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Effects of Practicing Yoga and Meditation on Cortisol Hormone Rhythm and Immunoglobulin A among Medical Students at the University of Pécs: A Mixed Method Study

PhD Thesis

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Doctoral School of Health Sciences

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Dedication

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In memory of my grandfather, Nanu Ji, and Guru Ji

To my father, Mother, whole family, lovely nieces (Amayra and Sehar), special friends (Sachal, Gauri, and Ann) for their unwavering and constant love and support. To my esteemed PhD colleagues, Dahabo and my closest sisters, Maha and Leman, whose intellectual companionship and unwavering support have been invaluable, and to all my friends and colleagues in the vibrant city of Pécs, who have made this chapter of my life so unforgettable, I extend my heartfelt appreciation.

I am profoundly grateful for every moment, every sacrifice, and every act of kindness that has contributed to this endeavor. Without the collective belief and support of these extraordinary individuals, this accomplishment would have remained a distant dream.

Finally, with the utmost humility and respect, I dedicate this dissertation to my students, whose eagerness to learn and unwavering participation in this research have been a constant source of motivation. I hope this work helps students manage stress and promotes their well-being throughout their lives.

Acknowledgement

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TABLE OF CONTENT

Abbreviations

APA: American Psychological Association

BSI: Brief Symptom Inventory

BMI: Body Mass Index

BWV: Brain Wave Vibration Procedure

CG: Control Group

CONSORT: Consolidated Standards of Reporting Trials

CT: Combined training group

DASS-21: Depression Anxiety Stress Scales-21

DERS: Difficulties in Emotion Regulation Scale

ECLIA: Electrochemiluminescence Immunoassay

EIA: Enzyme Immunoassay

FBG: Fasting Blood Glucose

GSY: Goodbye Stress with Yoga

HbA1C: Hemoglobin A1c

HbA1C_IFCC: Hemoglobin A1c (International Federation of Clinical Chemistry)

HDL: High-Density Lipoprotein

HPA: Hypothalamic-Pituitary-Adrenal

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

ISQ: Illness Symptoms Questionnaire

LDL: Low-Density Lipoprotein

MAAS: Mindfulness Attention Awareness Scale

MET: Metabolic Equivalent of Task

MVPA: Moderate-to-Vigorous Physical Activity

Non-HDL: Non-High-Density Lipoprotein

PA: Physical Activity

PNEIMED: Psychoneuroendocrinoimmunology-based meditation

PSQI: Pittsburgh Sleep Quality Index

PSS: Perceived Stress Scale

RCT: Randomised Controlled Trial

RIA: Radioimmunoassay

SCN: Suprachiasmatic Nucleus

SD: Standard Deviation

SVS: Six-Item Subjective Vitality Scale

T2DM: Type 2 Diabetes Mellitus

WHO: World Health Organization

WHOQOL: World Health Organization Quality of Life

Introduction

University students face a pretty demanding transition from adolescence into early adulthood (Dai, Chen, & Sharma, 2023). This brings significant psychological and physiological changes, including elevated stress (Hogan & Astone, 1986). During this period, students feel independence by leaving home, lacking parental supervision, changing in close relationships, adjusting to culture with new people, and facing many situations that create stress (Dai et al., 2023). There are various contributing factors attributed to the stress experienced by college students (Karyotaki et al., 2020). Evidence shows that compared to the general population, medical students have poor health status (Abdulghani, AlKanhal, Mahmoud, Ponnamperuma, & Alfaris, 2011; Bergmann, Muth, & Loerbroks, 2019). This is explained by medical students having higher levels of academic stress and psychosocial pressure (Karyotaki et al., 2020). According to the American Psychological Association, around 87% of university students reported education as their primary source of stress (Association, 2020).

Among medical students, chronic stress is a critical concern, especially its effect on academic performance and the student's overall health (Abdulghani et al., 2011). If we look at the educational curriculum of medical students, it is characterized by very demanding, high expectations from peers and faculty, and frequent assessment; these characteristics significantly contribute to elevating the stress level among medical students (Minhas et al., 2019). Evidence shows that approximately 63.1% of the medical students reported high levels of academic stress, which increased more during the exam period and led to anxiety and depression symptoms (Husnain, 2017). The pressure of the training increases with contributing factors such as parental expectation, fear of failure, and challenges faced during training (Melaku, Mossie, & Negash, 2015). Additionally, coming to medical school involves many lifestyle changes, including adapting to a new environment, separation from close relationships, and more time consumed in training, which also elevates stress levels (Siraj et al., 2014).

The research presented that chronic stress can lead to many health conditions and habits, such as substance abuse, impaired quality of sleep, and higher levels of suicidal thoughts among students(Salam et al., 2015). The collective effect of these factors can cause a decrease in mental health, including students reporting hopelessness, panic attacks, and anxiety (Puthran, Zhang, Tam, & Ho, 2016; Salam et al., 2015). It is essential to note that gender differences in the stress response are of concern as female medical students moreover report increased stress levels compared to male students (Moutinho et al., 2017). This result also highlights the coping mechanisms between the genders, including how stress is managed and perceived (Moutinho et al., 2017). The stress intensity is elevated as the medical student progresses with the year of training, especially when they have more clinical practice (Ibrahim & Abdelreheem, 2015; Siraj et al., 2014).

The highly competitive and continuously demanding environment of medical colleges can exacerbate these stressors, leading to psychiatric symptoms such as anxiety, depression, and burnout (Malathi &

Damodaran, 1999). It is important to address these stressors and provide adequate support and resources to help students cope with the demands of their education. Research conducted in Sweden has indicated that 12.9% of medical students experience symptoms of depression, and 2.7% have attempted suicide due to stress (Dahlin & Runeson, 2007). These findings highlight the seriousness of the issue. It is very important to understand the negative impact of chronic stress on the mental health of students is not limited to academic achievement or goals alone. Still, it can lead to a negative impact on their overall well-being and health (Dahlin & Runeson, 2007). By prioritizing students' mental health and well-being, universities and medical colleges can create a supportive environment that fosters academic success and promotes a healthy and fulfilling life.

It is shown that chronic stress can alter the hypothalamic-pituitary-adrenal (HPA) axis, which causes higher secretion of cortisol in individuals (Mbiydzenyuy & Qulu, 2024).

Cortisol hormone is a form of hormone from the steroid family called Glucocorticoid. It is a neuroendocrine hormone which can be measured by saliva (Nicolson, 2008). Secretion of the cortisol hormone is regulated by the nervous system, and its production is directly controlled by the brain, which can be affected in the response to Internal and external triggers (Thau, Gandhi, & Sharma, 2023). Cortisol is a master hormone responsible for regulating the physiological function around the 24-hour light/dark cycle (N. Smyth, Hucklebridge, Thorn, Evans, & Clow, 2013). This cycle is known as the Circadian rhythm. It can be defined as a periodic pattern that takes 24 hours, where the light and dark cycles synchronize the environment with biological function (Selfridge, Moyer, Capelluto, & Finkielstein, 2015). The circadian system coordinates physiological and behavioral responses to the environment in a way that an individual's body acts like a finely harmonized clock. The suprachiasmatic nucleus (SCN) located in the hypothalamus plays a key role as the master regulator of this clock, coordinating 24-hour rhythms in both the body's central and peripheral physiological functions, including other brain regions and peripheral tissues (Mohd Azmi et al., 2021). Cortisol hormone affects nearly all tissues and organs of the body, and it serves as a critical component of the circadian system by regulating various biological cyclical functions (Mohd Azmi et al., 2021). Cortisol status can be considered an objective biological indicator of the stress response and has been linked to a multitude of health conditions (Benarroch, 2008).

Although cortisol is important for managing acute stress (Gaab et al., 2003), elevated cortisol levels are closely linked to chronic stress, which has significant effects on the body, such as increased body weight, higher blood glucose levels, hormonal imbalance, sleep disorders, and insulin resistance, which collectively raise the risk of many non-communicable diseases (Jones & Gwenin, 2021; Lee, Kim, & Choi, 2015; N. Smyth et al., 2013). Regular chronic stress is strongly linked to many mental health conditions, such as anxiety, depression, impaired cognitive function, and poor quality of sleep (Manchia et al., 2022). It has been proved that medical students are vulnerable to high cortisol levels (Murphy, Denis, Ward, & Tartar, 2010). Additionally, they suffer in many situations with emotional challenges,

for example, witnessing suffering, dealing with patients, etc (Dyrbye, Thomas, & Shanafelt, 2005). Cortisol hormones influence the body's immunity; the effect can be acute and chronic on the immune system, especially immunoglobulin A (Elenkov, Webster, Torpy, & Chrousos, 1999). However, acute stress improves immune function by releasing pro-inflammatory cytokines, temporarily improving immune defence (Daniela et al., 2022). At the same time, chronic stress can lead to immunosuppression, which means dysregulation of the immune system, including a non-adaptive immune response (Munford & Pugin, 2001). A higher level of cortisol in the body is associated with disturbing the balance of cytokine production, which impairs the body's ability to decrease effective immune response against possible pathogens (Chrousos, 2000).

Due to medical student's chronic stress, they are more at risk of this disturbance in immune response, which makes them more vulnerable to infections and many health issues (Rosiek, Rosiek-Kryszewska, Leksowski, & Leksowski, 2016). Immunoglobulin A is one of the important components of the immune system, which is found in mucosal areas of the body and plays a vital role in preventing the colonization of pathogens on the mucosal surface (Chrousos, 2000; Woof & Kerr, 2006). Evidence shows that high-level IgA results in better immune function and lower health problems, especially if the individual suffers from chronic stress (Bellosta-Batalla et al., 2018). Prolonged exposure to chronic stress and high levels of cortisol indicate lower levels of IgA (Viena, Banks, Barbu, Schulman, & Tartar, 2012). It is essential to investigate further the relationship between cortisol hormones and IgA among medical students due to the impact of their higher chronic stress levels on overall health and well-being. Educational institutes face challenges in creating a supportive environment for medical students to help them reduce their chronic stress levels (Sundarasen et al., 2020). Yoga is classified as a combined mind-body intervention that integrates all the components of an individual's lifestyle, thus fostering overall well-being (Bhargav, 2018). Yoga is one of the best complementary medicines as it helps relax the body and mind, relieves stress, helps with weight loss, regulates blood circulation, and boosts the metabolism of the body (Chauhan et al., 2024). In the traditional yogic text, there are mentioned some Yogic postures specifically impact on reproductive organs, the uterus and ovaries, and also on digestion (S. Mishra & Dash, 2017). The system does not primarily revolve around focusing on the purpose of the treatment objective (D. Singh, Kishore Chaturvedi, Singh, & Kandan, 2022). However, evidence shows that consistent yoga practice promotes and maintains health conditions and manages disease.

