METHODS

5.0 METHODS

The present study work analyzed the working memory performance and associated prefrontal cortex oxygenation, resting state heart rate variability and psychological conditions – rumination and perseverative thinking, following 12 weeks of yoga practice in T2DM patients. The n-back task was administered to assess the performance of working memory. The prefrontal oxygenation was assessed using the near infrared spectroscopy (NIRS) technique. The heart rate variability was analyzed through the electrocardiogram (ECG). The Rumination Response Scale (RRS) and Perseverative Thinking Questionnaire (PTQ) were used to assess rumination and perseverative thinking respectively. Also, correlation analysis was done between (i) working memory performance and prefrontal oxygenation, (ii) heart rate variability and prefrontal oxygenation and (iii) working memory performance and psychological conditions. The methodology of the study has been described in the following sections:

5.1 PARTICIPANTS

Participants, both male and female, with ages ranging from 35 to 65 years, diagnosed with type 2 diabetes mellitus based on established criteria including medical history, medication use, glucose levels (Mayfield, 1998) and undergoing conventional treatment were targeted.

5.1.1 SAMPLE SIZE

The sample was calculated based on the previous study (Gothe et al., 2014) on the effect of yoga on executive function. The partial $\eta 2$ value of 0.08, and an alpha level of 0.05, with 2 groups and 3 measurements were used to estimate the sample size using the G*power program. The optimal total sample size estimated was 44. With the assumption of dropouts (~20%) during the study, the total sample size planned was 54.

5.1.2 SELECTION AND SOURCE OF PARTICIPANTS

Participants were recruited through advertisements sent via social media, and posted in diabetic clinics, community centers, and residential apartments. All the participants were from Bangalore city in India. The participation for this study was entirely voluntary and participants were free to discontinue the study. Figure 2. depicts the CONSORT flow diagram of this study.

5.1.3 INCLUSION CRITERIA

Participants meeting all the below criteria were included in the study.

- Diagnosed with T2DM for more than 2 years
- Both male and female, aged between 35 and 65
- Not practicing yoga or any type of exercise in the last 6 months

