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CLINICAL TRIAL REPORT

Common Yoga Protocol Increases Peripheral Blood CD34+ Cells: An Open-Label Single-Arm Exploratory Trial

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Purpose: Physical inactivity can be a cause of various lifestyle disorders including atherosclerosis, diabetes, hypertension, and cardiovascular diseases (CVDs). Lifestyle modification by the inclusion of Yoga and similar activities has shown beneficial effects on disease prevention and psychological management. However, the molecular mechanism at the cellular level is unknown. This study aims to identify the molecular response at systemic level generated after three months of Common Yoga Protocol (CYP) practice.

Methods: A total of 25 healthy adult females were recruited for this study (25 to 55 years). After the drop out of 6 participants at baseline and 2 participants after 1 month; blood samples of 17 participants were assessed. Blood samples were assessed for lipid profile, CD34+ cell enumeration and angiogenesis markers (ie, VEGF, Angiogenin and BDNF) at baseline (before intervention), after one month and after three months of Common Yoga Protocol (CYP) practice. The psychological health of the participants was assessed at baseline and after three months of CYP practice. The psychological tests used were General Health Questionnaire (GHQ), State-Trait Anxiety Inventory (STAI), Trail Making Test A & B, Digit symbol test, Digit symbol substitution test.

Results: After 3 months of intervention, blood samples of 17 participants were collected and following results were reported (1) percentage of CD34+ cells increased significantly after 3 months of CYP practice (from 18.18±7.32 cells/μL to 42.48±18.83 cells/μL) (effect size: W, 0.40; 95% CI, $p = 0.001$) (2) neurogenesis marker, ie, BDNF showed a significant change with time after 3 months of CYP intervention (effect size: W, 0.431, 95% CI; $p = 0.002$), (3) HDL showed an increasing trend (non-significant) after three months of CYP practice (53.017±1.28 mg/dl to 63.94±5.66 mg/dl) (effect size: W, 0.122; 95% CI; $p = 0.126$) (4) General Health score (10.64 ± 3.53 to 6.52 ± 3.12) (effect size: d, 0.98; 95% CI; $p = 0.001$) along with visual and executive function improved (69.94±26.21 to 61.88±28.55 (time taken in seconds)) (effect size: d, 0.582; 95% CI; $p = 0.036$), also stress and anxiety showed reduction (effect size: d, 0.91; 95% CI; $p = 0.002$) (5) a significant positive correlation was found between: HDL with VEGF ($r = 0.547$, $p = 0.023$) and BDNF ($r = 0.538$, $p = 0.039$) after 3 months of intervention; also, a significant positive correlation was found between VEGF with BDNF ($r = 0.818$, $p \leq 0.001$) and Angiogenin ($r = 0.946$, $p \leq 0.001$), also, BDNF was also positively correlated with Angiogenin ($r = 0.725$, $p = 0.002$) at both 1 month and 3 months after intervention. Also, VEGF and BDNF showed a significantly negative correlation with stress and anxiety questionnaire after the intervention.

Conclusion: The current study provides insights into the molecular response to CYP practice at systemic level. The results suggest that CYP practice indeed increased CD34+ cells in peripheral blood and BDNF also showed a significant change after the intervention. An overall improvement in general health and psychology of the participants was also observed.

Keywords: common yoga protocol, stem cells, angiogenesis, psychology, lipid profile

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Introduction

Physical inactivity can have serious implications leading to various lifestyle-related disorders and the incidence of these disorders increases with age. This may occur due to the dysregulation of the metabolic and molecular pathways, which may cause Non-Communicable Diseases (NCDs) either via overactivation or via inhibition of the molecules involved. To cite an example, in cancer overactivation of angiogenic mechanism occurs while via inhibition there is a reduced blood vessel development, halted stem cell proliferation and cardiac complications. The reason for these conditions can be physical inactivity along with other contributing factors. It has been shown that one-third of the adult population in the world is leading a sedentary life with insufficient physical activity.[1–3] In India, this population is also high, and women are more prone to physical inactivity.[4] As per a report published in 2019, on a global average, women are more physically inactive in comparison to men (31·7% for inactive women vs 23·4% for inactive men),[5] which makes women more prone to developing NCDs, with an impact on their quality of life[6] and cognition,[7,8] these studies underscore the importance of awareness and practice of physical activity among women globally.

Angiogenesis is described as the sprouting of new blood vessels from preexisting ones, and this process is mediated through molecular signals like VEGF and Angiogenin.[9] Angiogenesis is known to influence neurogenesis mechanism mediated via cross-talk between VEGF and BDNF.[10,11] Alteration in angiogenesis-related pathways is pivotal to the development of lifestyle disorders like cancer, diabetes, hypertension, atherosclerosis, stress and depression which is cross linked to the dysregulated angiogenesis.[12–17] Physical activity is known to primarily regulate angiogenesis molecular mechanism and therefore may help in prevention of NCDs.[18,19] Increased vascular density after exercise enhances cognition and quality of life.[19] The major molecular players involved in this mechanism are markers of angiogenesis (VEGF and Angiogenin),[20–22] Neurogenesis (BDNF),[23] Lipid profile,[24] stem cell mechanisms (CD34+ Hematopoietic stem cells (HPCs))[25] which lead to the development of new cells in the system and these altogether lead to an overall development of better health.

CD34+ cells are a type of hematopoietic stem cell (HPCs) with the potential of developing into endothelial cells.[26,27] These bone marrow-derived cells have been found in circulating peripheral blood, and their role in pro-angiogenic therapies has been studied extensively.[25] Circulating HPCs enhance the regenerative potential of blood and tissue cells, more specifically in circulation, which shows that these correlate with vascular endothelial function.[28] Studies have also shown that a deteriorating number of CD34+ along with angiogenic markers increases the risk of cardiovascular diseases (CVDs) reflecting reduced vascular capacity.[25] Also, physical exercise has been known to enhance the mobilization of CD34+ cells into circulation.[29]

Lipid metabolism is associated with CVDs like atherosclerosis, coronary heart disease, etc.[30–32] An increase in lipid metabolites above the normal range can inhibit the process of angiogenesis and further lead to blockage in arteries which is a significant cause of CVDs.[33] Together, these molecular responses to physical activity are known to influence and improve general health and cognition.[34,35]