Studies have shown that yoga positively impacts insulin resistance, lipid profile, stress anxiety, and glycaemic control (V. P. Singh & Khandelwal, 2020). It has been proven that stress is the leading cause of many diseases 65. It continuously disturbs the entire body's mechanism with the dysregulation of hormones by producing higher cortisol levels, leading to many health conditions (Ding et al., 2021). If we look at the sequence of yoga practice, it works in the opposite direction and brings balance to the dysregulated hormone by reducing cortisol secretion (Padmavathi et al., 2023). Additionally, the influence of yoga is proven on the autonomic nervous system, which is very important in regulating the

metabolic process (Udupa & Sathyaprabha, 2018). Yogic practices, such as pranayama meditation and asana, may enhance vagal activity, influencing autonomic flexibility 47.

Yoga practice has been associated with increased cytokines such as interferon-c and interleukin-12, which also improves the immune system (Lim & Cheong, 2015), which may help in improving metabolic function and restoring hormonal balance (Çetinoğlu & Pehlivan). Hence, yoga should not only be considered complementary to daily routine but also a beneficial approach to improve clinical outcomes due to chronic stress in people. This study just not only showed the importance of yoga clinically but also the need for yoga intervention. Research has shown that yoga can benefit the phases of the menstrual cycle by improving the balance of the neuroendocrine axis and bringing physical and psychological well-being (Daniela et al., 2022). Moreover, the effect of yoga has shown a positive impact on physiological changes, including blood glucose levels, haematological variables, and hormonal status (Pandi-Perumal et al., 2022). It is also proven that yoga improves immunity levels and helps in managing chronic diseases, for example, respiratory disorders, metabolic syndrome, endocrine disorders, obesity, cancer, etc (Madan, Sembhi, Khurana, Makkar, & Byati, 2023). Studies also demonstrated that regular yoga practice regulates the secretion of hormones in the body, for example, cortisol, renin, glucose, epinephrine, and norepinephrine; these hormones play very crucial roles in maintaining the body's function (Kanchibhotla, Subramanian, & Singh, 2023; Kumorojati, Alfie, & Warseno, 2020).

Overall, evidence indicates that regular yoga practice can positively impact hormonal and metabolic pathways, resulting in many health benefits for diverse populations and people who suffer from chronic stress.

The Theoretical framework of the dissertation

Research demonstrates various theories of chronic stress management, cortisol hormone balance, and immune response, which shows the complexity of this concept according to scientific understanding. Regarding cortisol hormone levels and Immunoglobulin A levels, the study shows that chronic stress response presents both complex and linear theoretical models at the community level or individual levels (Barakian, Hajisadeghi, Keykha, Mohammadbeigi, & Karimi, 2021; Sutherland et al., 2019). The study uses biomedical models to interpret stress or chronic stress as a higher level of cortisol hormone and physiological dysfunctions. Although this provides notable insights into the physiological impact of chronic stress, it might be reductive and doesn’t highlight the broad psychosocial factors that affect chronic stress and can cause several health outcomes (Doane & Adam, 2010).

In brief, the holistic approach demonstrates the correlation of social, psychosocial, and biological factors. Research has also proven that the biopsychosocial model can significantly improve cortisol

hormone rhythm and IgA levels; the biopsychosocial model includes environmental influences, psychological distress, and lifestyle choices, such as practicing yoga intervention and meditation(Kumorojati et al., 2020). This framework emphasizes the interconnectedness of psychological distress, environmental influences, and lifestyle choices in shaping stress responses. To comprehensively explore these psychosocial dimensions in the context of yoga intervention, the current study employed a selection of established questionnaires.

Following this framework, it is crucial to educate medical students who face challenges due to high levels of stress. It is also important to assist educational institutions in providing stress management techniques that are easily feasible for medical students and less time-consuming. Medical students need to adopt a holistic approach that helps reduce chronic stress on their health, such as engaging in meditation, breathing techniques, and yoga practice(Warnecke, Quinn, Ogden, Towle, & Nelson, 2011). However, there is a gap in the literature exploring the effect of yoga and meditation on cortisol hormone IgA levels, a psychosocial parameter among medical students.

Using the above-mentioned theoretical framework, the multicausal model (Figure 1) was created and used to determine if a regular practice of yoga in students could modify the perception of psychological stress, salivary cortisol, and Immunoglobulin A.

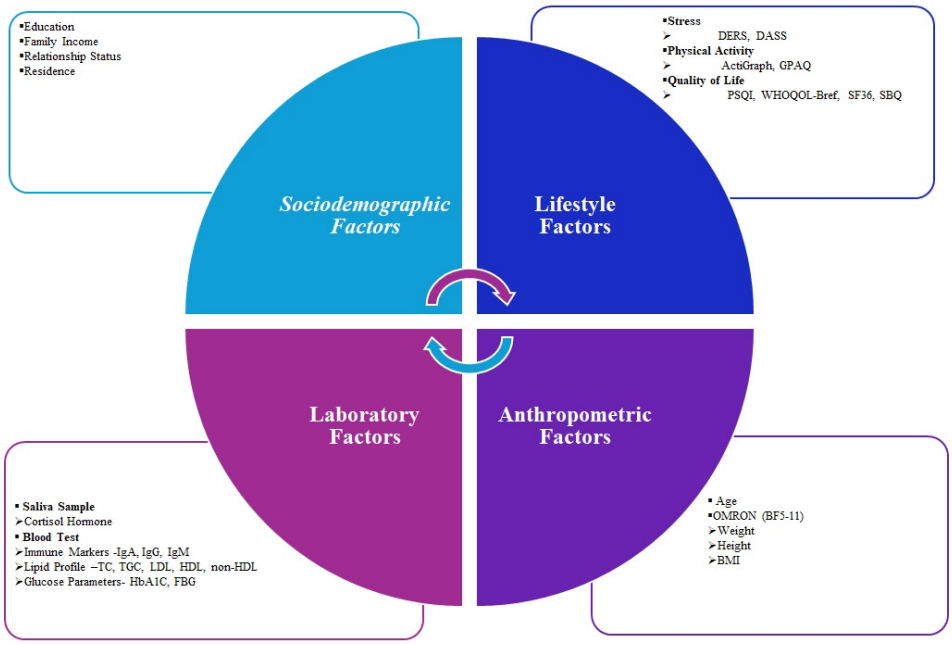

Figure 1: Multicausal model to achieve the objective of the current study.

*Note: Created by the author based.

Dissertation aims

The aim was to investigate the Effects of Yoga and Meditation on Cortisol Hormone Rhythm and Immunoglobulin A among medical students.

Specific aims

- To explore a qualitative synthesis of a systematic literature review of Cortisol and IgA Measurement after Yoga Interventions: A Qualitative Synthesis.
- To evaluate the effect of yoga on medical students to reduce the level of depression, anxiety, and stress: a pilot study.
- To explore the effect of 10 weeks of yoga intervention on Immune and metabolic parameters among medical students.
- To evaluate the effect of yoga intervention on medical students' mental health and well-being.
- To explore the effect of yoga intervention on medical students' salivary cortisol level: Randomised control trial.

Sub-study 1- Systematic literature review of cortisol hormone and IgA measurement after yoga interventions: a qualitative synthesis

1.1 Introduction

In recent years, yoga has gained popularity as an effective method for reducing chronic stress and improving overall well-being (Dai et al., 2023). This is the practice that involves physical postures with breathing techniques and various kinds of meditation (J. C. Smith, 2005). Yoga practice has an influential effect in regulating stress response and improving immune function; it has gained noticeable popularity in scientific research; however, findings are inconsistent (B. Mishra et al., 2024). Cortisol is a known steroid hormone produced by the adrenal glands and is the key marker of stress (El-Farhan, Rees, & Evans, 2017). The cortisol hormone is usually measured by using saliva samples and blood samples (El-Farhan et al., 2017). Similarly, the Immune parameter IgA is found in the mucosal area of the body and also plays a very important role in immune function and response; this parameter can reflect the effect of stress on the immune response of the individual (Staley, Conners, Hall, & Miller, 2018).

Moreover, yoga intervention studies focus on psychological parameters and have fewer measured biochemical parameters. A recent study from Jordan shows that yoga intervention helps significantly reduce the cortisol hormone of nursing students (Alhawatmeh et al., 2022). However, a lack of evidence is shown in the case of the effect of yoga on IgA among various populations. A systematic review (Yıldırım & Satılmış, 2022) by Yıldırım and Güngör highlights the impact of yoga intervention in

regulating the HPA (hypothalamic-pituitary-adrenal) axis. The HPA axis is responsible for the production of cortisol hormone, and it also has a potential effect on the immune function, which includes IgA levels (Jefferies, 1991). The study by Bower et al. shows that yoga intervention influences the sensitivity of glucocorticoid receptors and also decreases inflammatory signalling; this also shows that yoga intervention elucidates the mechanism of cortisol hormone and immune response (Bower et al., 2014).

The current systematic literature review aims to explore the most appropriate measuring method of cortisol hormone and IgA in the context of yoga intervention studies.

1.2 Methods/Design

The current Literature review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) 2020, (Parums, 2021) Figure 2. The search was conducted in four databases: PubMed, Embase, Cochrane Trials, and Web of Science.