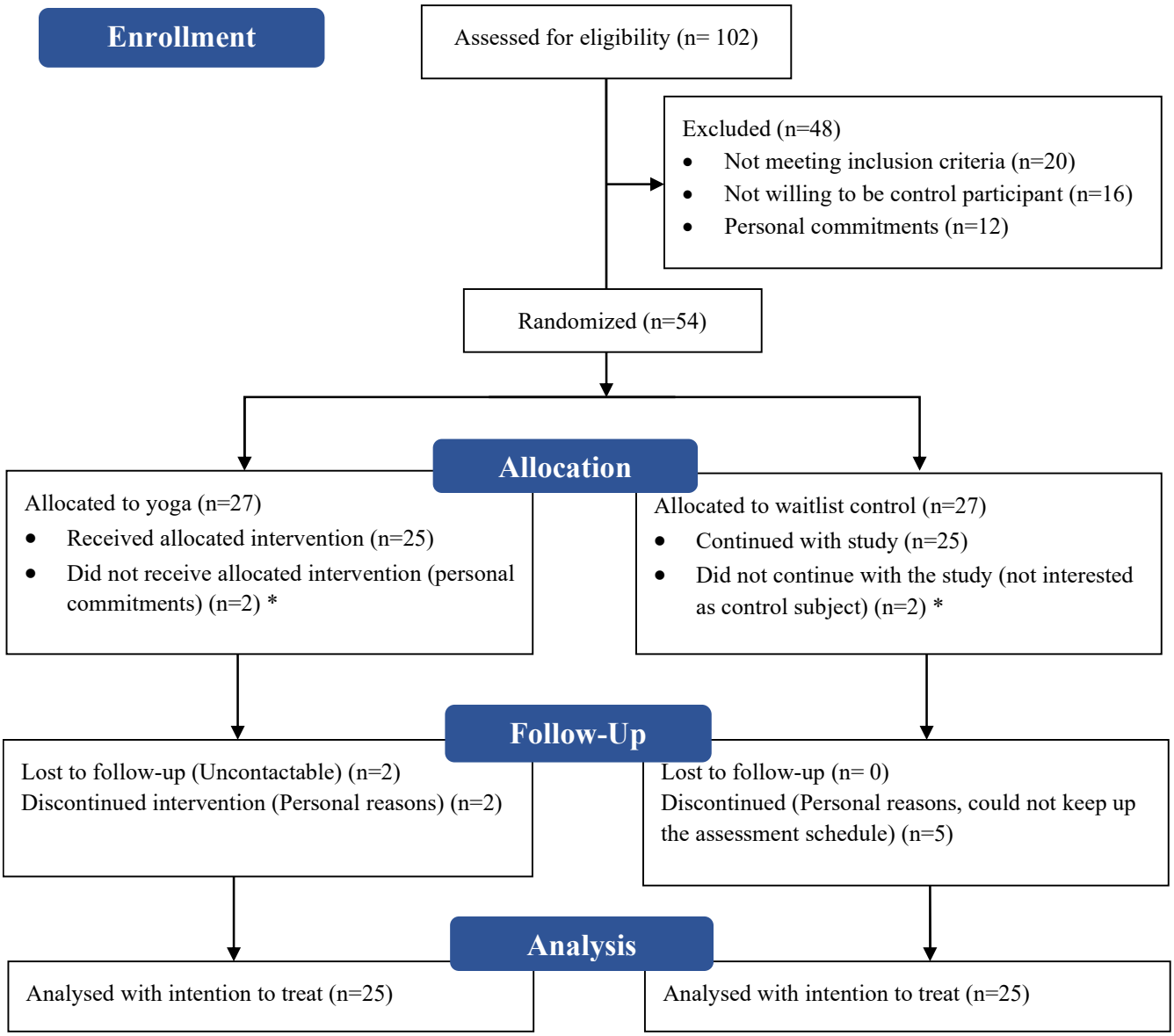
5.1.4 EXCLUSION CRITERIA

Participants meeting any of the below criteria were excluded from the study

- Diabetes related complications (ex: neuropathy, retinopathy, nephropathy)
- Uncontrolled hypertension, cardio vascular disorders and any other major health complications
- Cognitively impaired (Screening for cognitive functions was done using the Montreal cognitive assessment (MoCA) tool and the score was found to be below 26)
- Psychiatric or neurological disorders
- Obesity (BMI > 30)
- Tobacco, smoking, alcohol or any substance abuse
- Taking any other alternative and complementary medicine treatment

5.1.5 ETHICAL CONSIDERATION

The study was approved, prior to the study, by the institutional ethical committee of SVYASA University (No. RES/IEC-SVYASA-03/020/2016) (Appendix-1). Also, the study was registered with the Clinical Trials Registry-India (CTRI/2017/12/010936) (Appendix-2). The study protocol was explained to the participants and their signed consent was obtained (Appendix-3).



*No assessment done

Figure 2. CONSORT flow diagram

5.2 DESIGN OF THE STUDY

This study was a randomized controlled trial (RCT). The participants were randomized into two groups (1:1), the yoga and waitlist control groups based on the computer-generated random numbers using a random number generator software tool (www.randomizer.org). The randomization assignments were created by an investigator not involved in the assessment or delivery of the intervention. Figure 3. shows the schematic representation of the study design.

5.3 VARIABLES STUDIED

5.3.1 PRIMARY OUTCOME MEASURES

5.3.1.1 PFC Oxygenation

The cerebral oxygenation in the PFC region was assessed using fNIRS by measuring the levels of oxyhemoglobin (OxyHb), deoxyhemoglobin (DeoxyHb), and total hemoglobin (TotalHb) levels during resting as well as while executing the working memory task. The PFC region was further analyzed based on different regions within the PFC, namely dorsolateral PFC (dlPFC), ventrolateral PFC (vlPFC), ventromedial PFC (vmPFC), orbitofrontal cortex (OFC) and dorsomedial PFC (dmPFC). In this study, only OxyHb data was analyzed since it has a higher signal-to-noise ratio compared to DeoxyHb.

5.3.1.1.1 Specification of the fNIRS system

The PFC oxygenation was measured using NIRScout, a continuous-wave NIRS system, and NIRStar acquisition software (NIRx Medical Technologies, Brooklyn, NY). It determines changes in oxygenated and deoxygenated hemoglobin levels in the cerebral cortex using their different absorption spectra of near-infrared light. PFC brain activity was recorded using 8 NIRS source-detector pairs, each placed approximately 3.0 cm from one another within an elastic cap (Easycap) (Plate 1). In total, 24 channels were created from the source-detector arrangement, based on the 10–20 system (see Figure 4). A sampling rate of 7.825 Hz was used to record the data. Infrared lights operate at two wavelengths, 760 nm and 850 nm to detect oxygenated and deoxygenated hemoglobin levels. Automatic adjustments were made by the system to provide optimal light intensity.

