Effects of
Meditation on Human Well-being and Function

Clinical Protocol

Protocol # MED02

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Version 1.0

Principal Investigator: Dr. Tobias Moeller-Bertram

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Signature Page

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH GCP guidelines.

Site Investigator:*

Signed:

Date:

Name/Title

* The protocol should be signed by the clinical site investigator who is responsible for the day-to-day study implementation at his/her specific clinical site.

List of Abbreviations

EEG - Electroencephalogram

ECG - Electrocardiogram

GCP - Good Clinical Practices

ICH - International Council Harmonization

IRB - Institutional Review Board

COI - Conflict of Interest

Q&A - Question and Answer

PST - Pacific Standard Time

HRV - Heart Rate Variability

RCH – Remote Coherence Healing

1 Background/ Significance

This submission of MED02 is an extension of the currently approved protocol MED01 #1273003. Under this new protocol, we want to expand explorative data collection to cover two new areas of interest. One area is to collect pilot data on coherence healing (outlined in experiment A). Under this paradigm, meditators (Healers) utilize meditation to facilitate healing of a person (Healee).
The second area expands pilot data collection to Dr. Joe Dispenza's meditation community, independent from specific meditation events (outline in experiment C). Lastly, this new protocol builds upon the ability of collecting data during an event (outlined in experiment B).

Meditation has been used by cultures across the globe for millennia. In Eastern religions, meditation is common and has its roots in Hindu and Buddhist traditions and manuscripts dating back hundreds of years after Buddha's death.[1] Additionally, effects similar to meditation have also been described in Muslim[2] and Christian prayers, as well as spiritual reflection and chanting, suggesting that these practices are an integral part of human culture and existence.[1,3] In recent years, meditation as a therapeutic option and lifestyle choice has become increasingly popular in Western cultures. A paradigm shift is emerging in evaluating the relationship between spirituality and medicine. This shift is reflected by the increase in medical publications in the PubMed database containing the search term 'meditation'. Until the year 1970, below 2.2 manuscripts per year were published that contained this term. This average number increased by two orders of magnitude in recent years with an average of 26.4 between 1971 and 2000 and 244.6 from 2001 until present. Our specific interest is to establish and describe the molecular relationship between mind and body.[1]

Current manuscripts investigate several effects of meditation on human biology and pathology. Specifically, there is an emphasis on stress reduction and utilization of meditation as an adjunct treatment in chronic pain[4,5] and neuropsychiatric diseases such as anxiety and depression[6,7], sleep disorder[8,9], and post-traumatic stress disorder.[10] For example, the following molecular and physiological effects of meditation are described. In a study investigating inflammatory biomarkers in inflammatory bowel disease, psychological and physical symptoms as well as C-reactive protein were significantly reduced in a group that participated in a Breath-Body-Mind Workshop.[11] Another study investigated telomere biology in attendees of a 1-month long meditation event and concluded that training in an event setting may have effects on telomere regulation.[12] Cortisol is a biomarker that has been shown to be positively influenced in subjects practicing mindfulness.[9] Furthermore, influences of meditation on the heart-brain connection[13,14] and the epigenetic clock[15] have also been described.

Several studies have investigated brain waves measured with electroencephalography (EEG) in meditators and have looked at different meditation techniques. In one study investigating Vipassanna meditators, differences in brain wave recordings were found between novice, experienced senior and teacher meditators.[16] In Brahma Kumaris Raja yoga meditators it has been shown that experienced meditators can shift reliably and consciously between rest and meditation with enhanced theta power (4-8Hz).[17] Furthermore, an increased brain network integration and long-lasting changes in hippocampal functional topology during resting states have been described and associated with meditation.[18,19]

Popularity of Complementary and Alternative Medicine (CAM) such as Yoga, Tai Chi, Qi Gon, and meditation has been rapidly increased and many patients in all the world use spiritual healing and other CAM practices.[20] Spiritual actions and spirituality are intrinsic identities of humanity and potential healing forces.[21]

Given this wide variety of studies and techniques and the positive effects of meditation and mindfulness practices, well-controlled studies are difficult to perform in everyday settings. Furthermore, studies aiming to phenotype the meditative state and integrating read-outs evaluating behavior, physiology, and molecular biology are currently not available. Studies examining specific known end points have been conducted but introduce bias and are ultimately limited in their scope. Limited investigations have approached the mind:body:molecular connection in an unbiased manner to identify novel and unique markers.Studying a community using the same meditation principles and practice will allow to control for a lot of the above mentioned difficulties in studying meditation. In addition, nan event setting here poses the unique situation that matching/controlling for several parameters such as housing, diet, climate, circadian pattern, and daily routine is possible additionally to matching in parameters such as gender, age, and body mass index. This provides a rare opportunity for a very well-controlled pilot study to explore and ultimately phenotype meditative state in the human organism.