Yoga is a branch of physical activity that focuses on the mind and body, and evokes relaxation through stress and anxiety resistance techniques. It leads to an overall enhancement in physiological, psychological, and physical health. Studies have shown that the practice of Yoga reduces inflammatory markers, improves immune responses and T effector cell function, and improves the overall quality of life and psychosocial health.[35] Wu et al in 2020 reported an increased proportion of CD34+ cells after Innovative Mind-Body easy exercise.[36] Another similar study reported that sustained one-year Tai chi practice showed significant elevation in peripheral CD34+ cell number in young adults.[37] In our previous study, we have reported an increase in angiogenesis markers, ie, Angiogenin and VEGF after 1 month of Common Yoga Protocol (CYP) intervention along with and elevation in HDL, as an extension of previous study we wanted to explore the effects of CYP practice for a longer time duration, ie, 3 months and also wanted to explore and correlate the effects of CYP through angiogenesis and stem cell mechanisms.[38]

The risk factor for high disease frequency in females affecting their quality of life is the prevalence of sedentary lifestyle which is high worldwide and also in India. Therefore, we primarily aimed to identify the angiogenic response of Common Yoga Protocol, CYP[39] (a generalized yoga protocol introduced by Govt. of India on International Yoga Day for the general population) in sedentary adult women by evaluation of CD34+ cells, angiogenesis markers and lipid profile in

peripheral blood as angiogenic mechanism is the preliminary response of cell proliferation and growth. We also aimed to identify the psychological response to the involvement of CYP in daily lifestyle amongst these sedentary adult females. The study's hypothesis was to identify the response of inclusion of CYP in daily routine to improve and manage overall health and to identify the psychological aspects in response to CYP practice in sedentary adult women. This study was planned to identify the potential of CYP as an adjunct therapy in daily lifestyle for overall health management and decipher the molecular response associated with the practice of CYP in sedentary adult females.

Methodology

Study Design

This study is an open-label single-arm exploratory trial to investigate the effects of CYP practice for three months.

Subject's Characteristics

A total of 25 healthy female subjects (without any co-morbidity) between the age group of 25–55 years were recruited for this 3-month yoga intervention study based on their willingness to participate (Figure 1), after 3 months eight participants dropped out and 17 participants gave their follow-up samples. Recruitment of participants was based on self-reported sedentary lifestyle of the participants since last 1 year, those who were not performing any vigorous/moderate physical activity were recruited for the study (these details were acquired at the time of recruitment). The participants were residents of urban areas of Chandigarh city, India. The participant's primary language was Hindi, with an understanding of the basic English language. The education of all the participants was above secondary school.

Recruitment of participants was done between January 2021 and June 2021 (all the participants were recruited at different time points). All the recruited participants were informed about the purpose of the study and informed consent was obtained from all individual participants.

The participant's blood samples were taken at three time points: Baseline (before the intervention), after 1 month, and after 3 months. Blood obtained was used for lipid profile assessment, assessment of angiogenesis markers, and also for CD34+ cell enumeration. Ethical approval was obtained from the PGIMER, Chandigarh Ethical Committee (IEC No. IEC-03/2020-1541). The study was registered in CTRI (CTRI No. CTRI/2020/09/027747). The study complies with principles of Declaration of Helsinki.

Intervention

45–50 minutes of Yoga intervention (Common Yoga Protocol) (Table 1) was given to the participants 5 days/week for 3 months through online interface (Google meet). Common Yoga Protocol (CYP), which includes Loosening practices, Asanas (standing, sitting, prone and supine), Pranayama, and Meditation practice, was used[39] as an intervention. The intervention was given in the morning (6–6:45 am) and evening (5:30–6:15 pm) timings depending upon the suitability of the participant. Daily attendance of the participants was recorded.

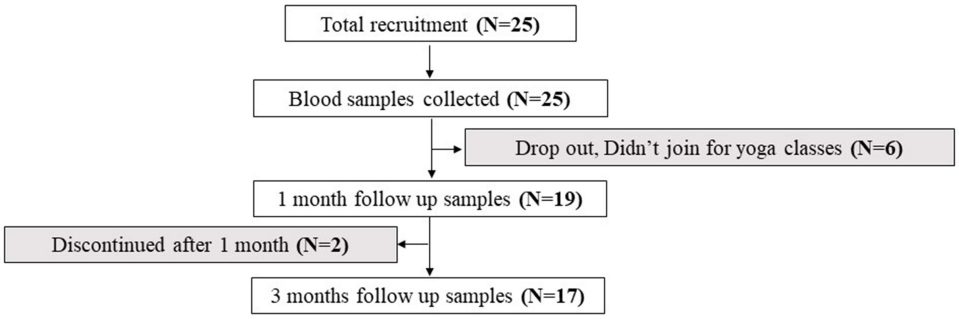

Figure 1 Participant information.

Table 1 Shows the Details of Common Yoga Protocol (CYP) Intervention

S. No.	*ASANAS*	Protocol
1.	Invocation/ Prayer (1 minute)	To enhance the benefits of practice.
2.	Loosening practices (5 minutes)	• Neck bending • Shoulder's movement • Trunk movement (*Katishaktivikasak*) • Knee movement
3.	*Yogasanas* (Yoga postures) (1 minute per asana)	**Standing postures** • *Tadasana* (Palm tree pose) • *Vrksasana* (Tree posture) • *Padahastasana* (The hands and feet posture) • *Ardhacakrasana* (The half wheel posture) • *Trikonasana* (The Triangle Posture) **Sitting postures** • *Bhadrasana* (The firm auspicious posture) • *Vajrasana* (Thunderbolt posture) • *Ardhaustrasana* (The half camel posture) • *Ustrasana* (Camel Posture) • *Sasakasana* (The Hare posture) • *Uttana mandukasana* (Stretched up frog posture) • *Vakrasana* (The spinal twist posture) **Prone postures** • *Makarasana* (The Crocodile posture) • *Bhujangasana* (The Cobra posture) • *Salabhasana* (The Locust Posture) **Supine postures** • *Setubandhasana* (The Bridge Posture) • *Uttanapada Asana* (Raised feet posture) • *Ardha Halasana* (Half plough posture) • *Pavanamuktasana* (The Wind releasing Posture) • *Savasana* (The Dead body Posture)
4.	Kapalbhati (3–4 minutes)	Forceful exhalation by contracting the abdominal muscles (30 strokes/round)
5.	*Pranayama* (2 minutes each)	• *Nadishodhana* or *Anulom Viloma Pranayama* (Alternate nostril breathing) • *Sitali pranayama* • *Bhramari pranayama*
6.	*Dhyana* (Meditation) (5–10 minutes)	For stress free deep relaxation and silencing of mind.
7.	*Sankalpa* (1 minute)	Commitment to be healthy, happy, peaceful and joyful human being.
8.	*Shanti path* (1 minute)	Prayer for happiness, health and peace for all.