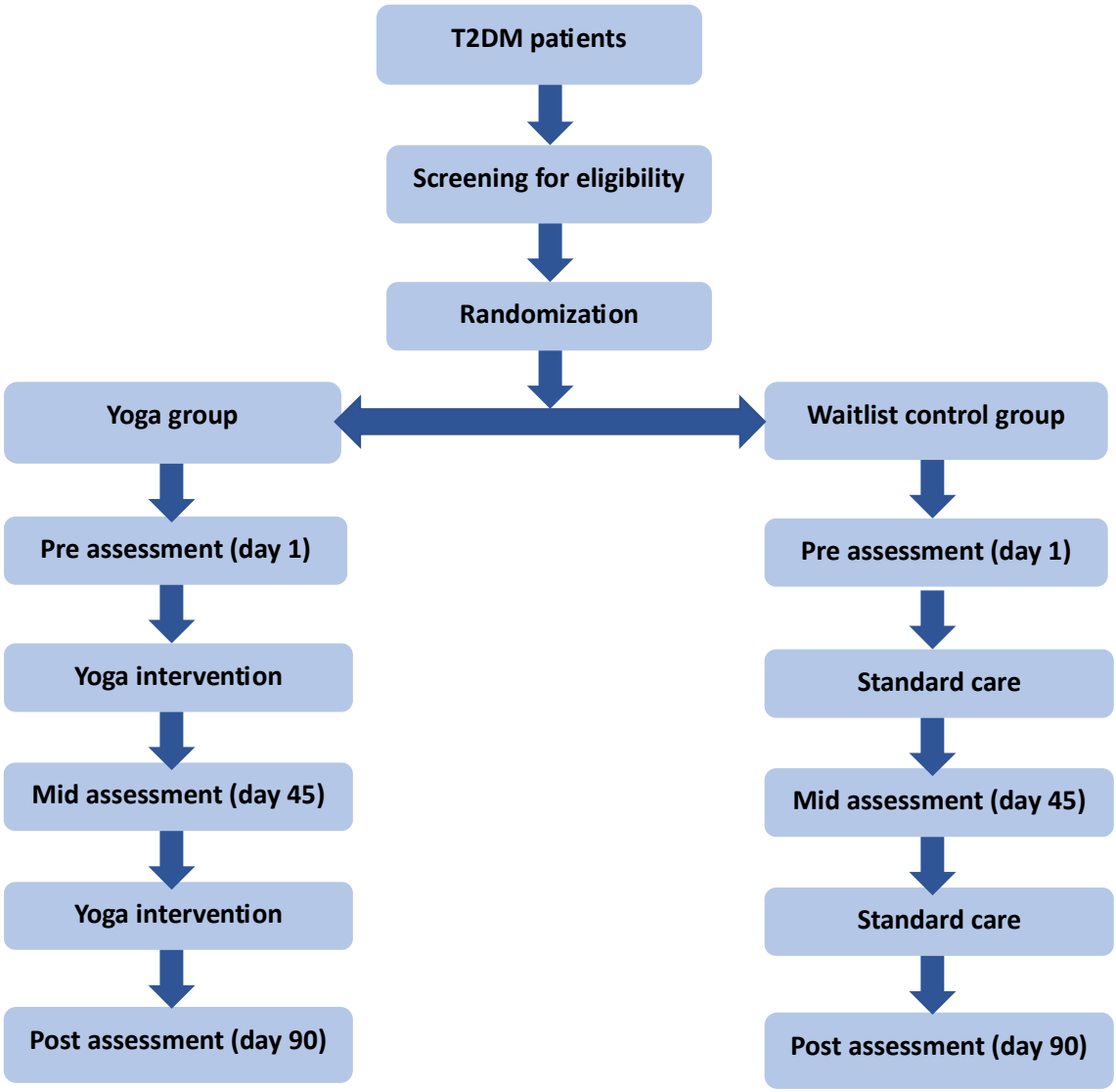
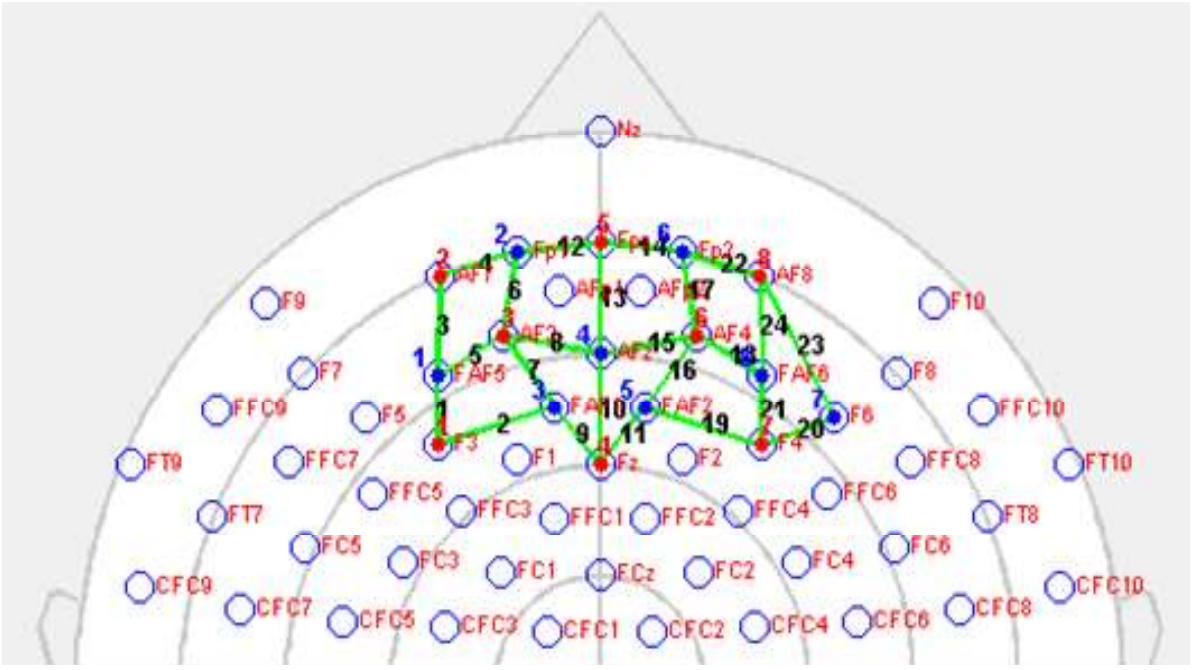


Figure 3. Schematic diagram of study design. T2DM participants were randomized into two groups (1:1), the yoga and waitlist control groups.