2 Objectives

The objective of this study is to describe the effects of meditation on human well-being and function and to learn in greater detail what effect meditation has on the human organism. We are proposing experiments exploring three areas of interest (experiments A, B, C) utilizing procedures, including bio-psychosocial readouts, physiologic, and biologic/molecular measurements. We therefore propose a combination of different experimental components, as follows:

Experiment A: Measuring the effects of meditation on the Healer and Healee. Healers include members of Dr. Joe Dispenza’s mediation community who participate in a healing event. Healees may include individuals who volunteer to receive a healing and may or may not be members of the Dr Joe Dispenza meditation community

Experiment B: Measuring the effects of meditation on the human organism. Here, we are interested in collecting data before, during, and after a meditation event (intervention) held by Dr. Joe Dispenza. We will compare Dr Joe meditators to controls and to meditators following other forms of meditation, including but not limited to mindfulness-based techniques, relaxation response, breath-based techniques, and prayer.

Experiment C: Measuring the effects of meditation within the meditation community. Here we are interested in collecting data from members of Dr. Joe Dispenza’s meditation community on human well-being and function.

Particularly, we are interested in collecting the following data:

1. Questionnaire data (i.e. demographics, medical history, PROMIS measures)
2. Physiological readouts (i.e heart rate variability {HRV}, Electroencephalogram {EEG} and physiological readouts from wearables like FitBit, Garmin or Apple Watch)
3. Biological data (i.e blood, plasma, serum, and saliva)
4. FLIP Video Capture (record a 1.5-3 min video at the end of each day of the meditation retreat reflecting on their experiences)

We are hoping to contribute significantly to the understanding of the effects of meditation on human well-being and function and to establish a meditation whole-organism phenotype.

We propose the following aims:

Aim 1: Explore effects of meditation on Healer, Healee and their interactions.

Aim 2: Evaluate and/or correlate brain waves (EEG) and physiological readouts (HRV, wearable data) of meditating subjects with their experience in meditation techniques including novice (less than 6 months experience) and experienced meditators (more than 6 months experience).

Aim 3: Explore potential effects of meditation on molecular biology (e.g. biomarker and -omic changes) between novice and experienced meditators and controls.

Aim 4: Explore correlation between EEG state, HRV, wearable readouts, and molecular findings.

3 Potential Risks

3.1 Risk Management

This study is anticipated to have minimal risk to subject safety.

Questionnaire:
The data collection, including demographic data, will be done by approved study personnel using paper or electronic format. For paper collections, all information will be de-identified and kept under double-secured filing systems. For electronic collections, we will be using HIPAA compliant software (i.e. SurveyMonkey, CRIO). Data will be de-identified and stored electronically in a password protected database. Access to the database will be restricted to key personnel of this study. As with all electronically stored data, a breach of security is a potential risk.

HRV / Wearable Recordings:
The HRV recording equipment is similar to recording a standard 3-lead ECG. Skin reactions to adhesives in the electrode are a theoretical risk. All wearable equipment is commercially available and cleared.

EEG Recordings:
During recording of the EEG, slight headaches in very sensitive individuals may occur. If wearing the EEG equipment on the head cannot be tolerated, the sampling procedure can be stopped immediately. Furthermore, a skin reaction to the electrode adhesive is theoretically

possible.

Biologicals:

Drawing blood always poses a risk of infection and mild discomfort. These will be mitigated by having the blood draws performed by experienced professionals using standard techniques. Saliva collections utilize non-invasive techniques. Collections of this data pose no additional risk.

3.2 Potential Benefits

There will be no direct benefits to participants in this study from the study procedures. However, the data obtained will contribute to future research into the molecular effects of meditation practices.

3.3 Risk/Benefit Assessment

The risks of participation are minimal and are offset by the value of the information obtained.

4 Eligibility

Since we propose to look at different cohorts under this protocol, as outlined under Experiment A, B, C, we will follow the same format for Inclusion/Exclusion Criteria.

4.1 Experiment A (Measuring the effects of meditation on the Healer and Healee)

4.1.1 Inclusion Criteria

Potential subjects must meet all inclusion criteria:

1. Subjects must be a participant in a Dr. Joe Dispenza healing event to qualify as a Healer.
2. Subject must volunteer to receive a healing in a Dr. Joe Dispenza healing event to qualify as a Healee.
3. Subject or an assigned representative must be willing and able to read and provide Informed Consent in English language.
4. Subjects must be 18 years of age or older.

4.1.2 Exclusion Criteria

Potential subjects must not meet any exclusion criteria:

1. Unable or unwilling to provide Informed Consent.
2. Subjects with any medical condition that by discretion of the Principal Investigator would be disqualified.

4.2 Experiment B (Measuring the effects of meditation on the human organism)

4.2.1 Inclusion Criteria

Potential subjects must meet all inclusion criteria:

1. Subjects must be 18 years of age or older.
2. Subjects must be a participant in a Dr. Joe Dispenza meditation event.
3. Subject or an assigned representative must be willing and able to read and provide Informed Consent in English language.

