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Effect of 12-Week Sequential Yoga Protocol (Pranayama-Core Asanas) on Neuro-Endocrine-Metabolic Axis in Young Students: A Randomized Controlled Trial Evaluating Sympathetic Tone, Anxiety, and Remnant Cholesterol

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ABSTRACT: **Introduction**: Chronic stress activates the neuro-endocrine-metabolic axis, leading to sympathetic overactivity, anxiety, and adverse lipid profiles including elevated remnant cholesterol. Yoga may modulate this axis, but integrated evidence is limited.

Objective: To evaluate the effect of a 12-week sequential yoga protocol (pranayama and core asanas) on sympathetic tone (via Cold Pressor Test and Hand Grip Test), anxiety (HAM-A), and remnant cholesterol levels in young students.

Methods: A randomized controlled trial was conducted on 94 students (18-25 years) allocated to yoga (n=47) or control (n=47) groups. The yoga group received 30-minute sessions, 5 days/week for 12 weeks. Assessments were performed at baseline and 12 weeks.

Results: The yoga group showed significant reduction in HAM-A scores (10.6±3.56 vs. 22.8±4.97; $p<0.001$), improved sympathetic responses (CPT-SBP: $p=0.001$; CPT-DBP: $p<0.001$; HGT-SBP: $p=0.003$; HGT-DBP: $p<0.001$), and favorable lipid changes including reduced remnant cholesterol (calculated as TC minus HDL-C minus LDL-C) from 23.0±15.2 to 15.1±12.4 mg/dL ($p<0.001$).

Conclusion: A 12-week sequential yoga protocol positively modulates the neuro-endocrine-metabolic axis, reducing sympathetic tone, anxiety, and remnant cholesterol in young students.

1. INTRODUCTION

The neuro-endocrine-metabolic axis represents an integrated network connecting the nervous system, endocrine glands, and metabolic pathways. Stress activates this axis through the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system, leading to catecholamine release, cortisol elevation, and subsequent metabolic dysregulation (1). In young students,

particularly medical professionals, chronic academic stress perpetuates this activation, manifesting as heightened sympathetic tone and anxiety disorders (2).

Anxiety disorders affect approximately 301 million people globally, with prevalence exceeding 4% of the world population. Concurrently, dyslipidemia contributes to 3.6 million annual deaths from cardiovascular disease, with remnant cholesterol emerging as an independent risk factor (3,4). Remnant cholesterol—comprising very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) particles—is particularly atherogenic and sensitive to stress-induced metabolic changes (5).

Yoga, derived from the Sanskrit root "yuj" meaning to unite, offers a holistic approach to rebalancing minds, intellect and self-control (6). Unlike isolated exercise, sequential yoga protocols integrating pranayama (breathing techniques) and core asanas (postures) may directly modulate vagal tone, reduce sympathetic outflow, and influence hypothalamic-pituitary axis function (7). Previous studies have demonstrated yoga's benefits on anxiety and traditional lipid parameters, but limited research has examined its integrated effect on the entire neuro-endocrine-metabolic axis, particularly on remnant cholesterol (8,9).

Knowledge Gap: No randomized controlled trial has simultaneously evaluated the effect of a sequential yoga protocol (pranayama followed by core asanas) on sympathetic tone, anxiety, and remnant cholesterol using a neuro-endocrine-metabolic axis framework.

Aim & Objective: To evaluate the effect of a 12-week sequential yoga protocol on:

Sympathetic tone assessed by Cold Pressor Test and Hand Grip Test, anxiety levels Hamilton Anxiety Rating Scale (HAM-A), remnant cholesterol and lipid parameters assessed using enzymatic methods.

Hypothesis of the study- Sequential yoga practice modulates the neuro-endocrine-metabolic axis, leading to reduced sympathetic reactivity, decreased anxiety, and improved remnant cholesterol levels.

2. METHODS

2.1 Study Design and Setting

A parallel-group, randomized controlled trial was conducted at GSVM Medical College, Kanpur, India, over one year (CTRI/2024/11/076747). Ethical approval was obtained from the Institutional Ethics Committee (EC/BMHR/2023/70, dated 20-10-2023).

2.2 Participants

Ninety-four healthy students of either gender were enrolled after informed consent.

Inclusion Criteria: Age 18-25 years; willing to provide informed consent

Exclusion Criteria: History of spinal injury or chronic illness; current drug or hormonal therapy; regular exercise (gym, sports); prior yoga/meditation experience.