Source Detector Channel

a

Channel no.	Anatomical area
3,4, 22,24	dlPFC/vlPFC
4,6,12,14,17,22	vmPFC & OFC
7,8,10,13,15,16	dmPFC (BA9)
1,2,19,20,21	dlPFC
9,11	dmPFC (BA6/8)

b

Figure 4. fNIRS measurement. a) fNIRS probe/optode (source and detectors) positions on the participant's headgear according to the 10-20 EEG system. b) Mapping of channels (source-detector pairs) to the region of interest (ROI)

a **b** **c**

Plate 1. a) fNIRS cap - housing for the optodes b) fNIRS amplifier and c) probe holder

5.3.1.2 Working memory performance: N-Back Task

Working memory was assessed using the n-back task (Kirchner, 1958). This task involves participants seeing a series of stimuli (such as letters, numbers, and shapes) and responding to whether the current stimulus is the same as those presented 'n' trials earlier. For example, in a 2-back task, participants would be asked to respond differently depending on whether the current stimulus matches a stimulus presented two trials ago or not. N-back tasks have been used in many studies to investigate working memory and cognitive control processes (Owen et al., 2005). For the behavioural outcome of the n-back task, for each participant, we calculated the percentage of correct responses as accuracy and the mean response time for all correct responses as reaction time. Figure 5 a) depicts the n-back task paradigm used in this study and Figure 5 b) depicts the structure of n-back task stimuli. In our experiment, the n-back paradigm consisted of 0-,1- and 2-back tasks presented sequentially with 60 seconds of rest time in between. Each n-back task consisted of 3 blocks separated by a 20-second rest period. Each block consisted of 20 stimuli with 20% target and 80% non-target stimuli. The stimuli used in the tasks were English alphabets in uppercase, presented on a 21-inch LCD placed at a distance of 60 cm. The response to the task was acquired through a response pad, which had predefined keys for 'yes' and 'no' responses, connected to a serial port of the computer used for administering the n-back paradigm.

The n-back task paradigm was designed and administered through SuperLab 6 software (Cedrus Corporation, San Pedro, CA, USA) while fNIRS recording was done. SuperLab 6 system logs at the runtime the task related information. The inter-stimulus interval was 1500 milliseconds and each stimulus was presented on the screen for 500 milliseconds. Instructions for 10 seconds were displayed before the task, informing the participant which n-back task was about to start. Participants were instructed to respond appropriately to each stimulus, depending on whether the stimulus is a target stimulus or not, by pressing either the "yes" or "no" keys respectively. The target in the 0-back task was the letter "X," whereas, in the 1-back and 2-back tasks, the target was any alphabet that was the same as the one that was shown immediately before it or two stimuli back, respectively.