Potential controls must meet all inclusion criteria:

1. Controls must be 18 years of age or older.
2. Controls must be willing and able to read and provide Informed Consent in English language.

4.2.2 Exclusion Criteria

Potential subjects must not meet any exclusion criteria:

1. Neurological diagnosis that could interfere/alter with EEG recordings such as epilepsy.
2. Subjects who had a heart transplant.
3. Subjects who have cardiac disease that could interfere with ECG recording such as but not limited to cardiac arrhythmia or disorder of cardiac conduction.
4. Subjects who have been diagnosed with a malignant disease that could interfere with blood parameters.
5. Subjects with any medical condition that by discretion of the Principal Investigator would be disqualified.

Potential controls must not meet any exclusion criteria:

1. Neurological Diagnosis that could interfere/alter with EEG recordings such as epilepsy.
2. Controls who had a heart transplant.
3. Controls who have cardiac disease that could interfere with ECG recording such as but not limited to cardiac arrhythmia or disorder of cardiac conduction.
4. Controls who have been diagnosed with a malignant disease that could interfere with blood parameters.
5. Controls with any medical condition that by discretion of the Principal Investigator would be disqualified.
6. Controls must not be a participant in a Dr. Joe Dispenza meditation event.

4.3 Experiment C (Measuring the effects of meditation within the meditation community)

4.3.1 Inclusion Criteria

Potential subjects must meet all inclusion criteria:

1. Subjects must be a member of Dr. Joe Dispenza meditation community.
2. Subject must be willing and able to read and provide Informed Consent in English language.
3. Subjects must be 18 years of age or older.

4.3.2 Exclusion Criteria

Potential subjects must not meet any exclusion criteria:

1. Unable or unwilling to provide Informed Consent.
2. Subjects with any condition that by discretion of the Principal Investigator would be disqualified.

4.4 Subject Withdrawal / Discontinuation

Subjects have the ability to withdraw from the study at any point. Data collected prior to the withdrawal of consent will be stored and potentially used in data analysis.

Subjects may also be discontinued from participating if the Investigator has reason to believe that they are not adhering to study requirements.

4.5 Subject Compensation

- Research subjects will not be compensated for participation in these studies.
- Research controls may receive a modest form of compensation for participation in these studies.

4.6 Alternatives to Participation

The alternative to participating in this research study is to not participate in the research study. Patients will receive no different treatment at the meditation event if they choose not to participate.

5 Subject Recruitment

Subject outreach will utilize Dr. Joe Dispenza's online presence (website, social media, etc.). Subjects will be recruited through prescreening emails to members of Dr. Joe Dispenza meditation community and/or registered event participants. These emails will briefly explain the research study and offer the subject the option to follow a link for pre-screening.

For pre-screening, potential subjects will visit a pre-screening questionnaire via a HIPAA compliant software (ie. SurveyMonkey) and be asked general pre-screening questions based on

the inclusion and exclusion criteria. The questionnaire will also collect basic data such as demographics and past medical history.

6 Study Design and Procedures

6.1 Study Procedures

6.1.1 Experiment A (Measuring the effects of meditation on the Healer and Healee)

Our exploratory study will focus on interactions among Healers and Healees. We are also interested in collecting data among Healers themselves.

6.1.2 Experiment B (Measuring the effects of meditation on the human organism)

Under this paradigm, we will collect data on the effects of meditation on the human organism. We therefore aim to collect data before, during, and after a meditation event that take place nationally and internationally on a regular basis. The environment of an event poses the rare opportunity of minimized confounding factors (i.e. diet, housing, sleep).

6.1.3 Experiment C (Measuring the effects of meditation within the meditation community)

Under this exploratory paradigm, we are interested in collecting data within members of Dr. Joe Dispenza's meditation community. Given the large size and diversity of this global community, practicing the same meditative practice, we will be able to generate a set of interesting pilot data.

6.2 Timeline

6.2.1 Experiment A (Measuring the effects of meditation on the Healer and Healee)

For qualifying subjects, consenting as well as data collection will be performed electronically prior to the event. For both Healers as well as Healees, we will collect a set of baseline measures, including demographics, medical history, and questionnaire data.

The baseline will be collected in a timeframe after consenting but before the first healing event. After the healing event, we will collect outcome date at different timepoints (i.e. immediately after healing event, 1, 3, 6, 9, 12 months out). These data will be collected electronically. If baseline data collection also included physiological and/or biological data, we may collect these follow-up data at the above time points as well.

6.2.2 Experiment B (Measuring the effects of meditation on the human organism)

The timeline, as outlined in Figure 1, is divided into pre-event, immediate pre-event, during event, immediate post-event, and post-event.

Pre-event: For qualifying subjects and controls, consenting as well as baseline data collection will be performed electronically prior to the event.