2.3 Sample size calculation: The minimum required sample size was calculated using OpenEpi software (version 3.01) (https://www.openepi.com/) using the module for comparing means of two independent groups. Based on a pilot study (n=10 per group), we assumed a medium effect size (Cohen's d = 0.58) for the primary outcome (HAM-A score), with a two-tailed α of 0.05 and power (1-β) of 0.80. The calculation yielded 42 participants per group. Anticipating a 10% dropout rate, we enrolled 47 participants per group (total N=94). The achieved power post-study for the primary outcome was >99.9%, and for secondary outcomes ranged from 65% to 98%.

2.4 Randomization and Blinding

Participants were randomized using a block design (1:1 allocation) into yoga group (n=47) and control group (n=47). Outcome assessors were blinded to group allocation. Participants and instructors could not be blinded due to the nature of the intervention.

To control for confounding by non-yoga physical activity and to distinguish the specific effects of the structured yoga intervention from general lifestyle activity, weekly physical activity levels were monitored in both groups using the self-

administered International Physical Activity Questionnaire (IPAQ) short form, which quantified time spent in walking, moderate-intensity, and vigorous-intensity activities across domestic, occupational, and leisure domains, thereby isolating the unique contribution of yoga practice.

To control for potential metabolic confounders, dietary intake was monitored at baseline and post-intervention using a validated three-day food diary (two weekdays and one weekend day) administered at each time point, with participants recording all foods, beverages, and portion sizes, and daily macronutrient and caloric intake subsequently analyzed using standardized nutrition software (NutriPro) to ensure that observed group differences in lipid parameters and remnant cholesterol were attributable to the yoga intervention rather than dietary variation.

2.5 Intervention (Reported per TIDieR/STRICTA Guidelines)

Item	Description
Brief name	12-week sequential yoga protocol (pranayama-core asanas)
Why	To modulate the neuro-endocrine-metabolic axis
What (materials)	Yoga mats, printed protocol, and timer
What (procedure)	See Table 2 for the sequence
Who provided	Certified yoga instructor (CSJM University)
How	Face-to-face group sessions
Where	Designated hall, GSVM Medical College
When and how much	30 minutes/day, 5 days/week, for 12 weeks; sessions conducted at 8:00 AM
Tailoring	None (fixed protocol)
Modifications	None
Fidelity	Attendance greater than 90%, recorded daily

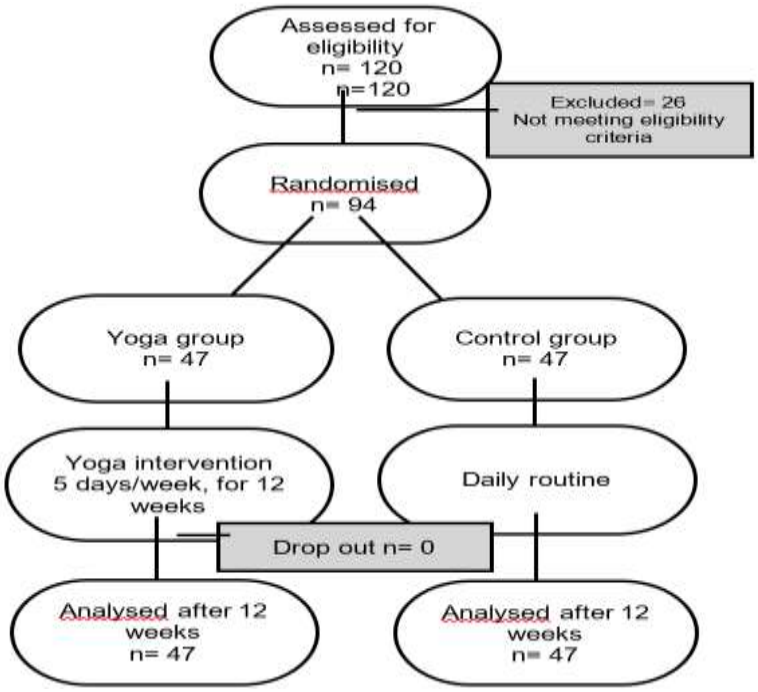


Figure 1: CONSORT diagram of study.

Table 1: Sequential Yoga Protocol (Pranayama → Core Asanas)

Component	Section	Duration	Physiological Target
Prayer	Opening	1 min	Mental centering
Kapalbhati	Pranayama Sequence	1 min	Sympathetic modulation
Bhastrika	Pranayama Sequence	1 min	Autonomic reset
Bhramari	Pranayama Sequence	1 min	Vagal activation
Rest	Pranayama Sequence	2 min	Integration
Tadasana	Core Asanas	1 min	Postural awareness
Triyak tadasana	Core Asanas	1 min	Lateral stretch
Naukasana	Core Asanas	5 min	Core activation
Suryanamaskar	Core Asanas	10 min	Dynamic sequencing
Shavasana	Core Asanas	2 min	Parasympathetic recovery

2.6 Control Group

Participants continued their usual daily routine with same dietary patterns without any structured exercise, yoga, or meditation program. Also, they were assessed time-to-time to confirm that they were following their usual routine and weren't experiencing any changes.

