *d* (effect size for t-test). A power analysis found that with an ITT sample of 64 (32 per arm), a two-tailed t-test with an alpha level of .05 will yield a statistical power of 79% to detect a large effect size of .70 and 50% to detect a moderate effect size (.50). Thus, if the effect size falls between moderate and large, power would approach 80%, since the evaluable sample should be between 64 and 80. Preliminary data suggested a moderate effect size of .54 for depression[34]. We predict a more robust effect size of at least .60-.70, as this is a larger sample recruited specifically for depressive symptoms.

Randomization: Our biostatistician will establish the blind and deliver it to the designated Research Coordinator. Randomization will be done by random permuted block design in blocks of 2 and 4. This method assigns subjects to each intervention group with equal probability, while at the same time generating a balanced design. The random permuted block randomization method also protects against bias due to temporal trends (e.g., experience of research staff, or seasonal effect). This randomization design should balance the heterogeneity of the subjects between groups.

9.3 Definition of Populations

A completer will be defined as a subject who attends: 1) a screen, 2) baseline, 3) at least one midpoint assessment (week-1, -3, or -5), and 4) a week-8 assessment visit. We will complete two analyses: 1) a completer analysis and 2) a modified ITT (MITT) analysis. The MITT will include those who have attended at least one post-baseline assessment visit. Our primary analyses utilize data from the IYG and DYG prior to the crossover.

9.4 Interim Analyses and Stopping Rules

We are not planning to conduct interim analyses due to the small sample size of this pilot study. The study is powered to require the full sample to assess efficacy. We do not anticipate any safety considerations that would warrant early discontinuation of the study. However, in the event that there are a high number of SAEs, the DSMB will guide the PI and study team on how to best proceed. Other study performance metrics (e.g., slow accrual, high losses-to-follow-up, and poor quality control) will be monitored by NCCIH and the DSMB.

9.5 Outcomes

The blinded assessors will collect the primary outcome of interest, IDS-CR. The Advisory Committee and DSMB will be unblinded.

9.5.1 Primary Outcome

Primary Outcome Measures

Acceptability Interview (primary aim I): A 19-question qualitative interview assesses perception of ease of participation, program likeability, and perceived benefits of the yoga program. Adapted from DeBoer et al.[34].
Inventory of Depressive Symptomatology (IDS-CR): A valid and reliable 30-item measure that is designed to assess severity of depression. The questions focus on neurovegetative and other depressive symptoms experienced over the past seven days. Higher scores indicate more severe pathology. A decrease of 50% or more in the IDS-CR score is considered to be a positive response to treatment, while a final score of 11 or less is considered typical remission.

9.5.2 Secondary Outcomes

Secondary Outcome Measures
Spielberger State-Trait Anxiety Inventory (STAI)[84].A 40-item commonly used measure that distinguishes between state vs. trait anxiety.
Hamilton Depression Rating Scale (HAM-D-28)[85]. A 28 item scale commonly used as an observational rating measure of depression presence and severity.
Medical Outcomes Study 36-item short-form survey (SF-36)[86]: Assesses bodily pain, general health perception, vitality, social functioning, physical and emotional impediments to role functioning, mental health, and includes an overall physical component scale (PCS) and mental component scale (MCS). Used in a trial of yoga for women in breast cancer treatment[87].
Quality of Life Satisfaction Questionnaire-Short Form (Q-LES-Q-SF)[88]: A measure of satisfaction and enjoyment in various domains.
Exercise Induced Feeling Inventory (EIFI)[89]: Assesses feeling states associated with physical activity: Positive Engagement, Revitalization, Tranquility, and Physical Exhaustion. Sensitive to changes over the course of yoga treatment[90].
The Perceived Stress Scale (PSS)[38]: Measures perceived level of stress.
University of Rhode Island Change Assessment Scale (URICA): Assesses participants' readiness to change.