Outcome Measures

Attendance Rate

Daily attendance of the participants was recorded.

Anthropometric Assessment

Age, weight, height, and BMI of the participants were recorded before and after the intervention.

Biochemical Assessment

One mL blood sample of the participants was assessed for lipid profile at baseline, after 1 month, and after 3 months of intervention.

Blood Serum Isolation

A fasting blood sample (approx. 3mL) was collected in a clot activator SST tube and was kept at room temperature for 30 minutes to allow clotting of the sample. The sample was processed at 2500 rpm for 30 minutes in a density gradient centrifuge. The upper yellowish layer was separated, aliquoted, and stored at −80°C.

CD34+ Cells Enumeration by Flow Cytometry

The flow cytometry sample preparation and gating strategy was based on published protocols (ISHAGE).

Briefly, 4mL of blood from the participants was collected in EDTA-coated vials. These vials were kept at room temperature for 45–60 minutes. The upper layer containing plasma was then layered on Hisep 1077 (Hi-Media) and centrifuged in a density gradient centrifuge at 1500 rpm for 30 minutes. The middle buffy layer containing PBMCs was then separated. From the separated PBMCs, approximately 1 million cells were suspended in 20 µL of Fc Blocker and kept at 4°C for 30 minutes. CD45 FITC and CD34 PE Fluorochrome labeled antibodies were added per requirement and incubated at 4°C for 1 hour. Washing of the cells with 1X PBS was then done, and finally, the pellet was resuspended in 200 µL of PBS and analyzed on FACS Calibur (BD Bioscience, USA) for 4 hours of processing.[40]

The cells were enumerated using ISHAGE guidelines. The acquisition was mainly based on forward, and side scatters analysis, including lymphocytes excluding debris. Manual gating procedure was followed keeping a negative (cells alone) control for each sample to avoid autofluorescence and false detection. After gating, CD34/CD45 dim cells were analyzed. ISHAGE guidelines were used to calculate estimates of CD34 concentration.

Elisa

Assessment of angiogenesis markers, ie, VEGF (Vascular Endothelial Growth Factor), Angiogenin, and BDNF (Brain-Derived Neurotrophic Factor) in blood serum of the participants was done by sandwich enzyme-linked immunoassay technique ELISA (Kinesis Dx). The procedure was followed as per the manufacturer's instructions. Briefly, after adding the samples, a biotinylated antibody was added, and the plate was incubated for 1 hour. The plate was then washed, the substrate was added, and the plate was again incubated for 10 minutes, after which stop solution was added. Reading was taken at 450 nm with an ELISA reader (Bio-Rad Laboratories). A standard curve was plotted for each experiment, and the respective protein concentration was calculated. $R^2 \geq 0.98$ was considered for the analysis.

Total protein assessment was done to normalize the concentration of the target protein. The Bradford method was used for total protein, and BSA was considered standard. Serum samples were diluted at a concentration of 400X. Coefficient of Variation (CV%) for total protein intra assay assessment was measured using formula $(\sigma/\mu)*100$. For ELISA, samples were assayed in singlets hence no CV% is reported for that.

Neuropsychological Assessment

Participants were assessed with neuropsychological tests before and after three months of intervention. The tests used were as follows: DST (Digit Substitution Test), which measures attention and verbal memory,[41] DSST (Digit Symbol Substitution Test), which measures the information processing capacity of the participant,[42] TMT A & B (Trail Making Test A & B) which measures the visual attention and task switching,[43] SLCT (Six Letter Cancellation Test).[42]

State-Trait Anxiety Inventory, which assesses the anxiety of the participant,[44] and the Short General Health Questionnaire (GHQ-12) to assess the participant for mental health and overall general health of the participant[45] were also administered to the participants. These tests were selected as these tests are quick, short, and reliable for research studies based on the general population.[46,47]

Statistics

Statistical analysis was performed by using SPSS 21 (IBM corp.). Shapiro Wilk test was used to test the normality of the data. Friedman test was used to analyze the non-parametric repeated measures data (lipid profile, CD34+ cell enumeration, and ELISA data). For the parametric pre-post data (psychological assessment, weight, and BMI) paired *t*-test was used. Correlation assessment was done using spearman's rho test to correlate the change between CD34+ cells, lipid

parameters, and angiogenesis markers after 1 month and 3 months of CYP practice. The effect size was reported using Kendall's W concordance coefficient for repeated measures of non-parametric data.

Using formula $W = X2/N(K-1)$, where W is Kendall's W value; X2 is the Friedman test statistic value; N is the sample size. k is the number of measurements per subject.

For the parametric pre-post data of psychological assessment along with weight and BMI assessment, Cohen's d effect size calculation was done using the formula

$$t/\sqrt{N}$$

Where t represents the t value, and N represents the number of participants.[48]

Results

Demographic and Anthropometric Characteristics

A total of 25 healthy females who met the inclusion criteria were recruited for the study. Six participants did not join the yoga classes and were thus excluded, and two discontinued after one month; therefore, 17 were assessed for the final analysis. The mean age of the participants was 40.82 ± 10.11 years.

All the participants were of Indian nationality recruited from Chandigarh city of India with education status of Higher Secondary School or above and an understanding of basic Hindi and English. The socio-economic status of all the study participants was above middle income.

The weight and BMI of the participants were assessed at baseline and after 3 months of CYP intervention, and it was observed that both weight (p = 0.019) and BMI (p = 0.017) reduced significantly after 3 months of intervention (Table 2).

Attendance Rate of the Participants for Intervention

For the present study, a total of 25 participants were recruited, out of which six did not join the yoga class after showing their willingness at the time of recruitment, and two others dropped out after 1 month of intervention. Two dropout participants attended classes even after 1 month for the next 15–20 days, after which they discontinued without giving any apparent reason. Participants with attendance ≥30 (43%) out of 70 classes were excluded from the study.