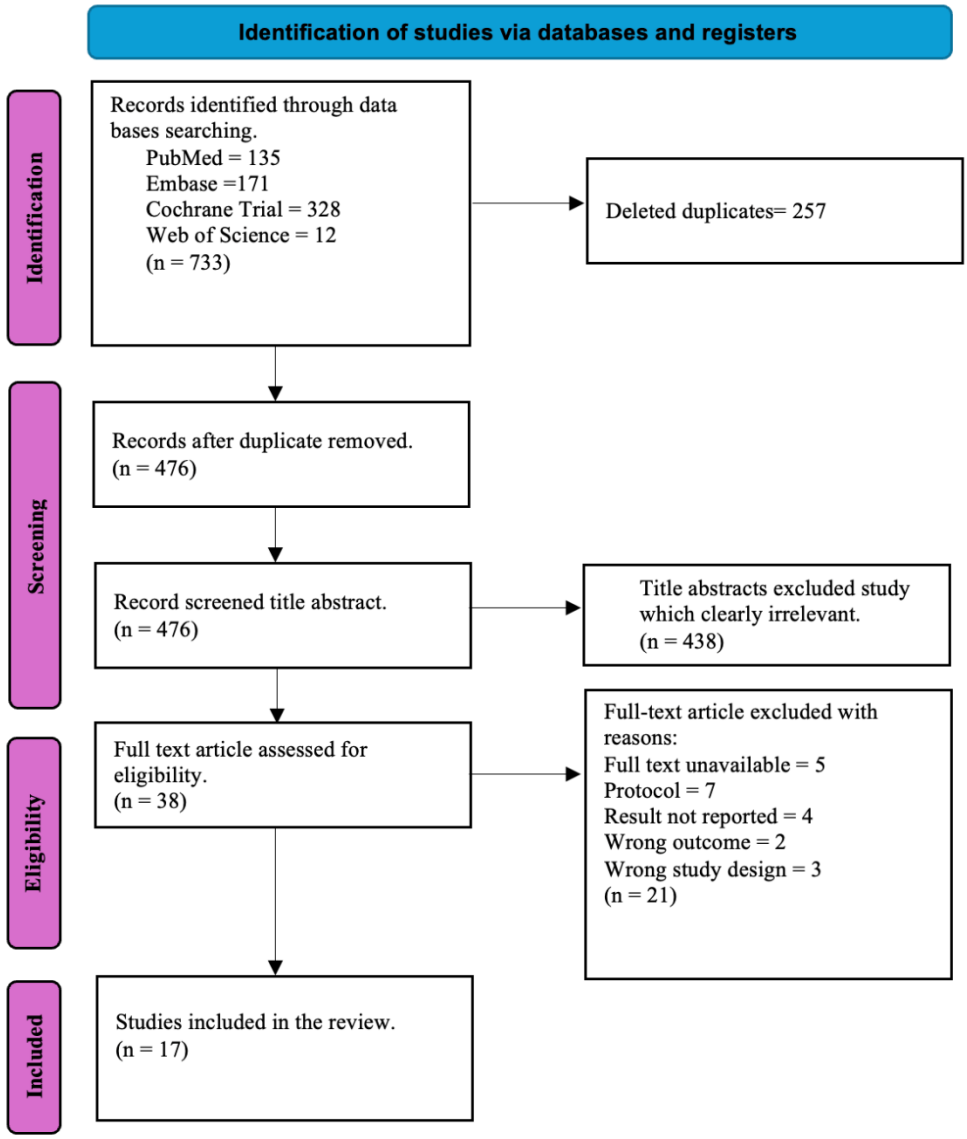

Figure 2: PRISMA 2020 flow diagram of the current systematic literature review.

The search was limited to the English language with no timeline restriction. Inclusion and exclusion criteria with a self-built search strategy are presented in Table 1. In addition, the search was conducted using the Google search engine to check for eligible grey literature.

Table 1: Criteria for Inclusion/Exclusion and Search Strategy used in the current Literature Review.

	Inclusion criteria	**Exclusion criteria**	**Search Strategy (MeSH terms and keywords)**
Intervention	All yoga form was included. Studies in which yoga therapy as an intervention was eligible for the review. No limitation to the timeline of intervention was given.	Studies have not specified yoga intervention.	Yoga [MeSH] OR Yoga [Title/Abstract] OR Yog* [Title/Abstract] OR Pranayama [Title/Abstract] OR Asana [Title/Abstract] OR Ashtanga [Title/Abstract] OR Ashtanga-based Yoga [Title/Abstract] OR Ashtanga Yoga [Title/Abstract] OR Bikram Yoga [Title/Abstract] OR Yoga Bikram [Title/Abstract] OR Kundalini Yoga [Title/Abstract] OR Yoga Kundalini [Title/Abstract] OR Iyengar Yoga [Title/Abstract] OR Hatha Yoga [Title/Abstract] OR Hatha [Title/Abstract] OR Viniyoga [Title/Abstract] OR meditation [MeSH] OR meditation [Title/Abstract] OR transcendental Meditation [Title/Abstract] OR Meditation, Transcendental [Title/Abstract] OR Sudarshan Kriya yoga [Title/Abstract] OR Kripalu yoga [Title/Abstract] OR Yin yoga [Title/Abstract] OR dharana [Title/Abstract] OR dhyana [Title/Abstract] OR samadhi [Title/Abstract] OR Isha kriya [Title/Abstract]
Comparison	The intervention group is compared to the control group, which doesn't receive any kind of therapy, which means the control group is with standard care and without any treatment or intervention. (If the trial includes multi-arms, we include any arm that meets the review inclusion criteria).		
Outcome	Cortisol Hormone and Immune parameters, including IgA.	No reporting or partially reporting outcome.	Cortisol Hormone [Title/Abstract] OR cortisol [Title/Abstract] OR stress hormone [Title/Abstract] OR Immunoglobulin A (IgA) (MeSH) OR IgA Antibody [Title/Abstract] OR IgA [Title/Abstract] or Glucocorticoid hormone [Title/Abstract] OR Hydrocortisone (MeSH) OR Immune system antibody [Title/Abstract] OR Secretory IgA (sIgA) [Title/Abstract] OR IgA antibody[Title/Abstract]
Study Design	Randomized control trial, Clinical Trial, and Experimental Study.	Any Observational Study.	Randomized controlled trial [MeSH] OR Controlled clinical trial [MeSH] OR Clinical trial [MeSH] OR Experimental study [MeSH] OR

Duplicates were removed using EndNote 21, and title abstract screening was conducted using Rayyan. The selection of the studies by title, abstract, and full-text screening was conducted by two independent reviewers; in the case of conflicts or disagreements, the third reviewer resolved each step by discussion. Data is presented in Table 2. by using a data extraction sheet which contains the information from all the included studies: 1) author's name, 2) year of study, 3) age of participant, 4) sample size, 5) study design, 6) outcome reported, 7) outcome assessment tool, 8) summary of results.

1.3 Results

In the Current Literature review, 733 citations were found after a systematic search in 6 databases (PubMed = 21, Embase = 45, Cochrane Trial = 54, Scopus = 83, Web of Science = 66, Clinical Trials

= 6). No relevant studies have been found other than these included studies from Google search. After removing duplicates, 476 citations were left, and 438 citations were excluded after performing title abstract screening. 38 studies were left and considered for full-text screening. After the full-text screening, 21 studies were excluded. In the end, 17 studies were included in the current review for qualitative synthesis. In total, 1007 participants were studied in the current review.

The result is summarised in Table 2, which includes comprehensive information on the included studies. Comparative RCT (Bowden, Gaudry, An, & Gruzelier, 2012) Involving 83 participants engaged in three interventions- BWV, Iyengar yoga, and mindfulness practice for 16 weeks showed significant improvement in psychological parameters, but all yoga groups did not show any improvement in salivary cortisol levels measured by ELISA assay kits. This result is contradicted by many included studies, such as the RCT trial by Hossam et al. (Alhawatmeh et al., 2022) showed that nursing students who participated in mindfulness practice for 5 weeks reported a reduction in serum cortisol; supporting these findings Kavita M.M et al. showed a significant reduction in serum cortisol among medical students who participated in yoga practice for regularly over six weeks (Kavitha, Chandrashekharayya, Anagha, & Kashinakunti, 2023). The variation in the findings can be understood by the different ways of measuring cortisol levels, different populations, and frequency of yoga practice. The current review also focuses on different measurement tools in yoga intervention studies for measuring serum cortisol, saliva cortisol hormone, and salivary immunoglobulin IgA in the included studies. We found a total of twelve studies that measure salivary cortisol utilizing eight distinct methods of measurement tools. which includes ELISA assay kits (Sarstedt saliva test containing a cotton wool swab) (Bowden et al., 2012), Luminescence immunosorbent assay (IBL-Tecan, Hamburg, Germany) (Szaszkó, Tschenett, Ansorge, & Nater, 2025), enzyme immunoassay (EIA) (Maheshkumar et al., 2022), Radioimmunoassay (Fan et al., 2024), Chemiluminescence immunometric assay involving competitive inhibition (West, Otte, Geher, Johnson, & Mohr, 2004), Electrochemiluminescence immunoassay (ECLIA) (Eda et al., 2018), Enzyme-Linked Immunosorbent Assay (ELISA) (Ozturk & Tezel, 2021), Enzyme immunoassay (EIA)(Bottaccioli et al., 2020), enzyme-linked immunosorbent assay (Raghavendra et al., 2009).

There were four included studies that measured serum cortisol levels which included ELISA reader (Gentaur/GDMS, Kampenhout, Belgium) (Alhawatmeh et al., 2022), Immunoassay method using Cortisol RIA KIT (Obaya et al., 2023), and Spectrophotometry (Kavitha et al., 2023). Only two studies were included that measured salivary IgA levels using enzyme-linked immunosorbent assay (ELISA) (Torkamani, Aghayousefi, Alipour, & Nami, 2018).

Table 2 Characteristics and key findings of included studies

Author & year	Participants	Interventions modality	Study Design	Duration	Outcomes	Summary of results
Deborah Bowden, (2011) UK	83 healthy participants (21 women, 12 men) Age 18-50	Brain Wave Vibration Procedure (BWV)- involved series of yogalike exercises which typically comprise Body and Brain Holistic Fitness class Iyengar Yoga Procedure- involved series of asana posture and breathing exercise Mindfulness Procedure- involved various mindfulness exercises, such as sitting and body-scan meditations and pair activity.	Comparative Randomized Control Trial	16 weeks	Mood - DASS21 Sleep- PSQI Vitality- SVS Mindfulness- MAAS Absorption- TAS Health- ISQ Memory- 2-Back Salivary cortisol- ELISA assay kits (Sarstedt salivettes containing a cotton wool swab)	Significant improvement shown in all interventions for mindfulness, while no changes occurred in health, memory, or salivary cortisol.
Hossam et al. (2022) Jordan	108 nursing students Experimental group- 54 (36 female and 18 male) Age 19.67+0.99 Control group - 54(28 female and 26 male) age 19.85+0.76	Experimental Group - Mindfulness meditation Control group- Usual Care	Randomized Control Trial	5 weeks	Trait mindfulness- MAAS Perceived Stress- PSS Serum Cortisol- ELISA reader (Gentaur/GDMS, Kampenhout, Belgium)	- Significant reduction in Serum Cortisol and Perceived stress scale
Kavitha M.M et al. (2021) India	80 Medical Students Age 18-20 years Experimental group- 40 (19 female and 21 male) Control group – 40 (20 female and 20 male)	Experimental Group – Regular 60-minute yoga and meditation practice provided by yoga instructor Control group- Usual Routine	Quasi Experimental Study	6 weeks	Pulse rate PR, Systolic blood pressure (SBP), Diastolic blood pressure (DBP), Respiratory rate (RR), Partial pressure of oxygen Serum cortisol Heart rate variability (HRV)	Significant decrease in serum cortisol levels and increased heart rate variability parameters in yoga practice group and meditation when compared to control group.
Fatemeh Fakharirad (2020) Iran	20 Coronary Artery Bypass Grafting patients CRT Group- 10 Participants with Age 64.3 ± 10.57 CT Group- 10 Participants with Age 61.1 ± 10.66	CRT Group - cardiac rehabilitation training group practice 3 times week for 60 minutes CT Group- combined training group include cardiac rehabilitation exercise with yoga practice 3 times week for 60 minutes	Quasi Experimental Study	8weeks	Salivary Cortisol Indices Salivary alpha-amylase salivary index	Significant effect was found in combined training for salivary cortisol parameters. Although this intervention did not show significant effect the alpha-amylase