2.7 Outcome Measures

Sympathetic Tone: Cold Pressor Test (CPT) and Hand Grip Test (HGT) measuring peak SBP and DBP responses (10,11).

Anxiety: Hamilton Anxiety Rating Scale (HAM-A), 14 items scored 0-4 (12).

Lipid Profile: Total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C using enzymatic methods. Atherogenic ratios (TC/HDL-C, LDL-C/HDL-C, TG/HDL-C) were computed to assess cardiovascular risk. (13)

Remnant Cholesterol: Calculated as TC minus HDL-C minus LDL-C (14).

2.8 Statistical Analysis

Data were analyzed using SPSS version 30. Normality was tested using Shapiro-Wilk test. Independent t-test was used for normally distributed data; Mann-Whitney U test for non-normal data (Table-3). Significance was set at $p<0.05$.

2.9 Limitations regarding statistical power

While the study was adequately powered for primary (HAM-A) and most secondary outcomes (CPT, HGT, TC, HDL-C, LDL-C, remnant cholesterol), the power for triglycerides was modest (65%) due to non-normal distribution and larger-than-expected variance. Future studies targeting triglycerides as a primary outcome should enroll at least 136 participants (68 per group) to achieve 80% power with a small-to-medium effect size (d=0.48).

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3. RESULTS

3.1 Baseline Characteristics

The yoga and control groups were well-matched at baseline with no significant differences in any demographic or anthropometric parameter. The mean age was 19.8 ±1.9 years in the yoga group and 19.6 ±1.8 years in the control group ($p = 0.602$). Body mass index was comparable between groups (yoga: 22.0 ±3.4 kg/m² vs. control: 21.8 ±3.2 kg/m²; $p = 0.768$). Although the yoga group had a higher proportion of males (40 vs. 7) compared to the control group (34 vs. 13), this difference was not statistically significant ($p = 0.124$). These data confirm successful randomization and baseline equivalence.

3.2 Neuro-Endocrine-Metabolic Axis Outcomes

3.2.1 Sympathetic Tone (Neuro Axis) and Anxiety (Endocrine Axis)

Table- 2

Parameter	Control (n=47)	Yoga (n=47)	p-value
Baseline CPT-SBP (mmHg)	141 ±5.95	144 ±8.43	0.061
Baseline CPT-DBP (mmHg)	85.5 ±3.21	86.9 ±5.91	0.168
Post CPT-SBP (mmHg)	136 ±4.88	132 ±7.31	0.001
Post CPT-DBP (mmHg)	86.9 ±2.55	82.4 ±4.15	<0.001
Baseline HGT-SBP (mmHg)	138 ±3.43	140 ±6.87	0.066
Baseline HGT-DBP (mmHg)	91.8 ±1.97	92.4 ±3.16	0.290
Post HGT-SBP (mmHg)	138 ±2.90	135 ±6.15	0.003
Post HGT-DBP (mmHg)	92.0 ±2.02	87.7 ±3.18	<0.001
Baseline HAM-A	19.7 ±6.10	17.8 ±5.26	0.107
Post HAM-A	22.8 ±4.97	10.6 ±3.56	<0.001

*p-value compares yoga post vs. control post; Independent t- test; *p <0.05 Significant; p <0.001 Highly Significant*; Baseline CPT-SBP (mmHg)= Baseline Cold Pressor Test Systolic Blood Pressure in mmHg*; Post CPT- SBP= post intervention cold pressor test Systolic Blood Pressure in mmHg*; CPT-DBP (mmHg)= Baseline Cold Pressor Test Diastolic Blood Pressure in mmHg*; Post CPT- DBP= post intervention cold pressor test Diastolic Blood Pressure in mmHg*; HGT= Hand Grip Test*; HAM-A= Hamilton Anxiety Rating Scale*.

Observation: The yoga group showed significantly attenuated sympathetic responses to both cold and isometric stress, indicating improved autonomic regulation (Table-2). Yoga significantly reduced anxiety scores, while controls showed worsening over 12 weeks.