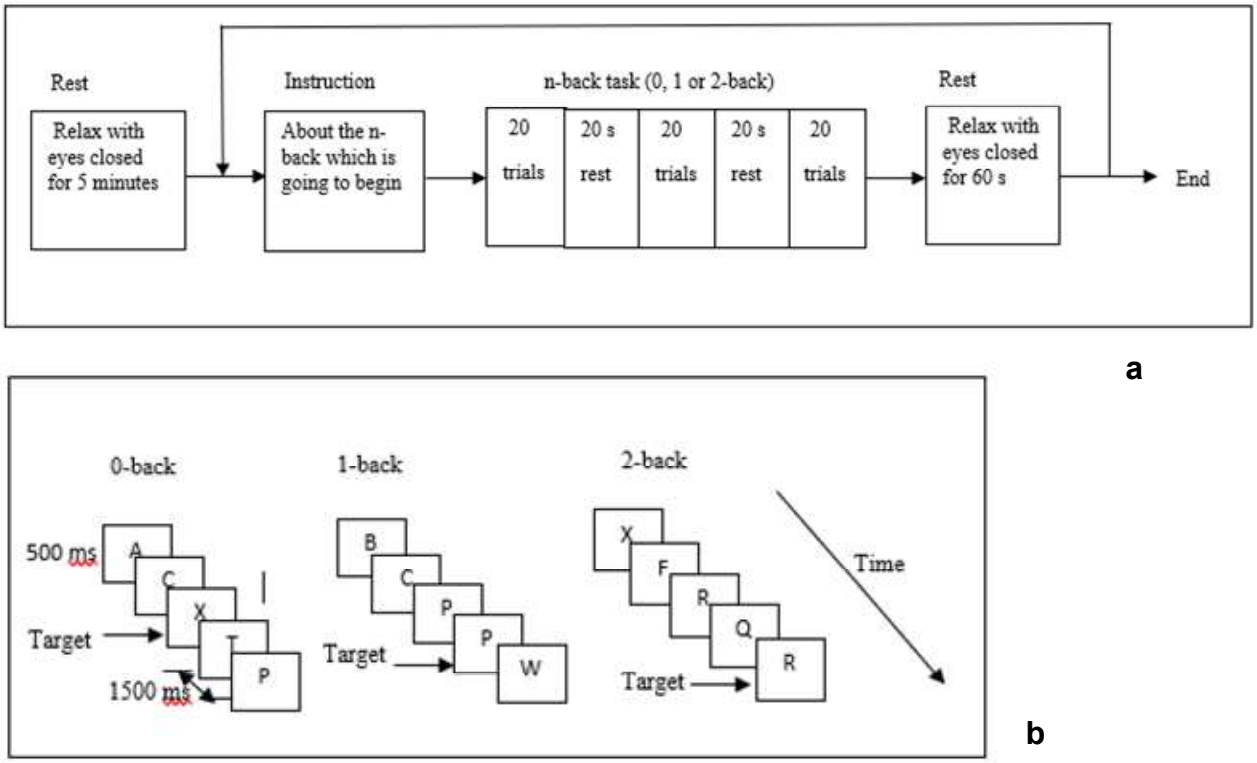


Figure 5. N-back task paradigm. a) N-back task sequence. b) N-back task conditions (0-,1-,2-back)

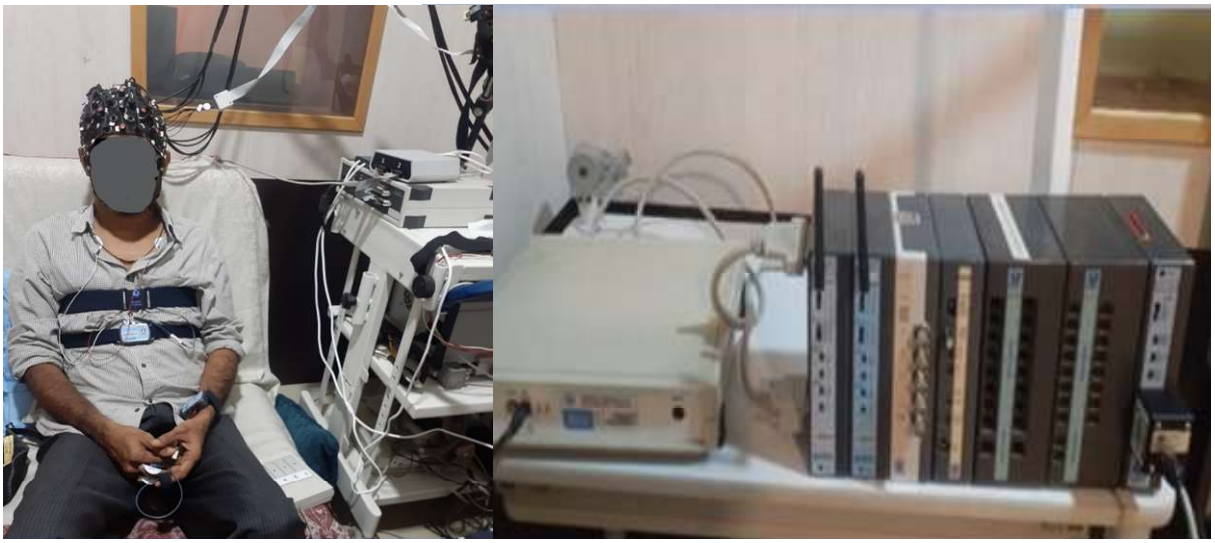


Plate 2. Setup for Heart rate variability assessment a) ECG probes b) MP 100 system

5.3.1.3 Heart rate variability

Short-term heart rate analysis was done to assess cardiac autonomic function. Five minutes electrocardiogram (ECG Lead II) recording was done using the BIOPAC MP100 acquisition system while the participant was at rest, sitting with eyes closed. The data was acquired at the rate of 1KHz. The frequency and time domain analyses were carried out offline. The HRV recordings and analysis were carried out pre and post-intervention as per the guidelines (Heart rate variability. Standards of measurement, 1996).

5.3.1.3.1 Specification of BIOPAC MP100 system

The ECG was assessed using MP 100 BIOPAC, a 4-channel polygraph system and Acqknowledge software (BIOPAC System Inc., U.S.A.). The ECG amplifier output can be switched between two modes -normal output and R-wave detection. R-wave detection produces a smooth pulse with every R-wave. With the R-wave mode, R-wave timing can be detected accurately even with extreme signal artifacts. A sampling rate of 1kHz was used for acquiring the data. Electrodes were placed on the left arm, right arm and left leg based on Einthoven's triangle principle to acquire ECG data. The setup for acquiring the ECG data is shown in Plate 2.

5.3.2 SECONDARY OUTCOME MEASURES

5.3.2.1 Rumination Response Scale (RRS) Score

The Ruminative Response Questionnaire, a self-report scale with 22 items (Treynor et al., 2003) that assesses the tendency to ruminate under depressive conditions was used to quantify rumination. Participants are required to grade, on a four-point scale, based on their style of response to depressive situations. Higher scores suggest the worst condition of rumination. The pre- and post-intervention RRS scores were analyzed. Also, a correlation analysis was done to check the association between the reduction of RRS scores (pre v/s post) and the improvement in n-back task performance (pre v/s post).

5.3.2.2 Perseverative Thinking Questionnaire (PTQ) Score

The 15-item Perseverative Thinking Questionnaire was developed based on a working definition of Repetitive Negative Thinking (RNT) that includes three core characteristics of RNT (repetitiveness, intrusiveness and difficulties to disengage) as well as two associated features -unproductiveness, capturing mental capacity (Ehring et al., 2011).