						salivary index
Fatma Ozlem Ozturk (2020) Iran	72 university students Intervention group- 36 students (28 female, 8. male) Control group- 36 students (29 female, 7. male)	Intervention Group- Laughter yoga session for 45 minute two times a week Control group- Usual Routine	Randomized Control Trial	4 weeks	Mental Health - BSI Salivary Cortisol level	Significant reduction in salivary cortisol and improve in mental health outcome
Anushree Devashish Patil et al. (2023) India	52 Infertile women with PCOS 26 Intervention arm 26 Control arm	The intervention groups received 90 min of structured yoga intervention for 3 months Control group - Usual Routine	Randomized Control Trial	12 weeks	Reproductive Hormonal (TSH, LH, FSH, SHBG, FAI,) tested using chemiluminescence technique AMH using paramagnetic particle chemiluminescence immunoassay Biochemical Serum Cortisol Fasting Blood Sugar (FBS), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Cholesterol, Postglucose Blood Sugar (PGBS) Serum Glutamic Pyruvic Transaminase (SGPT) Done using spectrophotometry.	Significant difference in the weight, basal metabolic rate, postglucose insulin, anti-Müllerian hormone, cholesterol, high-density lipoprotein, low-density lipoprotein, serum glutamic pyruvic transaminase, ultrasound parameters such as stromal thickness, follicle number per ovary, and QoL
Bence Szaszk´o et al (2024) Austria	116 participants (Hatha yoga intervention) 54 intervention 44 control 83 females; MAge = 25.03 years; SDAge = 4.18 years; range 18–40 years)	Intervention group (IG) took part in an eight-week Hatha yoga course led by four certified yoga instructors (MJob Experience = 4.00 years; SDJob Experience = 1.15 years; range 3–5 years of professional experience) Waitlist control group (CG) had the opportunity to take part in the same intervention after the conclusion of posttests	Randomized Control Trial	8 weeks	Subjective momentary stress-visual analogue scale (VAS) Salivary cortisol (sCort)-determined using luminescence immunosorbent assay (IBL-Tecan, Hamburg, Germany) Salivary alpha-amylase (sAA) activity was determined using an enzymatic photometric test	Significant reduction of subjective momentary stress but there was no change in diurnal salivary cortisol or Salivary alpha-amylase (sAA) levels.
Hany Ezzat Obaya et al (2023) Saudi Arabia	58 women with T2DM (mean age: 45.67 ± 2.92 years) n = 29; mean age [46.1 ± 2.7 years])	Two groups: 1st group participating in aerobic training; slow, deep breathing; and mindfulness meditation (AT + DMM group	Randomized Control Trial	6 weeks (three sessions per week)	Stress levels-using the PSS. Scores on the PSS range from 0 to 40 IPAQ-assess participants' physical activity levels	Group undertaking aerobic training combined with slow, deep breathing

	Aerobic exercise combined with n = 29; mean age [45.24 ± 3.14 years])	2nd Group undertaking aerobic training			Total serum cortisol concentration- Immunoassay method and using the Cortisol RIA KIT	exercises and mindfulness meditation showed significantly lower levels of FBG and cortisol levels
Maria Meier et al. (2020) Canada	35 participants (18-34 years) (mean age =23.77, SD age =4.48; 51% female)	Intervention group LY (n=11), a relaxation breathing (n=12) Control group (n=12) session prior to their exposure to the Trier Social Stress Test for Groups (TSST-G)	Randomized Control Trial	30 minutes	Salivary cortisol Salivary alpha amylase Subjective stress	Laughter Yoga reduced the amount of stress hormones that were released in response to the situation
K. Maheshkumar et al. (2021) India	26 participants (15 female; 11 male) (11-19 years) Intervention n=13 Control group n=13	Intervention group-Yoga group participants were trained to do Bhr. P for 45 min, thrice a week for six months Control group- usual routine	Randomized Control Trial	24 weeks	Salivary Cortisol levels-enzyme immuno-assay (EIA)	Yoga group exhibited a higher salivary cortisol Response A significant interaction with time (F (1, 88) = 316.5, p = .001, hp2 :0.91) and between the group x time (F (3, 88) = 2.83, p = 0.04, hp2: 2:0.8) was found after the intervention.
Yaxin Fan et al. (2024), China	32 participants-male college students (mean age ± SD = 21.33 ± 0.63) n=16 Intervention n=16 control	Intervention group-Mindfulness Meditation Integrative Body-Mind Training (IBMT) Control–Relaxation training (RT)	Randomized Control Trial	7 week	Salivary cortisol and testosterone concentrations were analysed by Radioimmunoassay	Increased cortisol and testosterone concentrations after acute stress in both groups, but testosterone rise was not associated with cortisol rise.
Jeremy West, B.A. at al. (2004), USA	69 participants African dance (n = 21) Hatha yoga (n = 18) Biology lecture (n = 30) 47 (65%) of the participants were women 22 (35%) were men Mean age 19,range of (17-24)	The major aim of this study was to investigate the effects of: Intervention: Hatha yoga and African dance on two psychological and biological markers of stress Control Group: An instructional biology class two psychological and biological markers of stress	Randomized Control Trial	3 weeks	Salivary cortisol-chemiluminescence immunometric assay involving competitive inhibition Perceived stress-14-item Perceived Stress Scale (PSS)	African dance and Hatha yoga signifi-cantly decreased perceived stress and negative affect, compared to the biology class Cortisol increased in African dance, de-

						creased in Hatha yoga, and did not change in the biology class.
Nobuhiko Eda et al. (2018), Japan	23 participants (age 60.4 ± 10.4 years; height 154.0 ± 5.2 cm; body mass 50.7 ± 6.4 kg; body mass index 21.5 ± 2.7 kg/m2; 2; body fat percentage 28.1 ± 5.4%)	The participants rested for 90 minutes on the first day and performed yoga stretching for 90 minutes on the second day, which occurred two weeks after the rest trial. The rest and yoga trials were performed at the same time of day by all participants (pre: 9:45 a.m., post: 11:30 a.m.)	Randomized Control Trial	2 weeks	Salivary SIgA-enzyme-linked immunosorbent assay (ELISA) Cortisol and testosterone - Enzyme Immunoassay (EIA)	The cortisol concentration and secretion rate were lower and testosterone secretion rate higher after yoga. Yoga stretching can reduce stress and enhance mucosal immune function in elderly women.
Fatma Ozlem Ozturk and Ayfer Tezel (2021), Turkey	75 participants (students) Intervention group-n=38 Control group n=37	Intervention group - took eight sessions of laughter yoga, that is, two sessions per week, Laughter is encouraged by various laughter exercises Control Group-Normal	Randomized Control Trial	4 weeks	Saliva samples of cortisol were tested using electrochemiluminescence immunoassay (ECLIA)	Significant decrease in the cortisol levels among intervention group
Fatemeh Torkamani et al. (2018), Iran	30 participants (mean age 44 ± 3 years)s (mean age 44 ± 3 years). Intervention group (n=15) Control (n =15)	Participants in both the groups attended a structured introductory lecture about mantra-meditation Intervention group participants did group mantra-meditation (repeating the mantra with loud voice) for about 20 min. Control group did not has a defined task. they were left passive in rest condition, sitting still on their chairs.	Randomized Control Trial	20 min	Saliva sample s-IgA level - enzyme-linked immunosorbent assay	A significant change in affect among experimental group subjects as compared to controls
Anna Giulia Bottaccioli et al .(2019) Italy	40 participants (students) Intervention group n=20 y (8 males and 12 females) Control group n=20 (6 males and 14 females)	Intervention group, IG - Twenty subjects were randomly assigned to 30 h of PNEIMED training (4 days, 30 h in total), and Control group, CG - twenty subjects were randomly assigned to 30 h of academic lessons	Randomized Control Trial	4 days	Salivary cortisol IG - enzyme-linked immunosorbent assay	a significant reduction in basal morning cortisol at the end of the PNEIMED course (post-test) compared to the salivary concentration at baseline (pre-test)
H. S. Vadiraja et al. (2009) India	88 participants (30 and 70 years of age)	Intervention group receive yoga (n = 44) prior to radiotherapy treatment.	Randomized Control Trial	6 weeks	Salivary cortisol- enzyme immunoassay (EIA) Anxiety and depression using the	Significant decreases in anxiety depression,

	Intervention group (n = 44) Control group (n = 44)	Control group (n = 44) brief supportive therapy prior to radiotherapy treatment.			Hospital Anxiety and Depression Scale (HADS)	perceived stress, 6 a.m. salivary cortisol, and pooled mean cortisol in the yoga group compared with controls.

1.4 Discussion

The current literature review included 17 studies according to inclusion criteria, which included diverse 1007 participants. The primary aim of the current review is to assess the different measurement tools in yoga research to assess the difference in cortisol hormone and IgA. However, the included studies showed variability in the findings, which can be understood by the higher level of heterogeneity in the study population and frequency of yoga intervention. Salivary Cortisol level was measured in 12 studies using various measurement tools. However, an ELISA kit was used in most studies. The finding of the salivary cortisol levels varies from study to study, but the reduction in cortisol levels was mostly reported after the yoga intervention. A study from Iran showed that university students who participated in laughter yoga interventions had a significant reduction ($p=0.02$) in salivary cortisol levels(Ozturk & Tezel, 2021). Salivary Cortisol level was measured in 12 studies using various measurement tools. However, an ELISA kit was used in most studies. Hanu Ezzat et al. showed a reduction in the level of salivary cortisol in the intervention group, which received aerobic exercise in combination of breathing techniques and meditation. This is supported by intensity and different types of physical activity, which can result in a reduction in salivary cortisol levels (Obaya et al., 2023). These findings suggest that different yoga postures, in combination with any other physical activity and breathing exercises, help reduce the cortisol hormone. However, the findings of the study can vary with the measurement tool used in the studies. Salivary cortisol is proven to be a reliable and recommended alternative tool to serum cortisol assessment, as salivary cortisol also reflects accurate results of stress levels (Saiyudthong, Suwannarat, Trongwongsa, & Srisurapanon, 2010). A study on nursing students showed a significant reduction $p<0.001$ in serum cortisol levels after 5 weeks of mindfulness meditation (Alhawatmeh et al., 2022). The findings are supported by the study conducted on medical students who attended regular 60-minute yoga interventions, which showed a significant reduction $p<0.005$ in the level of serum cortisol; however, it is noticeable that the frequency of regular yoga practice is higher in this study (Kavitha et al., 2023).