3.2.3 Lipid Profile and Remnant Cholesterol (Metabolic Axis)

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Table-3: Lipid Parameters and Remnant Cholesterol					
Parameter	Control (Baseline)	Control (Post)	Yoga (Baseline)	Yoga (Post)	p-value
TC (mg/dL)	160 ±24.1	171 ±23.9	162 ±27.8	150 ±20.8	<0.001
TG (mg/dL)	137 ±70.5	148 ±78.5	133 ±63.9	116 ±48.0	0.019
HDL-C (mg/dL)	50.7 ±5.61	47.8 ±5.21	48.8 ±9.02	52.2 ±9.11	0.005
LDL-C (mg/dL)	83.3 ±20.5	89.0 ±20.9	84.4 ±26.6	76.9 ±22.3	0.007
Remnant C (mg/dL)	26.0 ±16.8	34.2 ±19.4	23.0 ±15.2	15.1 ±12.4	<0.001
		Mann-Whitney U Test			
TG (mg/dL)*	—	128 (90-198)	—	98 (75-146)	0.019

*p-value compares yoga post vs. control post; Independent t- test; *p <0.05 Significant; p <0.001 Highly Significant; TC= total cholesterol*; HDL-C= high density lipoprotien*; TG= triglycerides*; Mann-Whitney U Test for TG

Table-4			
Parameter	Control (Post)	Yoga (Post)	p-value
TC/HDL-C ratio	3.58 ±0.71	2.87 ±0.52	0.002
LDL-C/HDL-C ratio	1.86 ±0.48	1.47 ±0.39	0.008
TG/HDL-C ratio	3.10 ±1.92	2.22 ±1.04	0.015

*p-value compares yoga post vs. control post; Independent t- test; *p <0.05 Significant; p <0.001 Highly Significant; TC= total cholesterol*; HDL-C= high density lipoprotien*; TG= triglycerides*

Observation: Remnant cholesterol decreased by 34.3% in the yoga group compared to a 31.5% increase in controls. After 12 weeks, the yoga group showed significantly improved atherogenic ratios compared to controls: TC/HDL-C (2.87 ±0.52 vs. 3.58 ±0.71; p = 0.002), LDL-C/HDL-C (1.47 ±0.39 vs. 1.86 ±0.48; p = 0.008), and TG/HDL-C (2.22 ±1.04 vs. 3.10 ±1.92; p = 0.015) (Table-3). Clinically, a TC/HDL-C ratio below 3.5 is desirable; the yoga group achieved 2.87 (desirable range), while controls remained at 3.58. A TG/HDL-C ratio exceeding 3.0 indicates increased insulin resistance; the yoga group reduced this ratio below threshold (2.22), whereas controls remained elevated (3.10) (Table-4). No baseline differences existed (p > 0.05), confirming that yoga favorably modified the atherogenic lipid profile.

4. DISCUSSION

This randomized controlled trial demonstrates that a 12-week sequential yoga protocol (pranayama followed by core asanas) significantly modulates the neuro-endocrine-metabolic axis in young students, evidenced by reduced sympathetic tone, decreased anxiety, and improved remnant cholesterol levels. Our findings provide integrated evidence for yoga as a holistic intervention targeting the interconnected stress-response pathways.

4.1 Neuro Axis: Modulation of Sympathetic Tone

The autonomic nervous system serves as the rapid-response arm of the neuro-endocrine-metabolic axis. Chronic stress perpetuates sympathetic dominance, leading to elevated blood pressure, tachycardia, and metabolic dysregulation (15). Our results showed significant attenuation of blood pressure responses to both cold pressor and hand grip tests in the yoga group (CPT-SBP: $p=0.001$; HGT-DBP: $p<0.001$).

The observed improvement can be mechanistically explained by pranayama-induced vagal activation. Kapalbhati and Bhastrika, rapid breathing techniques, have been shown to reset the medullary cardiovascular centers, while Bhramari (humming bee breath) directly stimulates the auricular branch of the vagus nerve (16). This is consistent with Shobana et al. (2022), who reported improved autonomic function after long-term yoga training (7). The sequential design—pranayama before asanas—may prime the nervous system for deeper parasympathetic engagement during postural practice.

4.2 Endocrine Axis: Anxiety Reduction

The HAM-A scores decreased dramatically in the yoga group (17.8→10.6) while increasing in controls (19.7→22.8), suggesting that yoga not only alleviates anxiety but may also protect against stress-induced worsening. The 40% reduction aligns with previous studies in medical students (8,9).

The endocrine mechanism likely involves downregulation of the HPA axis. Pranayama reduces cortisol secretion through feedback inhibition at the hypothalamic level, while asanas decrease sympathetic drive to the adrenal medulla, reducing epinephrine and norepinephrine release (17). The 12-week duration appears sufficient for neuroplastic changes in the limbic system, particularly the amygdala and prefrontal cortex, which regulate fear and anxiety responses (18).