Immediately pre-event: The data collection is organized into 4 stations.

Station 1:

- Confirming of consenting and baseline data (for those who did not use online option, consenting and baseline data will be done.)
- Vitals

Station 2:

- Physiological data (i.e. HRV, wearables)

Station 3:

- Physiological data (i.e. EEG)

Station 4:

- Biological data (i.e. saliva, blood)

During the event: Data collection during the event may include additional collection of questionnaire data, physiological data, and biological data.

Immediately post-event: The data collection is organized into 4 stations.

Station 1:

- Vitals
- Questionnaire data

Station 2:

- Physiological data (i.e. HRV, wearables)

Station 3:

- Physiological data (i.e. EEG)

Station 4:

- Biological data (i.e. saliva, blood)

Post-event: follow-up data may be collected electronically. Time points may include 1,3, 6, 9, 12 months post-event. If baseline data collection also included physiological and/or biological data, we may collect these follow-up data at the above time points as well.

Pre-event	• Screening Questionnaires • Consent • Baseline Questionnaire data	Online
Immediate Pre-event	• Consent and baseline questionnaire data (for those who did not utilize online) • Vitals • Physiological readouts (i.e. HRV, EEG, wearable data) • Biological data (i.e. saliva, blood)	In-Person
During event	• Questionnaire data • Physiological readouts (i.e. HRV, EEG, wearable data) • Biological data (i.e. saliva, blood)	In-Person
Immediately Post-event	• Questionnaire data • Vitals • Physiological readouts (i.e. HRV, EEG, wearable data) • Biological data (i.e. saliva, blood)	In-Person
Post-event	• Follow-up questionnaire data • Physiological readouts (i.e. wearables) • Biological data (i.e. saliva)	Online

Figure 1: Timeline

6.2.3 Experiment C (Measuring the effects of meditation within the meditation community)

In this exploratory paradigm, we will include studies collecting data among Dr. Joe Dispenza's meditation community. We will focus on gathering pilot data (i.e. questionnaires and wearable data) of long term effects of meditation on human well-being and function. The duration may span from several weeks to several months.

6.3 Experimental Protocol

6.3.1 Experiment A (Measuring the effects of meditation on the Healer and Healee)

Subject selection:

Healer: Members of Dr. Joe Dispenza's meditation community who meet inclusion/exclusion criteria qualify to participate. Depending on the healing event, we anticipate recruiting between 10 to 100 Healers.

For Healers, consenting as well as baseline data collection will be performed electronically prior to the event. The consent form will be provided to the selected Healers for review. The PI or designated research staff member will go over the consent form in person or via HIPAA

compliant web meeting platform (ie. Zoom). During this time, Healers will have ample time to have all questions and concerns addressed. We may also collect baseline measures including physiological readouts (i.e. wearables, HRVs) and/or biological data (i.e. saliva). During the healing event, we may collect physiological readouts (i.e. EEG, HRV), biological data (i.e. saliva) and questionnaire data. After the healing event, follow-up data will be collected electronically. Time points may include immediately after, 1,3, 6, 9 12 months post event. If baseline data collection also included physiological and/or biological data, we will collect these follow-up data at the above time points as well.

Healee: A list of Volunteers who are interested in participating in a healing event will be provided by Dr. Joe Dispenza's staff. From that list, participants who meet the inclusion/exclusion criteria will be recruited: we anticipate recruiting up to 100 to 150 participants.

For Healees, consenting as well as baseline data collection will be performed electronically prior to the event. The consent form will be provided to the selected Healees for review. The PI or designated research staff member will go over the consent form via HIPAA compliant web meeting platform (ie. Zoom). During this time, Healees will have ample time to have all questions and concerns addressed. We may also collect baseline measures including physiological readouts (i.e. wearables, HRVs) and/or biological data (i.e. saliva). Immediately prior to the healing event, Dr. Joe Dispenza guides the Healees through a meditation to prepare them for the healing event. During the healing event, Healees will listen to music. After the healing event, follow-up data will be collected electronically. Time points might include immediate after, 1,3, 6, 9 12 months post event. If baseline data collection also included physiological and/or biological data, we will collect these follow-up data at the above time points as well.

6.3.2 Experiment B (Measuring the effects of meditation on the human organism)

Subject selection: Pre-event, registered participants Dr. Joe Dispenza's event will be contacted (i.e. email) and made aware of the research opportunity. Registered participants who are interested will be able to follow a link to a pre-screening questionnaire. Members of Dr. Joe Dispenza's meditation community who pass pre-screening qualify to participate. Depending on the event, we anticipate recruiting between 10 to 100 subjects.

Control Selection: In addition, we will identify controls by asking participants if they are traveling with family/friends who will be at the event but will not participate in the event; these may be volunteers of the event, and/or family/friends of meditators participating in the event. Controls will be age and sex matched to match subjects. We anticipate recruiting between 5 and 20 control subjects. The consenting as well as some baseline data (i.e. Medical history, demographics) collection will be performed electronically prior to the event.