Psychological Mechanisms of Actions
Sleep Diary: Includes a Stanford Sleepiness Scale rating[91], in addition to tracking caffeine, alcohol, smoking, exercise and naps.
Pittsburg Sleep Quality Index (PSQI)[92]: A commonly used and well-validated self-rated measure of sleep-quality and disturbance.
The Five Facet Mindfulness Questionnaire (FFMQ)[93]: A self-report instrument that measures mindfulness; derived from factor analytic study of five independent mindfulness questionnaires.
Ruminative Responses Scale (RRS)[37]: A 22-item questionnaire, which assesses depressive rumination style.

Potential Moderator
Hot Yoga Beliefs Scale: Adapted from the *Acupuncture Beliefs Scale*[55], a 36-item self-administered instrument that to determine positive and negative beliefs about the study therapy. The data from this questionnaire can be used to assess how patient beliefs and

clinical improvement may impact each other. For this study, the scale's questions have been adapted to reflect heated yoga rather than acupuncture as the therapy of interest.

Physiological Mechanisms of Action

Salivary Cortisol[87]: Saliva will be collected using salivettes. To account for diurnal variation, four samples will be obtained prior to each cortisol assessment (nighttime [1 and 2] and waking [3 and 4]).

Inflammatory Markers: 30 mL of blood will be drawn per visit. 3 cc and 8 cc tubes will be used. Tubes will be centrifuged at 1500 rpm for 10 min at 4oC. Plasma will be transferred into 2 cc plastic tubes, capped and frozen at -80oC until processing. Levels of IL-6 and TNF-alpha cytokines will be assayed using commercially available radioimmunoassay or enzyme-linked immunosorbent assay at the Behavioral Neuroendocrinology Laboratory at the University of Colorado Boulder, led by Dr. Christopher A. Lowry. Hs-CRP will be assayed at Dr. Lowry's lab using immunochemiluminometric assay (ICMA). These three markers have been heavily implicated in the pathophysiology of depression [58, 94] and shown to improve with yoga[59]. Dr. Foster's add-on study, supported by her Reich award, will be performing RNA-sequencing and analysis of the neurosteroids allopregnanolone and progesterone. The RNA-sequencing will be performed by Genewiz, and the neurosteroid data will be processed by the PK/PD Bioanalytical Core Facility located at the UC Davis Medical Center.

9.6 Data Analyses

Statistical Analyses: Analyses will be conducted using SPSS. Full descriptive data will be produced. Baseline differences between the yoga and control group (demographic variables and depressive severity) will be assessed and controlled for as necessary. Two analyses will be completed: 1) an MITT and 2) a "completer" analysis. Primary analyses will only include data from the IYG and DYG prior to the crossover. We will conduct exploratory analyses including crossover DYG subjects to increase power.

Missing Data: We will test whether missing data occurred randomly across conditions, in which case it will be handled by replacing data with the scale or sub-scale mean. Where this is not feasible, missing data will be imputed through maximum likelihood estimation or multiple imputation. If missing data is not random, advanced statistical analyses taking into account assumptions of various tests will be conducted.

Adherence: number of heated yoga classes attended will be used as a covariate in the efficacy analyses to determine if participants who go to more classes demonstrate greater improvement in depressive symptoms.

Primary Aim I: To recruit and retain adults with depressive symptoms into a controlled trial of hyperthermic yoga, randomizing 40 participants to each the immediate yoga group (IYG) and delayed yoga group (DYG; control group) with seventy-five percent retention in both groups.

Hypothesis Ia: Seventy-five percent of the each group will complete a week-8 assessment.

Hypothesis Ib: Acceptability of hyperthermic yoga will be high on the Acceptability

Interview.

Screening logs will reveal the proportion of eligible screened individuals enrolled in the study. We will track factors that make participants ineligible or unwilling to participate. The goal is 75% retention, with enrollment defined as completing at least one post-baseline assessment visit. Attendance data will be characterized descriptively and compared between treatment arms by chi-squared analysis. Yoga attendance will be examined as a potential covariate in subsequent analyses. Participants who participated in Yoga will be re-contacted at completion of all patient study interventions for accuracy of Yoga attendance. Patient's will be contacted up to three times before they will be labeled as "Lost to Recontact" in the screening logs.