Salivary IgA was only measured in two included studies that showed significant ($P<0.05$) improvement in single mantra meditation sessions for 20 minutes (Torkamani et al., 2018). These findings should be considered with caution as they show temporary improvement in salivary IgA level, and it is unable to inform the sustainability of the effects. Nobuhiko Eda et al. showed in their study that 90 minutes of yoga intervention for two weeks showed significant improvement in salivary IgA levels among 23 adult

women (Eda et al., 2018). The duration of the intervention is very short, as it presents the sustainability of the effects of the yoga intervention. However, the study suggests that yoga and meditation interventions can influence participants' immunity. Both studies used the same measurement tools, enzyme-linked immunosorbent assay (ELISA), to assess salivary IgA levels. In the current literature review, it is noticeable that no study uses serum IgA levels to see the effect of yoga intervention on participants.

1.5 Conclusion

The present literature review examining the different measurement tools in the study focuses on the effect of different yoga interventions on the cortisol hormone and IgA levels. Most of the included studies used ELISA kits to measure salivary cortisol biomarkers, and most of the findings support that the effect of yoga intervention reduces the salivary cortisol level in the participants. Salivary IgA level is measured in two included studies, which showed significant improvement in the marker. However, the small duration of intervention can be explained as a temporary improvement in the IgA level. Addressing the potential benefits of the yoga intervention to reduce stress biomarkers and improve the immune marker highlights the need for further studies with a longer intervention duration to better understand the effects.

Sub-study 2- Effect of yoga in medical students to reduce the level of depression, anxiety, and stress: pilot study (Goodbye Stress with Yoga GSY)[1]

2.1 Introduction

Stress is very common among medical students (Kjeldstadli et al., 2006). From different regions around the globe, medical students have been found at risk of mental disorders, psychological stress, and a decline in life satisfaction (Kjeldstadli et al., 2006). Understanding the seriousness of this issue is crucial for comprehension and solution findings. It is important to state that this not only affects students' academic achievement but also affects their health. By prioritizing the mental health and well-being of students, universities and medical colleges can create a supportive environment that fosters academic success and promotes a healthy and fulfilling life.

The evidence suggests that yoga is a beneficial technique for reducing stress and promoting overall health and well-being (Alzahem, Van der Molen, Alaujan, Schmidt, & Zamakhshary, 2011; Dyrbye et al., 2005; Hartfiel, Havenhand, Khalsa, Clarke, & Krayer, 2011). It is an ideal practice for one's sustainable happiness, it can help to conceptualize individual potential, develop resilience, and elevate eudaimonic pleasure (Trulson & Vernon, 2019). Yoga has gained popularity for its effectiveness in helping and maintaining a healthy lifestyle. Numerous studies have demonstrated its efficacy, and it is widely utilized as a form of mind-body therapy in the treatment of various clinical conditions, including cancer, eating disorders, hypertension, and pain relief, etc (Anand et al., 2018; Büssing, Michalsen, Khalsa, Telles, & Sherman, 2012; Chong, Tsunaka, & Chan, 2011; Loudon et al., 2012).

The most recent systematic review conducted articles from January 2014 to November 2018 (F. Wang & Szabo, 2020), reported the positive effects of yoga on stress reduction in the healthy adult population. However, caution is advised in interpreting the results of this review because of the limited number of studies included, but most importantly, remission was reported in this review. Additionally, the included studies show the diversity of yoga and have more methodological problems to prove the effect of yoga considering the higher amount of stress exposure by this population. In the current study, we endeavour to employ a methodologically rigorous approach, and our paramount focus while conducting this study was to reduce potential bias.

The overall aim of this pilot study is to examine the potential role of yoga in the medical student's life to reduce stress, depression, and anxiety levels and improve the quality of life. Within this umbrella, we have many goals. First, to examine the necessity of intervention and whether 10 weeks of yoga intervention will be beneficial for students' overall mental health. Second, to investigate the variation

[1] Chauhan, S., Babu, A. M., Galgalo, D. A., Melczer, C., Prémusz, V., & Karsai, I. (2024). Effect of yoga in medical students to reduce the level of depression, anxiety, and stress: pilot study (Goodbye Stress with Yoga GSY). *BMC Complementary Medicine and Therapies*, *24*(1), 203.

in stress, depression, and anxiety levels before and after the yoga intervention. And third, to examine individual perception of quality of life in the recent days before and after yoga intervention.

2.2 Materials and methods

2.2.1 Study design

An interventional study was carried out with consecutive sampling using a self-administered questionnaire in paper-and-pencil format.

2.2.2 Sampling

Data collection was carried out of the medicine students of the University of Pécs, Hungary between February 15 to 13 of May 2022. Participants were involved in the study by taking their verbal and written consent, and both male and female participants from diverse international backgrounds and not living with their families participated in the study. The inclusion criteria for the study required participants to be currently enrolled in the Medicine Faculty of the University of Pécs. However, certain exclusion criteria were established. Students with specific health conditions, including recent injuries, chronic pain, congenital skeletal problems or significant arthritis, which could potentially pose safety concerns during participation in a yoga intervention, were excluded from the study.

All the participants who enrolled for the current research met the inclusion criteria and participated in the study, none met the exclusion criteria, additionally, there were no dropouts among the participants.

2.2.3 Study participants

All participants who registered in the physical training course with the name “Indian Yoga” at the University of Pécs, Hungary during the month of February -May 2022 were invited to participate in this current research. The research specifically included those students who expressed interest actively in the current study ($N = 28$) with an average age of 23.54 ± 4.36 years and a BMI of 22.42 ± 3.42 kg/m2. Each participant received comprehensive instruction and a detailed explanation of the study.

2.2.4 Intervention

The research spanned a period of 10 weeks, from February 2022 to May 2022, with 90 min of yoga sessions, occurring once a week. Each yoga session followed a structured format, consisting of a different activity as shown in (Table 3). The development of the yoga protocol, named “GSY Goodbye Stress with Yoga Protocol,” was a collaborative effort involving a certified yoga trainer, a medical researcher, and experienced yogis from India. Each part of the above-mentioned specific intervention is followed by 3 min of Corpse Pose (Shavasana). Study participants were instructed to not eat two hours before the yoga session. The yoga session took place in the gymnasium hall located at the University of Pécs. Before each session, the hall underwent a comprehensive cleaning and preparation process. The researchers provided yoga mats to the participants, ensuring that each mat was used exclusively by the same individual throughout the sessions. Yoga mats were fully sanitized and cleaned

before and after the yoga intervention. The session was conducted by a certified yoga instructor with 7 years of teaching experience.

Table 3 Description of Intervention

Sequences	Duration
Full body warm-up and light stretching exercise	15 Minute
Performing various yoga postures - including standing, sitting, prone and supine yoga postures (Asanas)	50 Minutes
Breathing exercises (Pranayama)	10 Minutes
Meditation	15 Minutes

2.2.5 Assessment scales

The variables of the research framework were analysed by a self-reported questionnaire. These questionnaires were filled out at the Yoga session before starting the intervention and after the 10 weeks of intervention. The questionnaire was used in paper pencil form and deeply explained before giving it to the participants to have more accuracy in the response. Sociodemographic characteristics were obtained by using questions regarding, age, weight, education level, country, source of funding, relationship status, practised yoga before, other physical activity, and BMI, psychosocial characteristics were assessed by measuring the domains of depression, anxiety, stress, and quality of Life.

2.2.5.1 Assessment of physical activity

To assess participant's physical activity levels, enable comparisons and analysis, using both subjective and objective assessment.

Global Physical Activity Questionnaires were used for Subjective Assessment and ActiGraph was used for Objective Assessment. Global Physical Activity Questionnaire GPAQ is used in validated English(Armstrong & Bull, 2006) to collect information on physical activity participation in the five domains (work-related vigorous, work-related moderate, travel, recreational vigorous, and recreational moderate) as well as sedentary behaviors comprising a total of 16 questions. Based on the GPAQ responses regarding activity intensity, duration, and frequency, the total MVPA was also calculated in MET-min/week. The Overall MVPA level was further categorized as low, moderate, and high according to WHO guidelines(Armstrong & Bull, 2006). ActiGraph GT3X-BT is ActiGraphy's flagship activity monitor, globally used by researchers to capture and document continuous, high-resolution data on physical activity and sleep/wake patterns. ActiGraph devices have demonstrated precision in estimating accurate PA in free-living environments. These devices have been employed in many large-scale

epidemiological studies across the world(Yano et al., 2019). These tools were used to serve as a baseline and outcome measure to evaluate the impact of the intervention.

2.2.5.2 Assessment of stress

To investigate the variation in stress, depression, and anxiety levels before and after implementing a yoga intervention.

The Depression Anxiety Stress Scale DASS 21 was applied in English (Norton, 2007). This scale is utilized to assess the stress, anxiety, and depression experienced by students prior to and following the Yoga intervention. It includes 21 items, these items are in 3 sets of self-reported instrument created to assess the emotional state of depression, anxiety, and stress. The DASS 21 adopts a dimensional approach to evaluate psychological disorders, rather than relying on categorical classifications. Each set Includes seven questions with total scores that range from Normal to extremely severe (Norton, 2007).

2.2.5.3 Assessment of quality of life

To Analyse the Individual perception of quality of life in recent days before and after Yoga Intervention.

World Health Organization Quality of Like BREF WHOQOL BREF tool is used in English version(Group, 1998). This is a self-administered questionnaire that includes 26 questions on the individual perception of the quality of life and health over the previous two weeks. These questions are separated into four domains (1) Physical health, (2) Psychological,3. Social Relationships and Environment. The scoring of the question is based on a 1–5 Likert scale where 1 represents “disagree” and 5 represents “Completely agree” (Group, 1998).

2.2.6 Ethical approval

The study was reviewed and approved by the Regional Research Ethics Committee as the Institutional Review Board Record number 9117- PTE 2022 University of Pécs. Before the initiation of any study-related procedures, participants provided written informed consent, indicated by their signature on the Informed Consent Form. The current study adheres to the principles outlined in the Declaration of Helsinki.