Table 2 Demographics and Anthropometric Details. Data Was Analyzed Using Paired *t*-Test. N = 17, **p <0.01

Demographics of the Participants					
Average age (in years)	40.82 ± 10.11				
Occupation	11 working professionals, 6- non professional				
Ethnicity	Asian Indian				
Nationality	Indian				
Education	Higher secondary school or above				
Primary Language	Primary Language: Hindi Understandable Language: English				
Socio economic status	All the participants belonged to middle income salary status (Monthly income ≥Rs 45,000/-)				
Co morbidity	2 had migraine				
Diet (self- declared)	4 non-vegetarians, 13 vegetarians				
	Pre (Mean ± SD)	Post (Mean ± SD)	*p* value	t value	Effect size (95% CI)
Weight (kg)	66.48 ± 7.82	65.32 ± 6.92	0.019**	2.607	0.16 (2.16 to 2.10)
BMI (kg/m^2)	26.36 ± 3.05	25.92 ± 2.82	0.017**	2.659	0.16 (0.090 to 0.80)

Daily attendance was recorded; 13 out of 17 participants attended more than 60% of the classes, whereas the other 4 participants joined 60% and 50% of classes (Figure 2).

Quantification of Absolute CD34+ Cell Count

After CYP practice, the CD34+ cell number increased over time. CD34+ cell number increased significantly after 3 months of CYP intervention (42.48±18.83 cells/μL) as compared to baseline (18.18±7.32 cells/μL) ($p = 0.001$), which is more than two times increase. However, this change was not observed at 1 month follow-up time point (Figure 3).

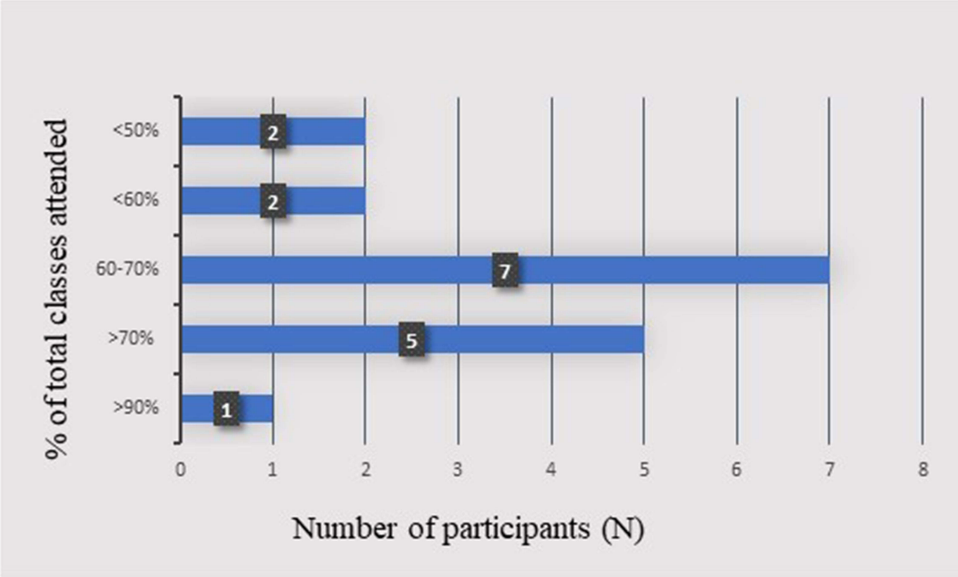

Figure 2 Figure showing attendance record of the participants for 3 months of intervention.

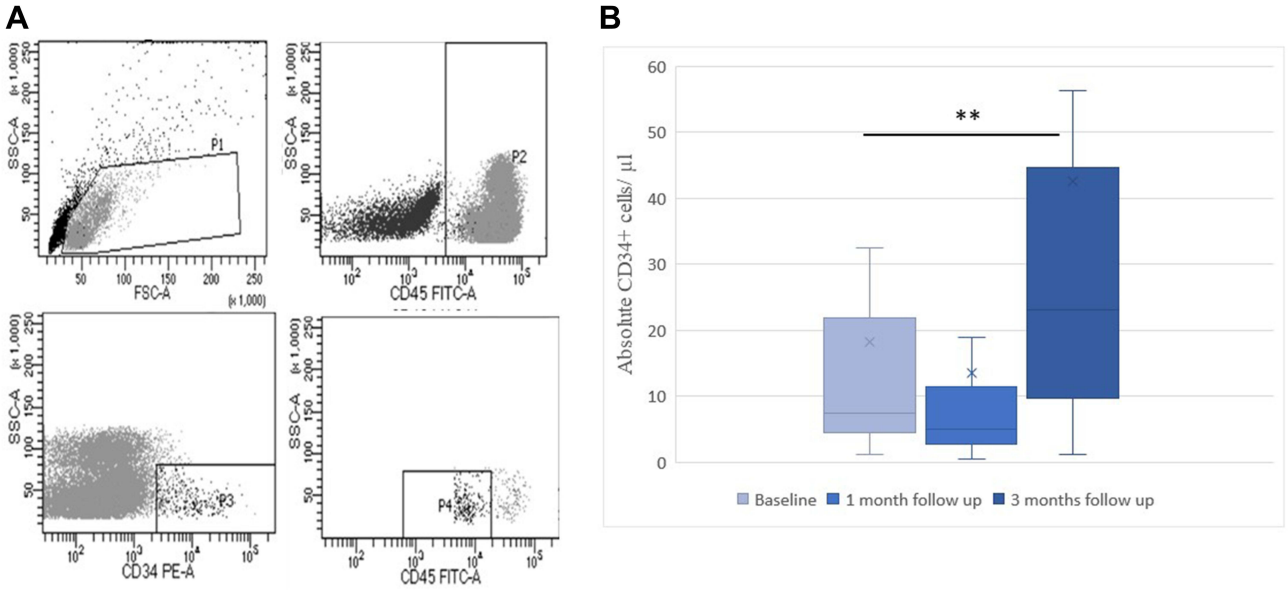

Figure 3 Enumeration of CD34+ cells before and after 1 month and after 3 months of CYP practice. (**A**) Scatter plot of enumeration CD34+ cells total blood cells, P1 shows total leukocyte population, P2 depicts the total CD45+ population, P3 is the CD34 population from the total CD45 population and P4 is the dim CD45+ (**B**) Box plot depicting quantified CD34+ cell population. **$p \leq 0.01$, Degrees of Freedom=2, Effect Size, W= 0.405.

Abbreviations: FSC, Forward Scatter; SSC, Side Scatter; PE, Phycoerythrin; FITC Fluorescein isothiocyanate.

Quantitative Biochemical Measurement

The lipid profile of the participants revealed no significant difference before and after the intervention. However, mean HDL (Baseline 53.01±5.28, 1-month follow-up 54.48±4.37, 3 months follow-up 63.93±23.33) has shown an increasing trend after 3 months of CYP practice (Effect Size, W = 0.122 (p = 0.126)) (Figure 4).