5.4 INTERVENTION

The validated integrated yoga protocol specific to T2DM was followed (Nagarathna et al., 2019). It included yoga postures (*asanas*), breathing exercises (*pranayama*) and meditation spanning over 60 minutes (Table 4 and Appendix-4). Earlier study validated and tested the yoga module. A review of classical and contemporary yoga texts was conducted to identify yoga practices. Following a review of research papers and yoga texts, a three-step yoga program for T2DM was developed. To validate the yoga program, a mixed methods approach integrating qualitative and quantitative inputs was used. Over three iterations, 18 experts contributed to the validation of the yoga program. A randomized controlled trial was conducted to test the yoga module. In our study, participants in the yoga group engaged in twelve weeks of yoga practice. A trained yoga instructor supervised yoga sessions on alternate days and self-practice was followed on other days for twelve weeks. Waitlist control group members were instructed not to engage in any form of exercise such as running, jogging, swimming, or lifting weights during the study period. The waitlist control group received yoga practice for four weeks upon completion of the trial.

5.5 PROCEDURE OF DATA COLLECTION

The participants were familiarized with a broad description of the procedure the previous day. The night before the assessment, they were advised to have a full night's sleep and abstain from drinking anything with caffeine. The next day, two hours after breakfast, the assessments were done in a dim, silent room with a temperature of around 24.0 ± 1.0◦C. The participants sat 70 cm away from a 15-inch LCD panel that displayed the n-back task stimuli. The participants were instructed to relax, with eyes closed, while a 5-minute resting state was recorded after the fNIRS headgear was secured. The resting state recording was followed by the n-back paradigm (0 -,1- and 2-back). The process took 45 minutes to complete. Electrocardiogram (ECG Lead II) recording was also done during the resting state. Every pre-, mid-, and post-intervention assessment was done using the same procedure. The participants were given a pretesting session (during pre-assessment) before the recording in which they practiced the n-back task, and they were only included after achieving a certain level of performance (75 % or more accuracy on all n-back conditions). Figure 6. represents the data acquisition setup and Plate 3. shows the subject under assessment.

Table 4. Yoga protocol for Type 2 Diabetes Mellitus (Nagarathna et al., 2019)

Sl.No.		Name of the Practice	Duration (minutes)
1		Starting prayer	1
2		Preparatory Practices (SukshmaVyayamas and Shithilikarana)	6
	a	Urdhva-hasta Shvasana (hand stretch breathing 3 rounds at 90°, 135°, 180° each)	
	b	Kati-Shakti Vikasaka (3 rounds each)	
		Forward and backward bending	
		Twisting	
	c	Sarvanga Pushti (clockwise and anticlockwise – 3 rounds each)	
3		Surya Namaskara	9
	a	10 step (fast mode) 6 rounds	
	b	12 step (slow mode) 1 round (a and b to be avoided by those with knee pain, cardiac problems, renal problem, low back pain, retinopathy and the elderly who are weak and not flexible; instead, they can do Chair Surya Namaskara, a modified version : 7 rounds	
4		Asanas (1 min per asana)	15
	a	Standing (1 min per asana)	
		Trikonasana, Pravritta Trikonasana, Prasarita pada-hastasana	
	b	Sitting	
		Mandukasana, Vakrasana/Ardhamatsyendrasana, Paschimatanasana, ArdhaUshtrasana	
	c	Prone	
		Bhujangasana, Dhanurasana followed by Pavanmuktasana	
	d	Supine	
		Jathara Parivartanasana, Pavanamuktasana, Viparitakarani	
		At the end, relaxation with abdominal breathing in supine position (vishranti), 10-15 rounds (2 min)	
5		Kriyas	3
		Agnisara: 1 min	
		Kapalabhati (at 60 breaths per minute for 1 min followed by rest for 1 min)	
6		Pranayama	9
	a	. Nadishuddhi (for 6 min, with antarkumbhaka and jalandharbandha for 2 s)	
	b	Bhramari (3 min)	
7		Meditation (for stress management for deep relaxation)	15
8		Resolve (I am completely healthy)	1
9		Closing prayer	1
		Total	60 mins