4.3 Metabolic Axis: Remnant Cholesterol Improvement

Traditional lipid parameters improved significantly in the yoga group. Notably, remnant cholesterol—a novel cardiovascular risk marker—decreased by 34.3% in the yoga group compared to a 31.5% increase in controls ($p<0.001$). Remnant cholesterol represents triglyceride-rich lipoproteins (VLDL and IDL) that penetrate arterial walls and promote atherosclerosis (5).

The metabolic mechanism involves multiple pathways. First, reduced sympathetic tone decreases hormone-sensitive lipase activity in adipose tissue, reducing free fatty acid mobilization and subsequent VLDL synthesis (19). Second, improved vagal tone enhances insulin sensitivity, reducing hepatic glucose output and triglyceride production. Third, stress reduction lowers cortisol, which otherwise promotesvisceral adiposity and dyslipidemia (20). These findings extend the work of Nagarathna et al. (2021) and Meher et al. (2023) by specifically quantifying changes in cholesterol levels (21,22).

4.4 Integrated Axis Interactions

The neuro-endocrine-metabolic axis operates as a bidirectional network, not as isolated components. Sympathetic overactivity (neuro) stimulates adrenal cortisol release (endocrine), which in turn upregulates hepatic apolipoprotein B production (metabolic), increasing remnant cholesterol. Yoga appears to interrupt this cascade at multiple points. The sequential protocol—pranayama first (direct vagal stimulation), then asanas (proprioceptive input to hypothalamic centers), then relaxation (parasympathetic consolidation)—may create synergistic effects exceeding any single component (23).

Table-5 Comparison with Existing Literature

Study	Population	Intervention	Key Findings
Ashtaputre (2015) (8)	Youth	General yoga	Anxiety reduction
Aman et al. (2022) (9)	Medical students	Yoga	Stress improvement

Table-5 Comparison with Existing Literature			
Kanchana et al. (2024) (24)	Young adults	Pranayama	Handgrip endurance
Subramanian et al. (2012) (25)	Students	Sudarshan Kriya & Pranayama	Lipid profile
Present study	Medical students	Sequential (pranayama→core asanas)	Neuro-endocrine-metabolic axis modulation + remnant cholesterol

4.6 Strengths of study

The study has several strengths. It used a randomized controlled trial design, which reduces bias. It looked at anxiety, sympathetic activity, and lipids, giving a complete picture. The study was done well with high adherence, with an attendance rate exceeding 90% for the 12-week intervention period, and no dropouts were recorded, making the data reliable. The researchers followed guidelines, making it easy for others to repeat the study.

4.7 Limitations and Missing Information

Several limitations should be addressed in future research. Direct measurement of cortisol, catecholamines, and HRV was not performed, limiting mechanistic insights. The absence of an active control group prevents attributing effects specifically to yoga. The 12-week intervention and lack of long-term follow-up restrict sustainability assessment; future studies should extend to 24-48 weeks. The predominantly male, single-center medical student sample limits generalizability. Regarding statistical power, while the study was adequately powered for most outcomes, power for triglycerides was modest (65%) due to non-normal distribution; future studies targeting triglycerides should enroll at least 136 participants (68 per group) to achieve 80% power (d=0.48). Finally, mechanistic studies examining gene expression, epigenetic modifications, and inflammatory markers (e.g., hs-CRP, IL-6) are needed to elucidate molecular pathways underlying yoga's effects on the neuro-endocrine-metabolic axis.

5. CONCLUSION

A 12-week sequential yoga protocol (pranayama followed by core asanas) positively modulates the neuro-endocrine-metabolic axis in young students. The intervention significantly reduced sympathetic tone ($p<0.001$), anxiety ($p<0.001$), and remnant cholesterol ($p<0.001$) compared to controls. These findings suggest that yoga can serve as an effective, low-cost, non-pharmacological intervention for stress-related neuro-endocrine-metabolic dysregulation. Yoga may be particularly valuable for young adults in high-stress academic environments, potentially reducing long-term cardiovascular risk.

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Declaration of AI Use

During the preparation of this manuscript, the authors used AI-assisted grammar and language correction tool Google Gemini solely for improving readability, grammar, and sentence structure. No artificial intelligence was used for data collection, statistical analysis, result interpretation, hypothesis generation, or scientific decision-making. All AI-suggested changes were reviewed and approved by the authors. The authors take full and exclusive responsibility for the accuracy, integrity, and originality of the work presented.

Additional Information

Author Contributions

Every author accept full liability for the content and have personally examined as well as accepted the final copy.

Declaration of Interest: None

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