Protein Expression Quantification

ELISA assessment for angiogenesis markers was done from blood serum samples obtained from the participants. VEGF and Angiogenin showed a non-significant trend of increment after 1 month and 3 months of CYP practice. BDNF, which is a marker of neurogenesis, showed a significant decreasing trend following the intervention (Effect size = 0.431, p = 0.002) (Table 3). CV% for intra assay total protein assessment was 8.96%.

Psychological Assessment

Psychological assessment of participants was done at baseline and after 3 months of intervention. Participants showed a significant improvement in general health score (Effect size, d = 0.98; p = 0.001) and a reduced anxiety score post-intervention (Effect size, d = 0.91; p = 0.002). Also, participants showed a significant improvement in Trail making test B (Effect size, d = 0.582; p = 0.036), a visual and executive function parameter. Participants also showed an increasing trend in SLCT, DST, TMT A (parameters of attention and information processing) tests, though not significant, which are parameters of attention and information processing (Table 4).

Correlation Analysis

To determine the effects of change in CD34+ cell count on Angiogenesis markers and lipid profile of the participants, we correlated the values obtained after 1 month and 3 months of CYP practice for all the parameters mentioned above. We found a positive correlation of VEGF with BDNF and Angiogenin after both 1 month (Table 5) and after 3 months

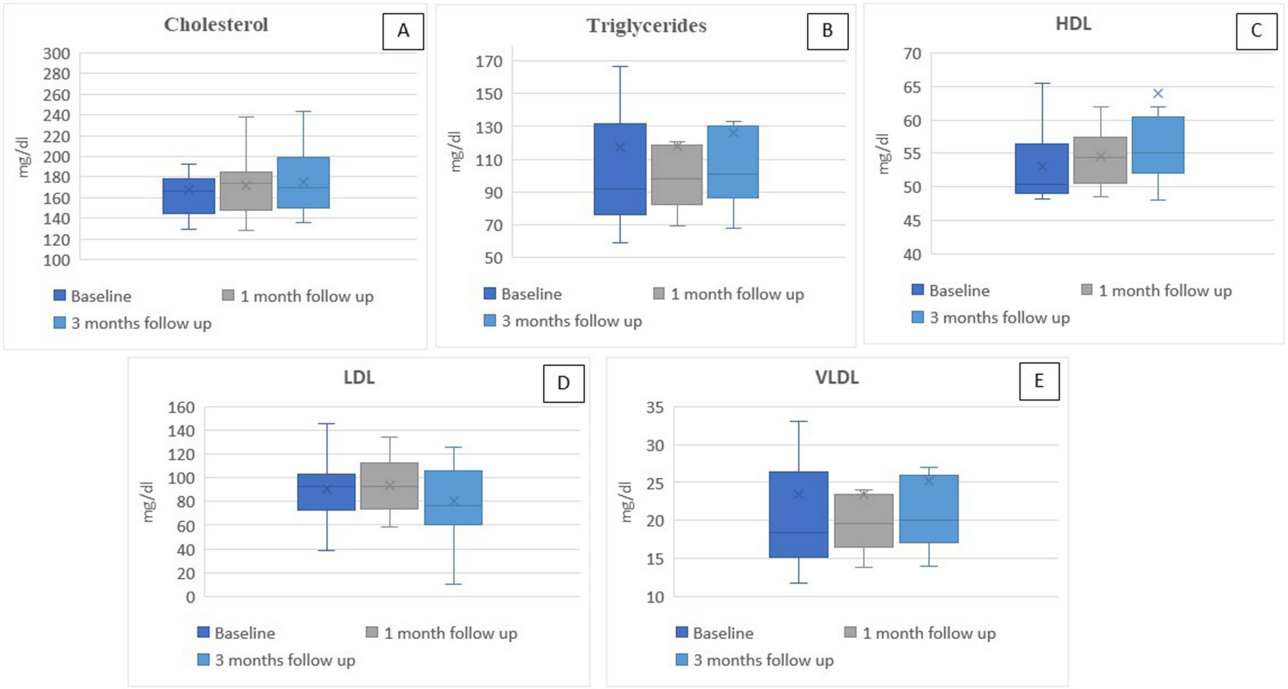

Figure 4 Box plot of Lipid profile at baseline, after 1 month and after 3 months of CYP practice (**A**) Cholesterol, Degree of Freedom = 2; Effect size, W = 0.027 (p = 0.630). (**B**) Triglycerides, Degree of Freedom = 2, Effect size, W = 0.064, p = 0.336. (**C**) HDL, Degree of Freedom = 2; Effect size, W = 0.122 (p = 0.126). (**D**) LDL Degree of Freedom = 2; Effect size, W = 0.010 (p = 0.838). (**E**) VLDL Degree of Freedom = 2, Effect size, W = 0.064, p = 0.336. Data was analysed by using SPSS Friedman K related samples test, N = 17.

Abbreviations: HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; VLDL, Very Low-Density Lipoprotein; CYP, Common Yoga Protocol.

Table 3 Table Showing Levels of Angiogenesis Markers, ie, VEGF, Angiogenin and BDNF at Baseline, After 1 Month and After 3 Months of CYP Practice. Data Was Analyzed Using SPSS Friedman Related Sample KS Test. N = 17, **p = 0.002

	VEGF (pg/µg)			Angiogenin (pg/µg)			BDNF (ng/µg)		
	Baseline	After 1 Month of CYP Practice	After 3 Months of CYP Practice	Baseline	After 1 Month of CYP Practice	After 3 Months of CYP Practice	Baseline	After 1 Month of CYP Practice	After 3 Months of CYP Practice
Median	30×10^{-4}	45×10^{-4}	39×10^{-4}	71×10^{-5}	69×10^{-5}	70×10^{-5}	14×10^{-6}	13×10^{-6}	7×10^{-6}
Minimum	3×10^{-4}	18×10^{-4}	16×10^{-4}	15×10^{-5}	30×10^{-5}	20×10^{-5}	8×10^{-6}	6×10^{-6}	4×10^{-6}
Maximum	7×10^{-2}	7×10^{-2}	6×10^{-2}	14×10^{-3}	23×10^{-3}	19×10^{-3}	12×10^{-5}	15×10^{-5}	12×10^{-5}
25th percentile	2×10^{-3}	3×10^{-3}	2×10^{-3}	39×10^{-5}	49×10^{-5}	36×10^{-5}	93×10^{-7}	80×10^{-7}	55×10^{-7}
75th percentile	20×10^{-3}	12×10^{-3}	14×10^{-3}	33×10^{-4}	32×10^{-4}	54×10^{-4}	58×10^{-6}	56×10^{-6}	40×10^{-6}
Chi square	1.412			1.529			12.933		
***p* value**	0.494			0.465			0.002**		
Degree of freedom	2			2			2		
Effect size (Kendall's W)	0.042			0.045			0.431		