2.2.7 Data analysis

Statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, ILUSA). For the baseline data, descriptive statistics were used. Data were presented as percentage (%) and frequency (N) for categorical variables, while continuous variables were presented as mean ±SD. Shapiro-Wilk test was used to test the normality of the data. The Wilcoxon Signed Ranks Test was utilized to assess the mean differences between the pre-and post-data. Paired sample correlation was used to investigate the correlation in the pre-and post- data, and Spearman’s Rank Correlation test was also applied. The significance level of $p<0.05$ was considered in each case.

2.3 Results

2.3.1 General characteristics

The major socio-demographic data of the study participants are shown in Table 4. The mean age of participants is 23.54± 4.36 years and a BMI of 22.42±3.42 kg/m2. The medium height of participants is 165 cm (155–188) and the medium weight of participants is 60 kg (48–98). 78.6% of females participated and 21.4% of male participated in the current study. 82.1% of the participants were living in the city at the time of the study. 42.% of the participants have scholarships for their financial support.

Table 4: Sociodemographic characteristics of the study populations

Characteristics	Frequency (%)
Age Group	
<20	6 (21.4)
21-25	15 (53.6)
> 26	7 (25.0)
Mean age (years)	23.54 ± 4.36
Gender	
Female	22 (78.6)
Male	6 (21.4)
Residence	
City	23 (82.1)
County	3 (10.7)
Village	2 (7.1)
Financial Support	
Self-Funding	16 (57.1)
Scholarship	12 (42.9)
Source of Income	
Family	15 (53.6)
Scholarship	13 (46.4)
Anthropometrics	
Height (cm)	165 (155- 188)
Weight (kg)	60 (48-98)
BMI (kg/m^2)	22.42 (3.42)

*BMI- body mass index, cm- centimetre, kg-kilogram, kg/m2-kilogram/square meter.

2.3.2 Physical activity

Data was collected on the baseline activity level by using (Global Physical Activity Questionnaire, Table 5) and accelerometers (ActiGraph GTXbt) to compare the difference between subjective assessment and objective assessment. Participants reported higher levels of physical activity MVPA (Moderate-to-Vigorous Physical Activity) 483.39±470.91 min/week when assessed using GPAQ

(Global Physical Activity Questionnaire) tool, compared to 275.25 ±128.06 min/week when measured using the ActiGraph GTXbt.

Table 5: Mean and Standard deviation of (GPAQ) Global Physical Activity Questionnaire after 10 weeks of yoga intervention in participants

		Work			Transport	Recreation			MVPA total	Sedentary
	N= 28	Vigorous	Moderate	MVPA		Vigorous	Moderate	MVPA		
Pre	Mean	0.36	109.28	110.00	216.90	61.40	33.50	156.40	483.30	473.80
	Median	0.00	0.00	0.00	200.00	25.00	5.00	95.00	405.00	480.00
	SD	1.90	475.50	475.50	193.60	99.60	41.10	200.30	470.90	174.40
	IQR -L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	120.00
	IQR-U	10.00	252.00	252.00	840.00	360.00	120.00	720.00	2520.00	840.00
Post	Mean	61.42	170.70	293.50	304.28	112.67	82.50	305.85	601.40	440.30
	Median	0.00	0.00	0.00	180.00	40.00	15.00	15.00	205.00	480.00
	SD	180.70	677.80	767.65	363.63	182.57	147.03	430.84	987.35	190.00
	IQR -L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00
	IQR-U	600.00	3570.00	3570.00	1500.00	750.00	600.00	1530.00	3570.00	840.00
Z		-1.60	-0.84	-1.36	-0.85	-1.00	-0.87	-1.19	-0.64	-0.16
p		0.11	0.40	0.17	0.35	0.32	0.38	0.23	0.53	0.43

*N: number of participants, IQR -L: Interquartile range -Lower, IQR -U: Interquartile range -Upper MVPA: moderate to vigorous physical activity.

Response of participants before and after yoga intervention on moderate-vigorous physical activity (MVPA) in the work domain changed from 110.0±475.5 min/week before yoga and 293.5±767.65 min/week after yoga respectively. Participants responded on MVPA in the recreational domain 156.4±200.3 min/week and 305.85±430.84 min/week. Participants responded that they spend before and after the yoga intervention on average with active transportation, for example walking or cycling 216.9±193.6 min/week and 304.28±363.63 min/week. They spent 473.8±174.4 min/week and 440.3±190.0 min/week in sedentary activity. However, there is no statistical improvement reported after the yoga intervention in MVPA in work domain ($p = 0.173$), MVPA in recreational domain ($p = 0.231$), total MVPA ($p = 0.525$), transportation activity domain ($p = 0.355$) and in sedentary activity domain ($p = 0.434$).

2.3.3 Changes in the perception of quality of life

Based on the World Health Organization Quality of Like BREF (WHOQOL-BREF, Table 6) which examined general quality of life (QoL), The mean and standard deviation of the physical health domain in the intervention group pre and post was 50.97±11.10 and 59.07±9.27, their response in the quality of life domain was 80.71± 17.62 and 82.14 ± 13.71, participants respond in Psychological health domain score was 56.79±9.90 and 68.25±6.18, their response in environment health domain score was76.16± 13.66 and 77.12± 13.92 and the response in social relation domain score was 62.48 ±23.22 and 71.07±

20.09 respectively. Students who participated in the yoga intervention showed statistical significantly improved in physical health domain ($p = 0.001$) and psychological health domain ($p < 0.001$). They showed improved in quality-of-life domain ($p = 0.678$), social relation domain ($p = 0.754$) and environmental domain ($p = 0.060$), but not statistically significant.

2.3.4 Changes in stress, anxiety, and depression

Based on the (Depression Anxiety Stress Scale DASS-21, Table 6). The mean and standard deviation of depression reported in participants before and after 10 weeks of yoga intervention for pre and post was 10.00 ± 10.60 and 7.21 ± 8.56, their anxiety reported in participants was 8.57 ± 10.09 and 5.50 ± 7.42, and participants stress reported before and after the intervention was 12.79 ± 10.73 and 9.64 ± 9.71 respectively. Depression and anxiety score significantly decrease after the yoga Intervention ($p = 0.019$ and $p = 0.049$). Stress score decreased after yoga intervention but not significantly ($p = 0.078$). Change reported in all measure stress anxiety and depression after 10 weeks of Yoga Intervention. These findings collectively indicate that 10 week of yoga intervention have positive effects on medical students, leading to improvements in their depression, anxiety, and stress levels.

Table 6: Mean and Standard Deviation of DASS 21 Depression Anxiety and Stress 21 score and WHOQOL BREF before and after 10 weeks of yoga intervention in participants

WHOQOL-Bref	**Mean ± SD**			
	Pre	**Post**	**Change**	**p value**
Physical Health domain score	50.97 ±11.10	59.07±9.27	-8.50 ± 12.56	0.001*
Quality of Life domain score	80.71 ± 17.62	82.14±13.71	-1.43 ± 17.99	0.678
Psychological Health domain score	56.79 ± 9.90	68.25 ± 6.18	-11.46 ±10.06	<0.001*
Environment Health domain score	76.16±13.66	77.12 ± 3.92	0.96 ± 15.12	0.060
Social Relation domain score	62.48 ± 23.22	71.07±20.09	-8.59 ± 14.98	0.754
DASS 21	**Mean ± SD**			
	Pre	**Post**	**Change**	**p value**
Depression	10.00 ± 10.60	7.21 ± 8.56	2.83 ± 6.19	0.019*
Anxiety	8.57 ± 10.09	5.50 ± 7.42	3.07 ± 7.90	0.049*
Stress	12.79 ± 10.73	9.64 ± 9.71	3.14 ± 9.08	0.078

2.4 Discussion

The current study findings demonstrated that yoga is a potential technique to reduce depression, anxiety, and stress and to improve the quality of life in medical students. According to evidence depression is the primary cause of disability on global scale, and stress, anxiety depression is the significant contributor to the worldwide burden of disease(Abdulghani et al., 2011). Many people experience comorbidities that certainly affect their quality of life(De Manincor et al., 2016). Education in medical school is distinctive the most stressful, which certainly causes an increase in stress, anxiety, and

depression(Abdulghani et al., 2011). Medical universities usually struggle to introduce student support programs that can help them reduce stress, depression, and anxiety and improve their quality of life(Bustamam, Theresa, & Wahyuningsih, 2020).

The present study evaluates a 10-week yoga intervention among medical students. The BMI, anxiety, stress, depression, and perception of quality of life were evaluated. A significant decrease in depression and anxiety was observed following 10 weeks of yoga intervention compared to baseline levels. Students responded and showed significant satisfaction in all four domains: psychological health, environmental health, physical health, and quality of life. This preliminary investigation suggests a positive effect of yoga on the student's stress support management.

2.4.1 Physical activity

In our current pilot study, we reported that during the 10-week of yoga intervention, medical students did not significantly ($p = 0.525$) change their physical activity habits as participants don't change their regular lifestyle according to GPAQ data except the yoga sessions. According to this finding the improvement in mental status (depression and anxiety) and quality of life may be resulted by yoga and not by other lifestyle changes.

Including yoga and other form of physical activity in their assessment, have moreover inconclusive results for physical activity outcome assessment (Ramos-Jiménez et al., 2009). This observation is consistent with an experimental study conducted in India by U.S Ray at al., 54 participants of this study was a group of engineering fellowship course trainees. The primary aim of the study was to observe the effect of yogic practices on the physical and mental health of the participants.

This study indicated that the yoga group maintained their physical activities in a mixed group way which means when yoga and other type of physical activity performed by the participants (US RAY et al., 2001). This kind of results are not clear to explain the effect, because the presence of yoga and other influencing factor parallelly. In our current study we explained that the improvement in mental status (depression and anxiety) is due to yoga intervention.

Our study also highlighted that there should be more objective assessment of PA to have more reliability of results reported. In our study participants significantly overestimated their activity level, when comparing objective and subjective data. We did not find any previous study that examined the effect of yoga and the level of the physical activity of the medical students or and other sample, and which can analyse the comparison between the objective assessment and subjective assessment of PA to have more reliability of results reported.