Primary Aim II: To collect the Inventory of Depressive Symptomatology – Clinician Rated (IDS-CR) scores at screening; baseline; and weeks-1, -3, -5 and -8 to evaluate the efficacy of hyperthermic yoga for depressive symptoms.

Hypothesis II: The IYG will have greater decreases in Inventory of Depressive Symptomatology – Clinician Rated (IDS-CR) scores after 8-weeks compared to the DYG.

Response over 8-weeks defined by 50% or greater decrease on IDS-CR total; remission ≤11 on IDS-CR total. Continuous variables will be analyzed by generalized mixed effect modeling, which impute missing values based on maximum likelihood estimates of missing parameters, allowing analysis of all MITT participants. A time-by-condition interaction will be analyzed to test the intervention's efficacy. Relevant potential covariates (e.g., attendance) will also be examined.

Primary Aim III: To collect (a) physiological and (b) psychological mediators of hyperthermic yoga in the IYG and DYG group at screening; baseline; weeks-1, -3, -5 and -8 to evaluate treatment response in the IYG compared to the DYG.

Hypothesis IIIa: Salivary cortisol, and inflammatory markers (i.e., Tumor Necrosis Factor [TNF-α], Interleukin-6 [IL-6], and C-Reactive Protein [CRP]) will significantly decrease, following 5- and 8-weeks of hyperthermic yoga in the IYG compared to the DYG.
Hypothesis IIIb: Perceived stress, mindfulness, and rumination will improve by 5- and 8-weeks in the IYG compared to the DYG.

Latent growth curve modeling will assess potential mechanisms of the treatment effect. The slope (change over time) of the mediator variables will be regressed upon the independent variable (condition). The slope of the dependent variable will also be regressed upon the slope of the mediators. This model will permit assessment of the change in mechanisms as it predicts the change in the dependent variables.

Secondary Aim I: To collect measures of *quality of life, general health, physical functioning, decreased perceived stress, anxiety, and sleep* in the IYG and DYG at screening; baseline; and weeks-1, -3, -5 and -8 to evaluate hyperthermic yoga for secondary outcome measures.

Secondary Hypothesis I: The IYG will have significant improvements in quality of life, general health, physical functioning, perceived stress, anxiety, and sleep compared to the DYG.

These will be analyzed by the same methods as outlined for Primary Aim II (above).

Secondary Aim II (exploratory): To evaluate the long-term effects of the yoga intervention (1-month post intervention), while controlling for continued yoga participation.

Secondary Hypothesis II: Subjects who choose to continue the yoga practice, either in the IYG or DYG, will have increased effects at 1-month post intervention, per the primary and secondary outcome measures compared to the week-8 assessment.

Within-participant treatment effects will be modeled via Generalized linear modeling, assessing depressive symptoms (and other secondary outcomes) at 1-month post-intervention compared to week 8.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data will be collected on the specified CRFs. The Research Coordinator will be responsible for collecting all self-report and other non-doctoral level staff CRFs. We will designate a blinded Coordinator (not the primary Research Coordinator for the study) to manage the assessment data vis-a-vis quality checking, entering it in the database, and assisting with analysis, as needed. Doctoral level staff conducting clinician-ratings and/or medical assessments will be responsible for completing the appropriate CRFs. Blinded assessors will complete an IDS-CR, the primary study endpoint. All forms will have a participant ID with no other identifying information, as specified in section 11.3 below.

10.2 Data Management

Self-report data provided by study subjects will be reviewed by blinded study staff for completeness and quality (such as multiple answers when one response is allowed) and all attempts will be made to solve problems prior to the subject leaving the premises. Any incomplete data, illegible, or questionable data provided by blinded assessors will be resolved through feedback with the clinician, within one day following the study visit. This study will utilize REDCap (Research Electronic Data Capture), a browser-based, metadata-driven EDC software solution and workflow methodology for designing clinical and translational research databases. REDCap is secured with password protection. Individual subjects' information will be entered into the database using study ID numbers. No personal identifiers will be stored.