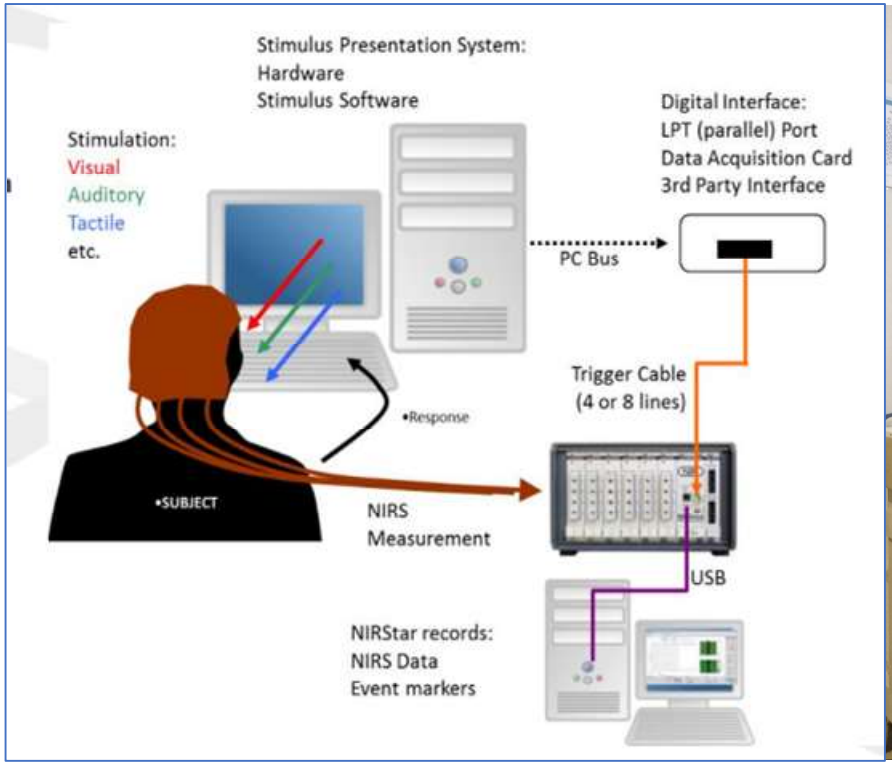


Figure 6. Schematic representation of fNIRS experimental setup.
(Reference: fNIRS experimental guide, © 2019 by NIRx Medical Technologies)

Plate 3. Participant under assessment.

5.6 DATA EXTRACTION

5.6.1 PFC OXYGENATION

The nirsLAB, a MATLAB-based analysis tool was used to analyze the data. (https://www.nitrc.org/projects/fnirs_downstate). The raw fNIRS signals were first converted to optical density changes and then transformed into changes in OxyHb and DeoxyHb using the modified Beer-Lambert law. A differential path length factor (DPF) with the value of 7.25/6.38 (760 nm/850 nm) was applied (Zhao et al., 2002). Channels with a coefficient of variation greater than 15% were excluded. In this study, only OxyHb data was analyzed since it has a higher signal-to-noise ratio than DeoxyHb. We employed a general linear regression model (GLM) to analyze channel-wise hemodynamic responses. The autoregressive iteratively reweighted least squares (AR-IRLS) method, introduced by Barker et al. (Barker et al., 2013) was followed to obtain the estimated amplitudes of OxyHb (β coefficients) for each channel. The changes in OxyHb in different ROIs were obtained by averaging the β coefficients of channels corresponding to the ROI. The changes in OxyHb in different n-back task conditions were measured relative to the resting condition during the assessment session.

5.6.2 WORKING MEMORY PERFORMANCE: N-BACK

The n-back task paradigm was administered while fNIRS recording was done. SuperLab 6 system logs at the runtime the task related information. The correctness of response (correct or not correct) to each task stimulus and the time taken to respond to each stimulus were logged. The percentage of correct responses (accuracy) and the mean response time for all correct responses (reaction time) were calculated for each participant in a particular assessed session.

5.6.3 HEART RATE VARIABILITY

HRV was analyzed using Kubios analysis software, version 2.2 (www.kubios.com). The energy in the HRV series was analyzed in the following frequency bands: low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.5 Hz). The values for the LF and HF bands were expressed in normalized units, as per the standards.

The schematic representation of the data extraction and analysis of fNIRS, N-back and Heart rate variability is shown in Figure 7.

5.7 DATA ANALYSIS

Shapiro–Wilk tests were used to check the normality of the data. Demographic data including age, gender, education and handedness, and clinical data including HbA1c, disease history, Body Mass Index (BMI), MoCA score and SCL90-R scores were compared by an independent sample t-test or Mann-Whitney U test based on the normality of the data. Intention-to-treat analysis was followed, independent of dropout after the pre-intervention assessment. The approach of 'last observation carry forward' was used for missing data. A mixed ANOVA for within-subject factors (time, task conditions) and between-subject factors (intervention groups) was performed to analyze behavioural measures and oxygenation values. The *post hoc* pairwise comparison with Bonferroni correction was done with the significance criterion set at $\alpha=0.05$. Wherever Mauchly's Test of Sphericity was violated, the Greenhouse-Geisser correction was applied. Pearson's correlation analysis was done to check the association between an increase in oxygenation and an improvement in n-back task performance. IBM SPSS Version 26.0 (IBM Corporation, USA) was used for statistical analysis.