2.4.2 Stress

Our study found that the 10 weeks of yoga intervention changed stress (3.14 ± 9.08) $p = 0.078$ but data were not statistically significant, this finding is consistent with the study done by Allison R. Bond, that

11 weeks of yoga intervention, with three times a week shows changes in perceived stress (-0.05±0.62) but not statistically significant ($p = 0.70$) (Mathad, Pradhan, & Sasidharan, 2017). This is a contrast to previous randomised control trial study done on individualised yoga which shows that yoga intervention is statistically significant ($p < 0.01$) in reducing stress score. But the participants of this trial were general population with different age group (De Manincor et al., 2016). This finding suggests the effectiveness of intervention to reduce stress score. Other studies have demonstrated that yoga decreases the level of stress in college students which leads to positive effects on their psychophysiological level [35]. In the current study post data were collected during the exam period, and in which typically students has the higher level of stress(Schmidt, Pinney, & Werning, 2022), we concluded that the yoga intervention helps them to manage their stress level during exam period which also shows the necessity of intervention during exam period to improve individual's productivity.

2.4.3 Depression and anxiety

Studies on yoga demonstrated the effect of yoga in-home practice, it states that in terms of the broader community, it might have several benefits associated with mental health. With additional health benefits reduction of depression and anxiety is more over-reported from these studies(De Manincor et al., 2016). Studies that explored the effects of yoga and meditation on depression and anxiety differed in the type of population, study design, or length of interventions. However, there were many similarities with the current study. In a randomized controlled trial of mindfulness versus yoga, study showed that in the yoga group compared with the control group depression and anxiety scores decreased significantly ($p < 0.01$) (Falsafi, 2016). Study by Burns et al. was conducted on the effect of meditation for two semester period consistently on college students. This study focused on anxiety and depression, participants showed a significant decreased in the depression ($p < 0.000$) and anxiety score ($p < 0.007$) (Burns, Lee, & Brown, 2011). In the above studies we can see that despite have the different study design and different period of intervention all the studies showed that yoga can significantly decrease depression and anxiety score. The present study also showed the statistically significant to reduce the depression ($p = 0.019$) and anxiety score ($p = 0.049$) based on Depression Anxiety Stress Scale DASS 21.

2.4.4 Quality of life

According to the World Health Organization (WHO), quality of life can be defined as an individual's perception about his/her life in the context of value system, surroundings and culture in which they live and in connection or relationship to their goals, standards, concerns and expectation (Organization, 1996). The decrease in quality of life possible contribute to sleep disturbance fatigue and medication abuse and to elevate stress (Moldofsky, 2007).

Our study demonstrated that 10 weeks of yoga intervention can improve perception quality of life, for example, participants reported significant improvement in physical health domain score ($p = 0.001$),

psychological health domain score ($p < 0.01$) and environmental health domain score ($p = 0.06$), these findings is consistency with study done by Oken and colleagues (2006) which evaluated the effect of yoga on various populations where they found an improvement in the perception of quality of life, for example, physical ability (balance, flexibility) ($p = 0.01$), social functioning ($p = 0.06$), and physical functioning ($p = 0.015$) in the 90 min /per week yoga intervention for 6 months. However this result is reported in the older adult age group (65–85 years) (Oken et al., 2006). In summary we concluded that despite any age group yoga intervention has the potential to improve the quality of life in all the age groups.

2.5 Limitations

The current study has several limitations. There was a lack of control group, including control group would help to ascertain whether the outcome of research or observed changes were due to intervention of other factors. A small sample size reduced the statistical strength of the research. In addition, we lack of follow up data on the psychological well-being students as current study was only for one semester duration. Another possible limitation which should take into consideration is that students voluntarily chose to enrol in study courses and were therefore self-selected group. Thus, students who participated in the research may have possessed unique interests and characteristics that differed from those of other students. This suggests that further studies are required with an active control group, a larger sample size, and with a longer duration of intervention weekly to have more clarity and generalize the findings.

2.6 Conclusion

Beyond the substantial interest in the intervention, our pilot study was able to provide validation for the efficacy and necessity of the yoga intervention with our preliminary survey. It demonstrated that 10 weeks of yoga intervention can result in a significant reduction in the perceived level of anxiety, depression, and stress. Despite collecting the post research data during exam period participants shows stress reduction and along with this it shows yoga can significantly improve the quality of life. At the same time, further detailed analyses with large sample size and a more objective research design are required to strengthen the findings.

Sub-study 3- Changes in immune and metabolic parameters by 10 weeks of yoga intervention among medical students[2]

3.1 Introduction

Increased stress levels among medical students contribute to an increased risk of cardiovascular and metabolic syndrome and a decrease in immune parameters, including Immunoglobulin A (IgA), Immunoglobulin G (IgG), and Immunoglobulin M (IgM) (Choi, 2020; Segerstrom & Miller, 2004). The body's natural reaction to stress is a "fight or flight" response, characterized by an increased level of hormone release, elevated blood pressure, and heart rate (Ovsiannikova, Pokhilko, Kerdyvar, Krasnokutsky, & Kosolapov, 2024). Preparation of the body for quick reaction from short-term danger, for example, in the case of medical students, exams, clinical responsibilities, or more high-pressure tasks. However, these activations can be problematic if they continue over time(Dhabhar, 2018). Evidence shows that medical students face long-term stress due to their training demands, academic pressure, etc(Choi, 2020). This type of chronic stress does not need any physical response and possibly has a high level of health consequences (Mariotti, 2015). A substantial number of evidence has shown that psychological challenges can potentially change the features of the immune response(Segerstrom & Miller, 2004). IgM, IgA, and IgG are serum immunoglobulin, an integral component of the adaptive immune system (Brandtzaeg, 1996). IgA and IgG are long-lasting binding antibodies that provide mucosal immunity, while IgM is involved in tissue homeostasis and provides a rapid immune response (Hoffman, Lakkis, & Chalasani, 2016; S. R. Khan et al., 2021). Prolonged stress can decrease immune parameters, for example, IgA, IgG, and IgM, which results in weakening the body's defense against any type of infection. This may lead to medical students being more susceptible to health diseases (Assaf, 2013). Chronic stress can also cause elevated lipid profiles and disrupted glucose parameters (Chuang et al., 2010). These changes in metabolic health are potential risk factors for many chronic diseases, including cardiovascular disease, polycystic ovarian syndrome, thyroid problems, and type 2 diabetes (Patrizio et al., 2024; Sangaraju, Yepez, Grandes, Manjunatha, & Habib, 2022; Stefan, 2020). It is important to understand these challenges do not only have a severe impact on student's academic performance but also on their health. Systematic review shows adults suffering from anxiety and depression disorder improve their overall health through yoga intervention (Martínez-Calderon, Casuso-Holgado, Muñoz-Fernandez, Garcia-Muñoz, & Heredia-Rizo, 2023). Studies have clearly shown that yoga has a positive impact on insulin resistance, lipid profile, stress anxiety, and glycemic control (V. P. Singh & Khandelwal, 2020). Additionally, the influence of yoga is proven on the autonomic nervous system, which is very important in regulating the metabolic process (Shobana, Maheshkumar, Venkateswaran, Geetha, & Padmavathi, 2022). A recent meta-analysis (Dutta et al., 2021) shows a decrease in Fasting Plasma Glucose (FPG), low-density lipoprotein cholesterol (LDL-

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C), total cholesterol (TC), and triglycerides as well as HbA1c and body weight in people with T2DM practicing yoga exercise compared to those who engage in daily exercise (Dutta et al., 2021).

Although research has demonstrated a link between stress and poor health outcomes in medical students, and the potential benefits of different types of yoga practices, however, gaps remain. One of the gaps is the lack of comprehensive research exploring the combined impact of stress and yoga on the immune and metabolic parameters. It is well documented that stress can potentially alter immune markers and affect metabolic parameters (Choi, 2020; Segerstrom & Miller, 2004) , but there is very little evidence available on the concurrent effects of yoga intervention on both markers among medical students. However, the data shows that yoga can significantly alter the immune marker (Falkenberg, Eising, & Peters, 2018).

The Primary objective of the current study is to explore the effect of 10 weeks of yoga intervention on the Immune parameters IgA, IgG, and IgM among medical students. Based on the evidence, the secondary objective is to explore the metabolic parameters after 10 weeks of yoga intervention among medical students. Findings from the current study would help medical students improve their stress management and overall health.

3.2 Methodology

The current study is an experimental study carried out in the framework of a pre-post-test yoga intervention at the University of Pécs, Hungary. It analyses the effect of yoga intervention on immune and metabolic, among medical students.

3.2.1 Sampling

As noted above, the research population included medical students and was recruited through a convenient sample at the University of Pécs, Hungary, between September to December 2022. Based on the time-limited nature of the recruitment procedure, we did not conduct a power analysis for a predetermining sample size. The participants were encouraged to participate in the research study, the final sample size was determined by the number of students who volunteered to participate and later we obtained their written and verbal consent. Both male and female students from various years of study from the faculty of medicine participated. Additionally, to ensure the well-being and safety of all the students while participating in the intervention, certain inclusion and exclusion criteria were implemented. Inclusion Criteria- Students presently enrolled in the faculty of medicine at the University of Pecs could participate in the current study. Exclusion Criteria—Participants with certain health conditions, such as chronic pain, recent injuries, congenital skeletal abnormalities, chronic pain, musculoskeletal disorders, or significant arthritis, were considered for exclusion from the study. However, no participants were excluded, all the individuals who participated in the current study met the inclusion criteria. The included participants had no prior experience with yoga. Importantly, there were two dropouts among research participants during the intervention due to the transfer to another

university. The information and data of dropouts were considered during the analyses of overall study findings to ensure that the dropouts did not affect the result, thus ensuring the reliability of the findings.

3.2.2 Intervention

The 10-week yoga intervention was conducted from September to December 2022, with weekly 90-minute yoga sessions. Each session followed a structured format with different activities outlined in Figure 1. The "GSY Goodbye Stress with Yoga Protocol"(Chauhan et al., 2024) was developed collaboratively by a certified yoga trainer, a medical researcher, and experienced yogis from India. Each intervention part was followed by 3 minutes of Corpse Pose (Shavasana). Participants were asked to avoid eating for two hours before the session. The sessions were held in the University of Pecs in the Faculty of Science gym room, thoroughly cleaned and prepared beforehand. Researchers provided sanitized yoga mats, with each participant using the same mat for every session. A certified yoga instructor with 7 years of experience holding certification from Swami Vivekananda International Youth & Yog Research Council (INDIA) led the sessions

3.2.3 Population

Among the total participants, 81.1% were females and 18.9% were males in the current study. All medical students who registered with their interest in the physical education course named “Indian Yoga” were encouraged to participate in the current research. Out of 75 registered students, 37 students participated in the current research with a mean age of 21.84 ± 2.67 years and a BMI of 22.56 ± 3.85 kg/m2. Before participation, all students received thorough instruction and details of the study procedure and purpose.