Table 4 Table Showing Pre and Post Effects of Psychological Assessment. Data Was Analyzed Using SPSS Paired Sample *t*-Test. N = 17, *p < 0.05, **p <0.01

Psychological Assessment	Before Intervention (Mean ± SD)	After 3 Months of CYP Practice (Mean ± SD)	t value	Effect Size (Cohen's d)	95% CI		Degree of Freedom	*p* value
					Lower	Upper		
General Health Questionnaire score (GHQ-12)	10.64 ± 3.53	6.52 ± 3.12	4.07	0.98	1.97	6.259	16	0.001**
State Trait Anxiety Inventory (STAI)	29.23 ± 8.62	23.11± 3.40	3.78	0.91	2.69	9.542	16	0.002**
DST (Digit Span Test) Score (forward and backward)	16.88±5.06	16.70±4.52	0.65	0.15	−17.36	32.778	16	0.524
SLCT (Six Letter Cancellation Test)	30.82±12.26	32±13.23	0.23	0.05	−1.42	1.774	16	0.818
DSST (Digit Symbol Substitution Test) Time in seconds	241.17±87.5	233.47± 103.1	−0.49	−0.11	−6.27	3.917	16	0.631
TMT A (Trail Making Test A)	33.41±13.53	31.41±11.16	0.71	0.17	−3.93	7.932	16	0.485
TMT B (Trail Making Test B)	69.94±26.21	61.88±28.55	2.28	0.55	0.582	15.534	16	0.036*

(Table 6). Also, BDNF and Angiogenin were found to be significantly correlated. After 1-month, lipid parameters were found to be correlated with each other (Table 5). A positive correlation of HDL with VEGF and BDNF was also observed after 3 months (Table 6). Also, neuropsychological assessment parameter STAI showed a significant negative correlation with BDNF and VEGF (Table 6). Parameters of neuropsychological assessment were found to be inter correlated (Table 6).

Table 5 Table Showing Correlation Between the Change in CD34+ Cells, Lipid Parameters and Biochemical Parameters After 1 Month of CYP Practice. Data Was Analyzed Using SPSS Spearman Correlation Analysis. N = 17, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. the table alignment should be proper, the empty boxes can be deleted.

CD34 F1	ρ=1.000								
Cholesterol F1	ρ=−0.087 P=0.740	ρ=1.000							
Triglycerides F1	ρ=0.218 p=0.400	ρ=0.524* p=0.031	ρ=1.000						
HDL F1	ρ=0.342 p=0.179	**ρ=0.549** **p=0.022***	ρ=0.447 p=0.072	ρ=1.000					
LDL F1	ρ=−0.368 p=0.146	**ρ=0.888** **p≤0.001****	ρ=0.191 p=0.462	ρ=0.291 p=0.257	ρ=1.000				
VLDL F1	ρ=0.200 p=0.442	**ρ=0.533** **p=0.028***	**ρ=0.999** **p≤0.001****	ρ=0.438 p=0.079	ρ=0.201 p=0.439	ρ=1.000			
VEGF F1	ρ=0.262 p=0.309	ρ=0.120 p=0.646	ρ=0.196 P=0.451	ρ=0.053 p=0.841	ρ=0.050 p=0.848	ρ=0.197 p=0.448	ρ=1.000		
BDNF F1	ρ=0.189 P=0.499	ρ=0.173 p=0.537	ρ=0.300 p=0.277	ρ=0.048 p=0.864	ρ=0.154 p=0.585	ρ=0.302 p=0.274	**ρ=0.929** **p≤0.001****	ρ=1.000	
Angiogenin F1	ρ=0.257 P=0.319	ρ=0.155 p=0.554	ρ=0.324 p=0.205	ρ=0.082 p=0.754	ρ=0.070 p=0.790	ρ=0.326 p=0.201	**ρ=0.917** **p≤0.001****	**ρ=0.896** **p≤0.001****	ρ=1.000
	CD34 F1	**Cholesterol F1**	**Triglycerides F1**	**HDL F1**	**LDL F1**	**VLDL F1**	**VEGF F1**	**BDNF F1**	**Angiogenin F1**

Note: Parameters showing significant correlation is represented in bold.

Discussion

The present study demonstrates that the inclusion of CYP practice in the daily lifestyle routine provides physiological health benefits by enhancing the level of hematopoietic and endothelial progenitor cells, CD34+; and influences angiogenesis markers (VEGF, Angiogenin and BDNF). We have previously also reported a similar trend with respect to CYP practice in sedentary adults after 1 month.[38] We also report an improvement in general health, reduced anxiety and improved visual and executive function after 3 months, which could be attributed to the change in BDNF after 3 months of CYP practice and a negative correlation of STAI with BDNF and VEGF, other cognitive parameters did not show any significant improvement after CYP practice, which implies a longer duration of CYP practice could be assessed for cognitive parameters. Overall, these results depict an improvement in the physical, physiological, and general well-being of the participants with the inclusion of a standardized yoga protocol, ie, CYP, in the participants daily routine.

The average age of the participants was 40.82±10.11 years. In the Indian context, this age group of females has high prospects of high BMI and abnormal lipid profile,[49] indicating the need for lifestyle moderation. Studies have shown that women in their middle age are prone to more sedentary and leisure activities make them susceptible to various lifestyle-related disorders.[50,51] Furthermore, it has been seen that women in their middle age have deteriorated quality of life compared to men of the same age, which accentuates the need for lifestyle modification in women's lifestyles.[52,53]

In the present study, we have found a significant reduction in body weight (66.48 ± 7.82 to 65.32 ± 6.92) (p = 0.019) and BMI (26.36 ± 3.05 to 25.92 ± 2.82) (p = 0.017) after 3 months of CYP practice, the BMI of the participants has moved towards overweight from the obese category.[54] Since the recruited participants did not have any co morbid condition, their lipid profile did not show any significant change, except for HDL, which has shown an increasing trend, though not significant, it has a role in cardiovascular repair mechanism and is also associated with an increase in CD34+.[55,56]

CD34+ cell population was consistent with previous studies, which shows that the detection technique and enumeration method followed were similar to other studies.[57] A significant increase in the total CD34+ population in peripheral blood after 3 months of CYP practice shows an enhancement in the cells regenerative potential and angiogenesis.[36,58] Also, a decrease in CD34+ cells represent a marker of aging; we found the enhancement in CD34+ cells, which depicts a curtailed cellular aging.[56,59]

Table 6 Table Showing Correlation Between the Change in CD34+ Cells, Lipid Parameters and Biochemical Parameters After 3 Months of CYP Practice. Data Was Analyzed Using SPSS Spearman Correlation Analysis. N = 17, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001the table alignment is not proper.