Pre-event: Consenting: The consent form will be provided to the selected subjects for review prior to the event. The PI or designated research staff member will go over the consent form in person or via HIPAA compliant web meeting platform (i.e. Zoom). During this time, subjects will have ample time to have all questions and concerns addressed. We may also collect baseline

measures including physiological readouts (i.e. wearables, HRVs) and/or biological data (i.e. saliva).

Immediate Pre-event: Confirming of consent and baseline data (for those who did not use online option consenting and baseline data will be done). Vitals (i.e. height, weight, heartrate, temperature, blood pressure), physiological data (i.e. HRV, EEG, wearables), and biological data (i.e. saliva, blood) will be collected from all consented subjects and controls.

During event: Data collection during the event may include additional collection of questionnaire data, physiological data and biological data.

Immediate post-event: Vitals (i.e. height, weight, heartrate, temperature, blood pressure), physiological data (i.e. HRV, EEG, wearables), and biological data (i.e. saliva, blood) will be collected from all consented subjects and controls.

Post event: Follow-up data may be collected electronically. Time points may include 1, 3, 6, 9, 12 months post-event. If baseline data collection also included physiological and/or biological data, we may collect these follow up data at the time points above as well.

6.3.3 **Experiment C** (Measuring the effects of meditation within the meditation community)

We will be collecting data among Dr. Joe Dispenza's meditation community. We will focus on gathering pilot data (ie. Questionnaires and wearable data) on long term effects of meditation on well-being and function. Duration may span from several weeks to several months. Data collection time points may include baseline, 1, 3, 6, 9, 12 months post event.

6.4 Electroencephalography (EEG) and Quantitative electroencephalography (QEEG)

Electroencephalography (EEG, from Greek encephalon brain, writing graphs) is a method of medical diagnostics and neurological research for measuring the summed electrical activity of the brain by recording the voltage fluctuations at the head surface. The electroencephalogram is the graphical representation of these variations in the typical waveform. The EEG is a standard method used in neurology.

Quantitative electroencephalography is a further development that allows further conclusions regarding disorders by means of IT-technical procedures of the EEG data. In essence, here the amplitudes of frequencies are measured by means of the fast Fourier transform and set in relation to each other.

The QEEG data is intended to describe the change in brain activity and arousal for each participant.

For the analysis and data collection, three different arousal states will be recorded before and after the event. State 1 records the QEEG with eyes open without meditation/audiobook, state 2 records QEEG with eyes closed but without meditation/audiobook, and state 3 records QEEG with eyes closed listening to a short pre-recorded meditation by Dr. Joe Dispenza or a section of

an audiobook. All participants are instructed to sit as still and calm as possible to avoid artifacts. Participants will be filmed to allow for potential artifact tracking.

We will be utilizing two different EEG device manufacturers (details follow under 6.4.2 and 6.4.3)

6.4.1 EEG and QEEG – Procedure Details

Immediate Pre-event and Immediate Post-event procedure: We will measure brainwave data and acquire six recordings per participant.

Subjects: The subjects will be listening to a pre-recorded meditation from Dr. Joe Dispenza. Measurements will take place with eyes open, and eyes closed (one set as baseline before pre-recorded meditation, one set during pre-recorded meditation, and possibly one set after pre-recorded meditation).

Controls: The controls will be listening to a section of an audio book. Measurements will take place with eyes open, and eyes closed (one set as baseline before pre-recorded audio book, one set during pre-recorded audio book, and possibly one set after pre-recorded audio book).

During event: EEG recording procedure details: Prior to a meditation happening during the event, some subject's EEG may be measured. Following above outlined details, subjects will be fitted with the EEG cap. The subject will meditate, and the EEG will be recorded for the duration of the meditation.

6.4.2 EEG and QEEG – NeuroField Q21 device details

To obtain the EEG, subjects will be fitted with a 19+1 channel Q21 DC coupled EEG amplifier. (Channel: 19+1 (Non-Multiplexed). References: A1/A2 (ears). Ground: Channel 20. Input Range: +/- 100mV EEG, +/-5 VDC. Input noise: 0.25 V P-P, 0.1-10.0Hz. Gain: 50. Common Mode Rejection Ratio: >120dB from 0-60Hz. Single Pole Low Pass Filter: ~80Hz. Resolution: 24 bits. Sample Rate/Data Rate: +/-2.25 V. EEG Resolution: 0.02 Volts/LSB. EEG Channels Bandwidth: Software Controllable. DC Offset Measurement: Average DC Level. Sensor Contact Quality: Monitoring Via Offset Voltage. PC Connection: USB – CAN Optically Isolated. Software Compatibility: NeuroField64 & Neuroguide)

The Q21 is fast, quiet and accurate. When used with NeuroField EEG the amplifier can measure data from 0-128 Hz in real time. This amplifier acquires EEG in 21 analog to digital pipes (each channel has its own dedicated analog to digital conversion, thus avoiding the slew found in amplifiers using multi-duplex switching.) We may also collect event related potentials using NeuroField ERP which is a visual and auditory GO NOGO matching task, used to determine reaction time and processing speed, before and after the event.