The REDcap system will flag any remaining data quality problems (such as data values outside the expected range) during data entry; any problems will be resolved before the record is added to the database. Extensive quality control procedures for handling and assaying laboratory samples will be carried out to ensure complete and valid biological data. Early in the study and periodically throughout the period of data collection, data will be reviewed by the study Statistician for completeness, consistency, and other indicators of data quality in a

blinded fashion. Missing data will be described but will be inexplicitly imputed in all analyses by using only available data. All primary endpoint data will be quality-assured and assessed by blinded raters. Any errors in completion will be reviewed to determine if directions or procedures need to be altered. In this case, permission from the IRB will be requested to change any procedure. If it is determined that patients are misinterpreting any instrument and answering incorrectly as a result, they will be given proper clarification and instruction.

10.3 Quality Assurance

10.3.1 Training

All staff engaged in human subjects research are required to complete the Collaborative Institutional Training Initiative (CITI) human subjects training and refresher training every three years to be involved with human subject research.

Yoga instructors and studio owners at Bikram Yoga Boston and Bikram Yoga Cambridge will undergo Partners IRB approved modified CITI training by the PI.

10.3.2 Quality Control Committee

N/A

10.3.3 Metrics

Missing data will be handled as specified in section 9.6. Data entry will be double checked by another individual.

10.3.4 Protocol Deviations

Protocol deviations will be collected and documented on the Protocol Deviation Log by the Research Coordinator, overseen by the PI. The PI and at least one member of her mentoring team will review these deviations for potential amendment to IRB approved protocol.

10.3.5 Monitoring

The DSMB, NCCIH, the Partners IRB, and/or OHRP will be monitoring the protocol compliance, data quality, and consent forms.

10.3.6 Data Use Agreement

In addition to the previously described samples analyses, we intend to obtain a Data User Agreement in order to collaborate and share data with an undergraduate student being mentored by Dr. Foster, whose previously approved study utilizing both assessment measures and biological data from the current protocol. The student is completing an undergraduate thesis and is affiliated with UCSI University in Malaysia, and holds an RA position at Harvard Medical School. The fully processed and deidentified data from this protocol with Dr Foster will be used for a requisite to complete his dissertation and ultimately graduation. All data will be securely stored in MGH Dropbox and Harvard Medical School (HMS) O2 High Performance computing (HPC) cluster. The data will not be be transferred from the HMS O2 HPC cluster. Upon approval of the DUA, the fully processed data in the form of graphs and summary tables will be transferred to UCSI servers using a secure file transfer portal using HMS resources. All data associated with the DUA will be de-identified. The student will be monitored and mentored remotely by Dr. Foster. No data will leave the HMS cluster or Boston. The data being utilized is

from blood samples that are currently being processed for gene expression (RNA sequencing) and from clinician-rated primary outcome measures. No samples will be physically removed from the MGH premises**.**

11. PARTICIPANTS RIGHTS AND RESPONSIBILITIES

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the Partners IRB.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant and this will be documented in the participant's record.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All identifiable records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the NCCIH, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the Partners IRB, the NCCIH, the OHRP, or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

Advisory Committee: All mentors (Drs. Fava, Mischoulon, and Streeter) will meet quarterly as an Advisory Committee throughout the PI's K23 Award. The committee will provide guidance such that the following goals are met: 1) recruitment does not fall below 75% of projection, 2) treatment adherence is sufficient, and 3) authorship and future grant submission goals are met. Dr. Fava will oversee the PI's formal progress evaluations.

13. PUBLICATION OF RESEARCH FINDINGS

The study governance includes an Advisory Committee (see 12 above). Publication of the results of this trial will be governed by the policies and procedures developed by the Advisory Committee. Any presentation, abstract, or manuscript will be made available for review by the sponsor and the NCCIH prior to submission.

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