CD34 F2	ρ=1.000										
Cholesterol F2	ρ =- 0.083 P=0.750	ρ=1.000									
Triglycerides F2	ρ= 0.267 P= 0.300	ρ=0.254 P=0.326	ρ= 1.000								
HDL F2	ρ= −0.145 P=0.578	**ρ= 0.661 P=0.004****	ρ= 0.044 P= 0.866	ρ=1.000							
LDL F2	ρ= 0.065 P=0.804	ρ=0.381 P=0.132	ρ = −0.082 P= 0.754	ρ= −0.033 P= 0.899	ρ=1.000						
VLDL F2	ρ= 0.245 *P*=0.344	ρ=0.285 P=0.267	**ρ= 0.997 P≤.001*****	ρ= 0.074 P= 0.778	ρ= −0.065 P=0.804	ρ=1.000					
VEGF F2	ρ= −0.020 P=0.940	ρ=0.150 P=0.567	ρ=0.000 P=1.000	**ρ=0.547 P=0.023***	ρ= −0.163 P= 0.531	ρ= 0.005 P= 0.985	ρ=1.000				
BDNF F2	ρ= −0.275 P=0.321	ρ=0.146 P=0.603	ρ = −0.089 P= 0.752	**ρ=0.538 P=0.039***	ρ= −0.342 P=0.213	ρ= −0.086 P=0.761	**ρ= 0.818 P≤0.001*****	ρ=1.000			
ANGIOGENIN F2	ρ= 0.125 P=0.633	ρ=0.197 P=0.448	ρ= 0.088 P= 0.736	ρ=0.407 P=0.105	ρ= −0.061 P= 0.815	ρ= 0.086 P= 0.743	**ρ= 0.946 P≤ 0.001*****	**ρ= 0.725 P= 0.002****	ρ=1.000		
GHQ POST	ρ= −0.359 P=0.158	ρ= −0.257 P=0.319	ρ= −0.022 P= 0.932	ρ= −0.116 P=0.656	ρ= 0.082 P= 0.754	ρ= 0.002 P= 0.992	ρ= −0.305 P= 0.234	ρ= −0.480 P= 0.070	ρ= −0.449 P= 0.070	ρ= 1.000	
STAI POST	ρ= −0.118 P=0.651	ρ= −0.180 P= 0.489	ρ= −0.024 P= 0.928	ρ= −0.305 P= 0.234	ρ= 0.184 P= 0.480	ρ= −0.006 P= 0.981	**ρ= −0.483 P= 0.049***	**ρ= −0.585 P= 0.022***	ρ= −0.458 P= 0.064	ρ= 0.481 P= 0.051	ρ= 1.000

(*Continued*)

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Table 6 (Continued).

DSST POST	ρ= 0.104 P=0.690	ρ= 0.117 P =0.654	ρ=0.299 P=0.243	ρ= 0.260 P= 0.313	ρ= −0.038 P=0.885	ρ= 0.286 P= 0.267	ρ= 0.211 P= 0.416	ρ= 0.071 P= 0.800	ρ= 0.180 P= 0.488	ρ= 0.061 P= 0.816	ρ= −0.509* P=0.037	ρ= 1.000			
DST POST	ρ= −0.290 P=0.260	ρ=0.271 P=0.292	ρ= −0.152 P= 0.561	ρ= 0.197 P= 0.449	ρ= −0.018 P=0.946	ρ= −0.123 P= 0.638	ρ= 0.144 P= 0.581	ρ= 0.382 P= 0.160	ρ= 0.100 P= 0.703	ρ= −0.258 P= 0.317	ρ= 0.089 P= 0.734	**ρ= −0.711 P=0.001*****	ρ= 1.000		
SLCT POST	ρ= −0.243 P= 0.348	ρ= −0.172 P= 0.510	ρ= −0.278 P= 0.279	ρ= −0.478 P=0.052	ρ= 0.157 P= 0.549	ρ= −0.278 P= 0.280	ρ= −0.240 P= 0.353	ρ= 0.038 P= 0.894	ρ= −0.210 P= 0.419	ρ= −0.148 P= 0.570	ρ= 0.219 P= 0.399	**ρ= −0.786 P≤0.001*****	**ρ= 0.678 P=0.003****	ρ= 1.000	
TMT A POST	ρ= 0.244 P=0.346	ρ= 0.220 P= 0.397	ρ= 0.154 P= 0.555	ρ=0.324 P=0.205	ρ= 0.070 P= 0.791	ρ= 0.169 P= 0.516	ρ= 0.142 P= 0.588	ρ= −0.160 P= 0.569	ρ= 0.167 P= 0.521	ρ= 0.062 P= 0.814	ρ= −0.218 P= 0.401	**ρ= 0.742 P= 0.001*****	**ρ= −0.593 P=0.012***	**ρ= −0.861 P≤0.001*****	ρ= 1.000	
TMT B POST	ρ=0.075 P=0.775	ρ= −0.057 P= 0.827	ρ= 0.246 P= 0.341	ρ=0.255 P=0.323	ρ= −0.339 P= 0.183	ρ= 0.247 P= 0.338	ρ= 0.176 P= 0.499	ρ= 0.079 P= 0.780	ρ= 0.080 P= 0.760	ρ= 0.152 P= 0.560	ρ= −0.264 P=0.306	**ρ= 0.760 P≤0.001*****	**ρ= −0.662 P=0.004****	**ρ= −0.788 P≤0.001*****	**ρ= 0.735 P=0.001*****	ρ=1.000
	CD34 F2	**Cholesterol F2**	**Trigly cerides F2**	**HDL F2**	**LDL F2**	**VLDL F2**	**VEGF F2**	**BDNF F2**	**ANGIO GENIN F2**	**GHQ POST**	**STAI POST**	**DSST POST**	**DST POST**	**SLCT POST**	**TMT A POST**	**TMT B POST**

Note: Parameters showing significant correlation is represented in bold.