6.4.3 EEG and QEEG – BrainMaster device details

To obtain the EEG, subjects will be fitted with a 19 channel cap (COMBY EEG CAP, Pamel, hr) according to the International 10/20 System with linked ear montage (Fp1, Fp2, F7, F3, Fz,

F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2). COMBY EEG Caps are manufactured with gold plated electrodes and electrodes made from sintered silver chloride (Ag/AgCl), which are currently most suited for brain bio potential detection. They have low impedance and auto-potential. Their quality is reflected in excellent selectivity and stability. The EEG recordings will be recorded using BrainAvatar 4.6.4 software (BrainMaster Technologies, Inc, Bedford, OH). BrainAvatar incorporates technology including BrainMaster's Discovery hardware devices, extensive signal processing and graphics software, and an exclusive high-speed sLORETA (standardized low-resolution electromagnetic brain tomography) voxel processor and 3D image projector. BrainAvatar is a full-featured clinically useful system, registered with the US FDA (https://www.brainmaster.com/kb-entry/?id=559) for professional clinical use, clinical EEG device, for neurological use and with a CE mark in Europe as a medically qualified biofeedback, monitoring, and imaging system.

The EEG signals are then imported into the NeuroGuide v2.9.1 software (Applied Neuroscience, Inc., Largo, FL) for computation and analysis. Data will be visually inspected and artifacts (i.e., activity collected from the EEG not produced by the brain) removed. The EEG will be processed with linked ear montage and compared with the NeuroGuide normative database, and Z-scores will be obtained. For analysis, particular attention will be given to alpha and theta amplitudes. Specifically, increased frontal theta and low alpha, increased gamma and increased alpha and theta coherence are scientific correlates for meditative states and of interest.

NeuroGuide software (Applied Neuroscience) is an FDA Registered Database of Normative qEEG Values (510(K) Number K041263). It is a computer program that can compare the qEEG values of a given patient to the normative database and comparative produce brain maps.

Data will be averaged for the analysis across the recording epochs for each electrode (LE montage - Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2) to get the results of the absolute power (percentage of total power), power ratios (Ratio of the difference Frequency bands) and coherence (functional connectivity in human cortex, measuring between each electrodes).

6.5 Heart Rate Variability (HRV) and Wearables

Heart rate variability (HRV) is a measure of the naturally occurring beat-to-beat changes in heart rate/heart rhythms. It serves as a critical method for gauging human health and resiliency. HRV is a powerful, objective and noninvasive tool to explore the dynamic interactions between physiological, mental, emotional and behavioral processes. Researchers use HRV, as measured by an electrocardiogram (ECG) or pulse wave recording, to assess the state of the autonomic nervous system (ANS), which controls heart and breath rates, gastrointestinal tract movement and gland secretion among other internal bodily functions. Analysis of heart rhythm patterns also can provide an objective measurement of physiological coherence, a term that describes a state of high performance characterized by harmony in the body's oscillatory systems.

6.5.1 Heart Rate Variability (HRV) Procedure Details

Applicable to experiments A, B, & C:

Subjects participating in HRV data collection will receive in-person or online training by the PI or designated research staff on using the HRV device and will be provided with written instructions (see submitted appendix 6). In addition, for each day where HRV is collected, the subjects will provide brief information (i.e. time they went to bed, activity level, device pop-ups) (see submitted appendix 7)

Experiment B:

Immediate Pre-event and Immediate Post-event procedure:

This 3-step HRV assessment protocol will be performed on subjects:

1. Resting HRV assessment (5 minutes): Reflective of baseline HRV and cardiac coherence status

   Participant instructions: Please sit quietly for the next 5-minutes without talking, try to remain as still as possible without sacrificing comfort. Do not engage in intense mental or emotional activity. To help avoid falling asleep keep your eyes open. Do not meditate or use other similar practices. Just sit quietly as if you were wanting at the bus stop for the bus. (No gum chewing, or reading magazines etc.)

2. Stress Prep (3 minutes): Reflective of ability to self-regulate and induce heart coherence on demand.

   Participant instructions: During the next 3-minutes please do the following, as if you were preparing yourself mentally and emotionally for an important upcoming event or activity. Focus your attention in the center of your chest. Try and activate and experience a positive feeling such as care or appreciation for someone or some special place.

3. Deep breathing assessment (1 minute): Reflective of vagal tone capacity

   Participant is guided with a visual to breathe as deeply as they comfortably can at the pace shown on the screen (5 sec in / 5 sec out i.e. resonant frequency breathing).