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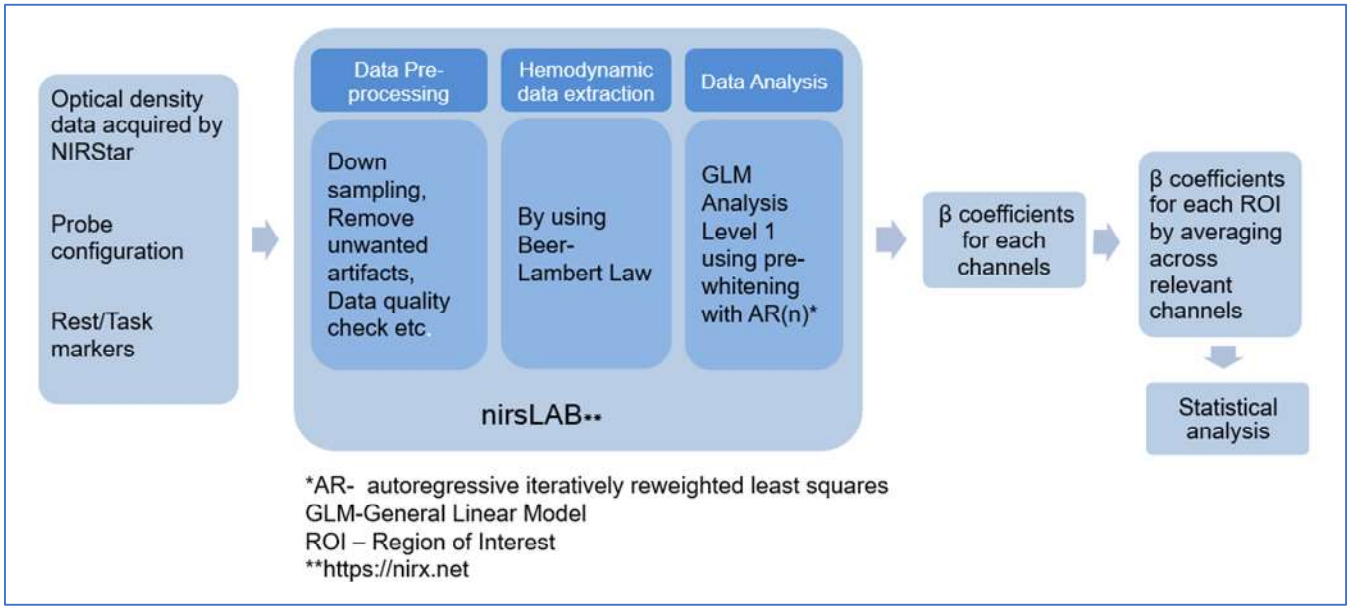

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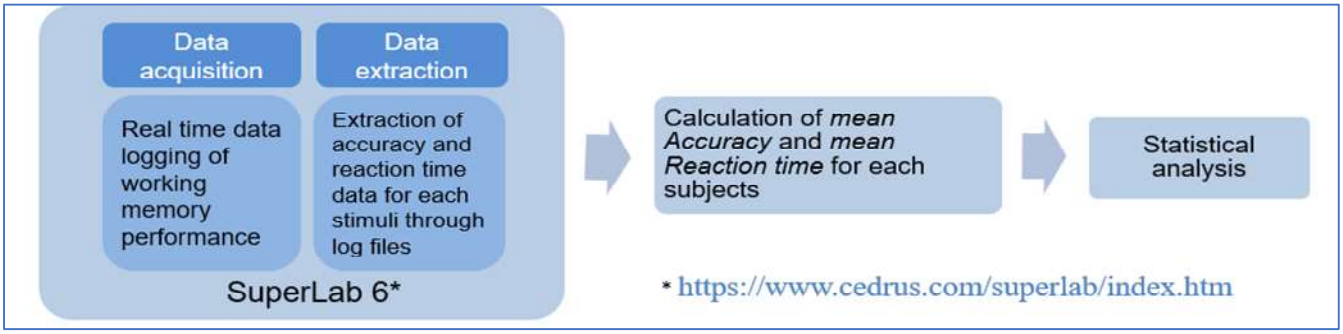

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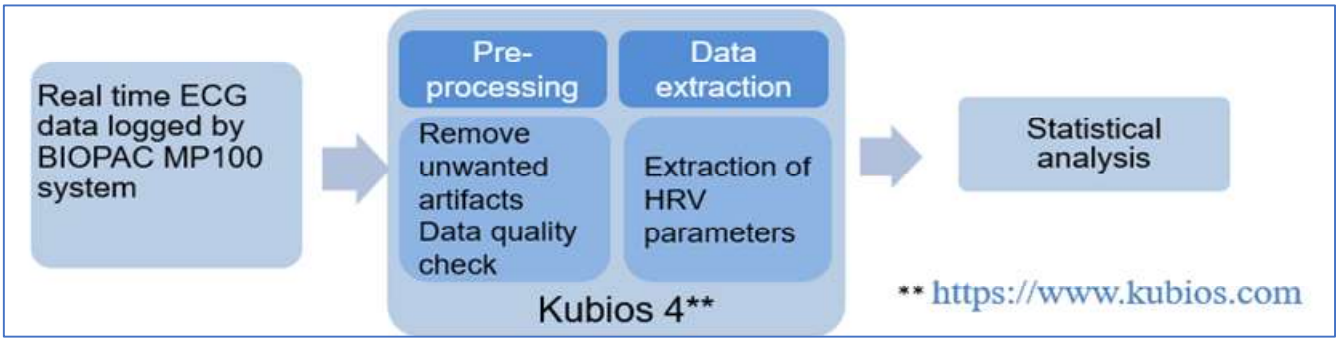

Figure 7. Schematic representation of the data extraction. a) fNIRS data extraction b) N-back task performance data extraction and c) Heart rate variability data extraction