Our study has found a significant positive correlation of HDL with VEGF and Angiogenin after 3 months of CYP practice, which signifies that CYP induces a mechanism of elevation in angiogenesis and cardiovascular repair. Furthermore, a positive correlation was also found between VEGF, BDNF, and Angiogenin after both 1 month and 3 months of CYP practice (Table 5 and Table 6) which signifies that the response is interrelated between these angiogenesis and neurogenesis molecules. No positive correlation was detected for CD34+ cells with any other markers analyzed. Furthermore, a negative correlation of STAI assessment with BDNF and VEGF was reported (Table 6) which signifies that with decrease in stress and anxiety after the CYP practice, an increment in VEGF and BDNF could be anticipated. However, studying these responses with a larger sample size and longer duration would depict more precise information.

Yoga may be beneficial with aging by increasing the CD34+ cells and angiogenesis, thereby reducing the risk of CVDs. Yoga may influence this response by immediate induction of intermittent hypoxia through breathing techniques and thereby sympathetic response and increasing blood flow at the time of practice.[37,60] Consequently, in the present study, we found a significant increase in CD34+ cells, and VEGF and Angiogenin followed an increasing trend after CYP for 3 months which is consistent with our previous study. BDNF showed a significant decreasing trend which may be due to the inverse response of resting BDNF levels to the long term of practice.[61]

The present study employs CYP as the standardized Yoga intervention (recommended by Govt. of India for International Yoga Day) as an adjunct inclusion to the daily routine of the recruited adult sedentary females who did not participate in any physical activity in their daily routine. Through this study we confer that CYP protocol mediates its health benefits through angiogenic mechanism via activating the endothelial stem cell niche and further activating the angiogenic molecular response to the practice of CYP. An enhancing level of HDL further enhances the angiogenic activation response via its function in cardiovascular repair. We also found an improvement in general health, reduced stress and anxiety score, and increment in information and visual processing cognitive function, however overall neuro psychology did not show any significant improvement after the intervention.

Overall, the current study shows that the practice of a validated and standardized 45–50 minutes of Yoga protocol, ie, CYP (which is freely available on AYUSH Ministry website) which includes the practices, can be performed by individuals of any age

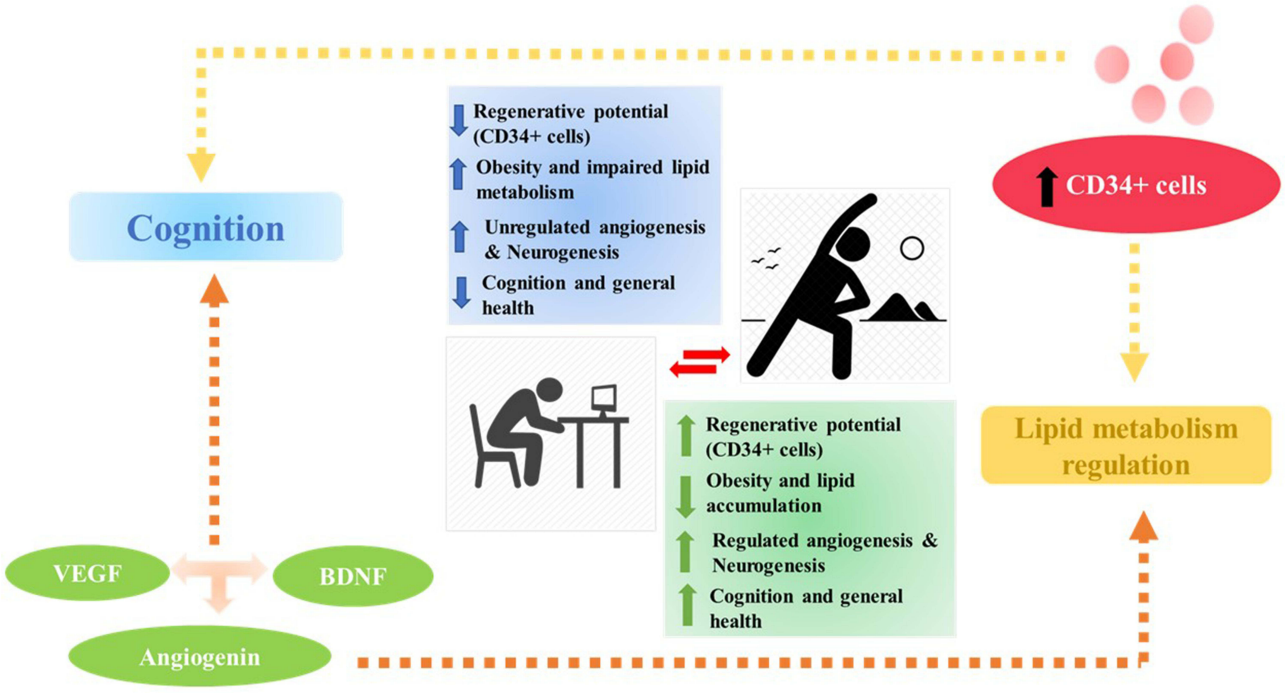

Figure 5 Schematic showing effects of Yoga induced through the regulation of neurogenesis and angiogenesis pathways via CD34+ cells number and Lipid metabolism. ↑ depicts increase, ↓ depicts decrease,----► depicts the pathway.
Note: the arrows in the figure are distorted and not properly alinged

(with no comorbid condition) as an adjunct in daily lifestyle. This can enhance the overall quality of life by boosting general health and improving cognition and also may be beneficial in prevention of NCDs (Figure 5). The possible mechanism of these benefits could be the intermittent hypoxic mechanism activated with the practice of Yoga which activates the stem cell niche from the bone marrow into the peripheral blood. This would also enhance the growth of blood vessels through angiogenesis activation.

Limitations

Small sample size and lack of a control group were the significant limitations of the present study.

Conclusion

We demonstrated that when sedentary adults included 45–50 minutes of Yoga practice in their daily lifestyle, it led to an overall physical, physiological, and psychological health benefits. These health benefits could be escalated through stem cell proliferation prompted by the intermittent hypoxia induced by the Yoga practice. Hence, including CYP as a daily lifestyle habit may provide health benefits and may prevent NCDs.

Data Sharing Statement

The authors confirm that the data confirming the findings of the study are available within the article. The raw data that supports the finding of the study can be made available from corresponding author (AA) on reasonable request.

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Disclosure

The authors report no conflicts of interest in this work.

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