During event: When collecting HRV data, the participants will be instructed to apply the HRV monitor at the beginning of the day. Propper fitting will be confirmed by research study staff. For multiple day data collections, the participants will receive new electrodes as needed.

6.5.2 Heart Rate Variabiltiy (HRV) Firstbeat Bodyguard 2 (BG2) device details

Materials and Supplies: Firstbeat Bodyguard 2 (BG2) wearable heart rate monitor. The BG2 provides accurate data for HRV analysis with a sampling rate of 1000Hz. Once HRV data is collected, it will be uploaded to a PC using the FirstBeat Technologies utility program called Firstbeat Uploader. The ASCII-text file is emailed to HeartMath and analysis of the data is

performed using DADiSP 2002 software and Microsoft Excel. Visual inspection for the quality of the recording is made prior to generating the Autonomic Assessment Report which gives a detailed look at several HRV indices including SDNN, RMS-SD, HF, LF, VLF, as well as circadian sleep cycles. (see attached appendix 8)

6.5.3 Heart Rate Variability (HRV) EmWave software details

emWave® Pro Plus provides an easy way to inexpensively add standardized HRV measures to your research or practice with two heart rate variability assessments that can be added to the emWave Pro software. These assessments can be used in a wide range of applications such as quantifying HRV levels in relationship to autonomic capacity, determining changes in HRV or coherence levels in research studies or interventions, and documenting physiological baseline shifts over time.

6.5.4 Wearables

Commercially available wearables (i.e. FitBit, Garmin, Apple Watch) are able to record physiological data including HRV, skin conductance, temperature, etc. This non-invasive method of capturing physiological data may be added to the proposed experiments.

For collection and analysis of wearable data, we also will utilize technology available through RAE Health. The first component of the RAE system is a wrist worn wearable sensor to continuously detect physiologic data, which are streamed continuously via Bluetooth connection to the RAE app. The current version of the app pairs with the Garmin Viosmart 4. The Vivosmart 4 is equipped with a barometric altimeter, tri-axial accelerometer, heart rate monitor, and pulse oxygenation sensor. The Viviosmart 4 has a touch screen interface, has up to 7 days of battery life, and functions as a standard fitness tracker.

The RAE mobile application is available for Android and iOS. Features of RAE app include geolocation based monitoring tools, and general health monitoring tools.

6.6 Laboratory work

Unbiased analysis of biological specimen: Serum, plasma, and saliva samples will be used for various assays that will examine changes in the biophysical properties of contents as well as the potential to assess the impact of the biospecimen on various assays of cell stress and metabolism. The overall approach to analyze the collected samples will be exploratory. The following assays are part of this approach.

NanoSite characterization: We will use small amounts of plasma/serum (<100uL) to assess extracellular particle size and quantity.

Electron Paramagnetic Resonance (EPR): We will use EPR to perform two assays. One will use specific lipid spin probes to assess changes in the membrane/lipid fluidity of particles floating in the plasma/serum. The second assay will measure ascorbyl radical as a global measurement of oxidative stress in a system. This value can be used to determine if there are whole body level changes in stress pre- and post- intervention.

Seahorse Metabolic Flux Assay: We will use plasma/serum to assess the impact of plasma/serum components on cellular effects on mitochondrial metabolism. We can assess these effects in a variety of different cell types that represent every organ in the human body. This can provide a non-invasive picture of what may be happening in cells pre and post treatment.

Metabolomic analysis: We will assess standard and lipid metabolite changes in plasma/serum with a core facility at UC Davis. This will give an unbiased assessment of factors that may be changing pre and post treatment. The analysis can then correlate to other measurements made.

Cell based assays: Measurements of cell death, apoptosis, growth, and changes in specific proteins will be performed.

Additionally, we intent to analyze concentrations of hormones (i.e. Cortisol, Norepinephrine, Cytokine profile) and biomarkers of the human stress response in the biospecimen.

6.7 Biospecimen, Collection, Handling and Storage

6.7.1 Saliva collection and Handling

The participants will be instructed not to eat or drink 30-45 minutes prior to specimen collection. Immediately prior to specimen collection, the participant is instructed to scrape inner cheek for 10-20 motions, and then submit saliva into collection tubes. We will collect up to 4 mL of specimen each collection. We may mix saliva sample with stabilizer to preserve specimen. The saliva collection tubes will be handled by a trained research staff member using universal precautions.

6.7.2 Blood Collection and Handling

Blood will be collected by certified personnel utilizing universal precautions for venous puncture. Blood will be collected utilizing purple top EDTA tubes (10cc), red top serum tubes (6cc), or blood RNA tubes (2.5cc). Total amount of blood per collection will not exceed 30cc. The blood collection tubes will be handled by a trained research staff member using universal precautions. Purple top EDTA tubes will be placed on wet ice immediately after collection and then centrifuged at 3000RPM for 10 minutes. After centrifugation, the plasma from Purple top EDTA tubes will be aliquoted into 1mL Eppendorf tubes. Red top serum tubes will rest at room temperature for 30 minutes, and then centrifuged at 3000RPM for 10 minutes. After centrifugation, the serum from red top tubes will be aliquoted into 1mL Eppendorf tubes. RNA tubes will remain at room temperature.

6.7.3 Biospecimen Storage

Biospecimen (ie. Saliva, blood) will be processed and handled with universal precautions and stored at -80 degrees Celsius in a locked freezer. The freezer will be equipped with emergency power and access will be restricted. All samples will be blinded, coded, and the code will be stored in a separate location.

6.8 FLIP Video/ Audio Capture

Subjects will be provided with written instruction in which they will be asked to 1) download the

FLIP app onto their iPhone or Android device, 2) create a unique account in the app, and 3) record a 1.5-3 min video at the end of each day of the meditation retreat reflecting on their particular experiences. Following video capture, the videos will be transferred to an online database and identified using study subject numbers. Each subject will have access to only their own videos. Access to study subject videos will be restricted to two senior collaborators affiliated with UC San Diego. After video and audio content analyses are complete, the data will be erased from the database by the UC San Diego collaborators.
Participants will be asked to record a short 1.5-3 minute video to describe their daily meditation experiences. Participants will be asked to download the FLIP app onto their cellular device to complete this recording. Data from these videos will allow us to assess personal transformation via verbal and body language at the end of each day and at the end of the retreat.

7 Data Collection and Management Procedures

Data collection will be performed through a HIPAA compliant software platform (i.e Clinical Research IO, a platform designed for Electronic Data Capture and eCRF development, or DropBox)

8 Data Analysis

Demographic characteristics will be analyzed by descriptive statistics. Survey data are collected on an ordinal 4-point or 5-point Likert scale and treated as continuous variables during analysis. The data is then analyzed to describe the distribution. If the data is normally distributed, we will follow up with utilizing parametric statistics. However, if the data does not follow a classic normal distribution, we will utilize other methods such as reporting the median instead of the mean for central tendency and further analyze our results with frequencies, contingency tables, chi-square-tests, Spearman rho assessment, or the Mann-Whitney U test. All other questionnaires will be analyzed according to published protocols.

9 Quality Control and Quality Assurance

To allow for high quality data collection, all study material will be pre-labelled whenever possible. During data collection days, a quality assurance person not directly involved in data collection will oversee the data collection process. The experimental protocol may include a full day for data collection set-up prior to data collection during the event This will assure that all necessary equipment is available and functioning. Once data is collected, standard data cleaning procedure will be applied, and data will be randomly audited.

10 Statistical Analysis

Data analysis will be organized by Vitamed Research LLC. Vitamed Research LLC. staff has 10+ years of experience with statistical and data analysis. We will perform statistical analysis with appropriate parametric and non-parametric methods as described under Section 8.

11 Regulatory Requirements

11.1 Informed Consent

Informed consent will be obtained either electronically or in person following 21 CFR Part 11. Study procedures will be explained, and subjects will have the opportunity to have their questions and concerns addressed by PI or designated research study personnel.

11.2 Subject Confidentiality

Subjects will be de-identified and will be provided a subject number. All potentially identifiable information will be stored in a separate database and linked by a key.

11.3 Unanticipated Problems / SAEs

Any unanticipated problems will be reviewed by the investigator. If the investigator determines that the problem or SAE is related to the study protocol, the event will be reported to the IRB in adherence with standard protocol.

12 Funding

This project is funded through the Gift-to-Give foundation.

13 Conflict of Interest

There is no financial conflict of interest between PI Dr. Tobias Moeller-Bertram and Gift-To-Give foundation. We plan on utilizing some event staff (volunteers of Dr. Joe Dispenza) to assist in set-up, break-down, and flow of events.

In addition, if any potential Conflict of Interest arises, it will be promptly reported to the IRB for review. If any COI is to arise without approval, the study will be reassigned to a new Principal Investigator.

14 Qualifications and Training

The investigator is triple board-certified MD and holds a Masters in Advances Science (MAS) Clinical Research. All Vitamed Research LLC. staff hold current training certificates in Good Clinical Practices.

15 Appendices

1. Informed Consent
2. Demographics Questionnaire
3. Medical History Template
4. PROMIS-29 Questionnaire
5. MEQ 30 Questionnaire
6. FirstBeat Fitting Cheatsheet
7. Daily HRV Log Template
8. FirstBeat – Technologies – BodyGuard 2
9. Coherence Healing Questionnaire
10. GAD-7
11. MSQ Questionnaire
12. PEG 3
13. PSS
14. Perma Profiler
15. PHQ-9
16. PANAS
17. Primal World Survey
18. SF-36
19. PCL-5
20. BPI

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