3.3 Reported Outcome

We have used the immune parameters, which are IgA, IgG, and IgM, as the primary outcome, as chronic stress results in a decrease in the level of Immunoglobulin, which leads to a reduction in the individual's immunity[53]. The secondary outcome measures were glucose, lactate, HbA1c, HbA1c_IFCC, cholesterol, non-HDL, HDL, LDL, triglycerides, depression, anxiety, stress, and sedentary behaviors.

3,3.1 Assessment of blood parameters

Fasting venous blood samples were collected from participants in the morning (7:00 AM to 8:00 AM); samples were drawn in suitable vacutainers; tubes containing potassium ethylenediaminetetraacetic acid (K-EDTA) were used to test hemoglobin A1c parameters. Tubes containing sodium-fluoride (NaF) were used for plasma glucose and lactate analysis, while native tubes were used to obtain serum in support of the routine laboratory blood tests. All samples were transferred to the laboratory within one hour, where plasma and serum were separated using centrifugation (15 min, room temperature, 1500 g).

Plasma hemoglobin A1c levels were measured on a Tosoh G11 (Tosoh Bioscience, Tokyo, Japan) ion-exchange HPLC Analyzer, serum parameters including glucose, lactate, cholesterol, HDL, LDL, triglyceride,were measured using the Cobas 8000 Modular Analyzer (Roche Diagnostics, GmbH, Mannheim, Germany) while immunoglobulin levels were measured on Dade Behring BNII Nephelometer System (Siemens, Marburg, Germany) in strict accordance to the manufacturer's recommended guidance.

3.3.2 Ethical declaration

All included participants provided their informed consent before any study-related procedure by signature on the consent form, which was explained verbally to each participant. The investigation of the current study conforms to the principles outlined in the Declaration of Helsinki. The National Research Ethics Committee reviewed and approved the study as the Review Board Record number 26914-5/2021/EÜIG National Public Health Center, Hungary.

3.3.3 Statistical Analyses

The distribution of data was tested using the Shapiro-Wilk test. Descriptive analyses were conducted to summarise the demographic factors of participants. Paired sample T-tests were used for the normally distributed variables to compare the pre-and post-intervention mean and standard deviation. The effect size of these variables was calculated using Cohen's d test. The effect size value was interpreted as (small 0.2, medium 0.5, and large 0.8) to assess the magnitude of the intervention on the reported outcome[54,55]. For not normally distributed variables, mean and standard deviation were compared for pre- and post-intervention variables using the Wilcoxon signed ranked test. These variables' effect sizes r were calculated using the formula $r = z/\sqrt{N}$, where z is the test statistic from the Wilcoxon Signed-Rank Test[56]. In this case, the interpretation of effect size value r is as (small 0.1, medium 0.3, and large 0.5) [54]. The significant level considered in each case was $p<0.05$, all statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA).

3.4 Results

3.4.1 Participants

Thirty-seven out of seventy-five participants expressed interest in participating in the current study. After collecting the baseline data and beginning the intervention, two participants were excluded due to lack of presence in the intervention. This results in thirty-five participants who completed the study. The characteristics of the study population are shown in Table 7.

Table 7: Characteristics of Study Populations

Characteristics	Frequency (%)
Anthropometrics	
Height (cm)	168 (154- 190)
Weight (kg)	65 (45-95)
BMI (kg/m^2)	22.56 (3.85)
Mean age (years)	21.84 ± 2.67
Gender (%)	
Female	30 (81.1)
Male	7 (18.9)
Residence	
Capital	31 (83.8)
City	2 (5.4)
County	2 (5.4)
Village	2 (5.4)
Major (%)	
1st year	16 (43.2)
2nd year	15 (40.5)
3rd year	6 (16.2)

*BMI- body mass index, cm- centimetre, kg-kilogram, kg/m2-kilogram/square meter.

The average of the included participants is 21.84 ± 2.67 years and a BMI of 22.56 ±3.85 kg/m^2. The medium height and weight of the participants are 168 cm (154- 190) and 65 kg (45-95), respectively. Among the total participants in the current study, 81.1% were female and 18.9% were male. 83.8% were living in the city, 5.4% in the capital,5.4% in the countryside, and 5.4% in the village. 43.2% of students from the first year, 40.5% from the second year, and 16.2% from the third year of their major participated in the current study. All the markers from pre- and post-data fell within the normal range. See Table 8.

Table 8: Mean and Standard deviation of Immune and metabolic parameters before and after 10 weeks of yoga intervention in participant

N =35	Normal Range	Pre		Post	
Variables		**Mean**	**SD**	**Mean**	**SD**
IgA g/l	0.700-4.000	1.90	0.85	2.20	2.35
IgG g/l	7.00-16.00	12.28	2.13	11.48	2.12
IgM g/l	0.40-2.30	1.08	0.41	1.15	0.42
HbAIC %	4.00-5.60	5.25	0.21	5.29	0.27
HbAIC _IFCC mmol/l	20-38	33.91	2.38	34.29	2.94
FBG mmol/l	3.90-6.00	4.88	0.47	4.99	0.31
Cholesterol mmol/l	0.00-5.20	4.41	0.84	4.38	0.71
TGC mmol/l	0.00-1.70	0.97	0.42	0.91	0.43
HDL mmol/l	>1.15	1.53	0.41	1.59	0.32
Non-HDL mmol/l	2.6–3.3	2.87	0.77	2.78	0.69
LDL mmol/l	0.00-3.40	2.57	0.69	2.66	0.65

*Abbreviations: SD, standard deviation; N number of participants, IgA – Immunoglobulin A, IgG - Immunoglobulin A, IgM- Immunoglobulin M, Glycated haemoglobin HbA1C, Fasting Blood Glucose FBG, Triglycerides TGC, High-density lipoproteins HDL, Low-density lipoproteins LDL SD standard deviation

3.4.2 Primary outcomes: Changes in Immune Parameters

Based on the blood test, The level of IgA increased significantly from a pre-intervention mean of 1.90±0.85 g/l to a post-intervention mean of 2.20±2.35 g/l (mean difference: 0.30±2.30 g/l, $p<0.001$) (Table 8 and Table 9). This increase was associated with a strong effect size ($r=-0.702$). The level of IgG showed a slight but significant decrease from a pre-intervention mean of 1.90±0.85 g/l to a post-intervention mean of 1.90±0.85 g/l (mean difference: -0.80±1.04 g/l, $p<0.01$). The effect size for this change was moderate ($d = 0.771$). Similarly, the level of IgM increased significantly from a pre-intervention mean of 1.08±0.41 g/l to a post-intervention mean of 1.15±0.42 g/l (mean difference: 0.07±0.11 g/l, $p=0.001$). The effect size for the change in IgM was small ($d= 0.002$).

Table 9: The mean change of Immune Parameters after 10 weeks of yoga intervention in participants

N =35	Change		Z value	Significance	Effect Size	
Variables	**Mean diff.**	**SD**		**Two-Sided p**	**Cohen's d**	**R value**
IgA g/l	0.30	2.30	-4.153c	<0.001	NA	-0.702
IgG g/l	-0.80	1.04	NA	<0.001	0.771	NA
IgM g/l	0.07	0.11	NA	0.001	0.002	NA

*Abbreviations: SD, standard deviation; NR, not reported, N number of participant, IgA – Immunoglobulin A, IgG - Immunoglobulin A, IgM- Immunoglobulin M, SD standard deviation, Significant at $P < 0.05$.

3.4.3 Secondary Outcome: Changes in Metabolic Parameter

Changes in Metabolic Parameter The level of HbA1C increased from 5.25±0.21 % to 5.29±0.27 % (mean difference -0.03±0.18 %, p = 0.360) (Table 8 and Table 10), with a small negative effect size (d -0.157). Similarly, HbA1C_IFCC increased from 33.91±2.38 mmol/l to 34.29±2.94 mmol/l (mean difference -0.37±2.06 mmol/l, p=0.293), with a small effect size (d -0.180). Fasting blood glucose (FBG) levels increased from a pre-intervention mean of 4.88±0.47 mmol/l to a post-intervention mean of 4.99±0.31 mmol/l (mean difference: 0.122±0.39 mmol/l, p=0.10), and the small effect size (r=0.278). Total cholesterol levels decreased from a pre-intervention mean of 4.41±0.84 mmol/l to a post-intervention mean of 4.38±0.71 mmol/l (mean difference: 0.04±0.37 mmol/l, p=0.554), with a small positive effect size (d 0.101). Triglyceride (TGC) levels decreased from a pre-intervention mean of 0.97±0.42 mmol/l to a post-intervention mean of 0.91±0.43 mmol/l (mean difference -0.05±0.31 mmol/l, p=0.298), and the effect size (r -0.176) was small and negative. HDL levels significantly increased from a pre-intervention mean of 1.53±0.41 mmol/l to a post-intervention mean of 1.59±0.32 mmol/l (mean difference: 0.06±0.19 mmol/l, p=0.005), with a negative effect size (d -0.474). LDL levels increased from a pre-intervention mean of 2.57±0.69 mmol/l to a post-intervention mean of 2.66±0.65 mmol/l (mean difference: 0.09±0.36 mmol/l, p=0.14), with a small negative effect size (d -0.251). Non-HDL levels decreased from a pre-intervention mean of 2.87±0.77 mmol/l to a post-intervention mean of 2.78±0.69 mmol/l (mean difference: -0.09±0.40 mmol/l, p= 0.214), and the effect size was moderate and positive (d=0.214). The result is shown in Table 10. (See Figure 3 for a visual abstract).

Table 10. Mean change of a metabolic parameter after 10 weeks of yoga intervention in participants

N= 35	Changes			**Significance**	**Effect Size**	
Variables	**Mean diff.**	**SD**	**Z-value**	**Two-Sided p**	**(Cohen's d)**	**r value**
HbAIC %	-0.03	0.18	NA	0.360	-0.157	NA
HbAIC _IFCC mmol/l	-0.37	2.06	NA	0.293	-0.180	NA
FBG mmol/l	0.12	0.30	-1.646b	0.100	NA	0.278
Cholesterol mmol/l	0.04	0.37	NA	0.554	0.101	NA
TGC mmol/l	-0.05	0.31	-1.040c	0.298	NA	-0.176
HDL mmol/l	0.06	0.19	-2.806b	0.005	NA	-0.474
Non-HDL mmol/l	-0.09	0.40	NA	0.214	0.214	NA
LDL mmol/l	0.09	0.36	NA	0.147	-0.251	NA

**Abbreviations: NR not reported, N number of participants, Glycated hemoglobin HbA1C, Fasting Blood Glucose FBG, Triglycerides TGC, High-density lipoproteins HDL, Low-density lipoproteins LDL, SD standard deviation, Significant at $P < 0.05$.

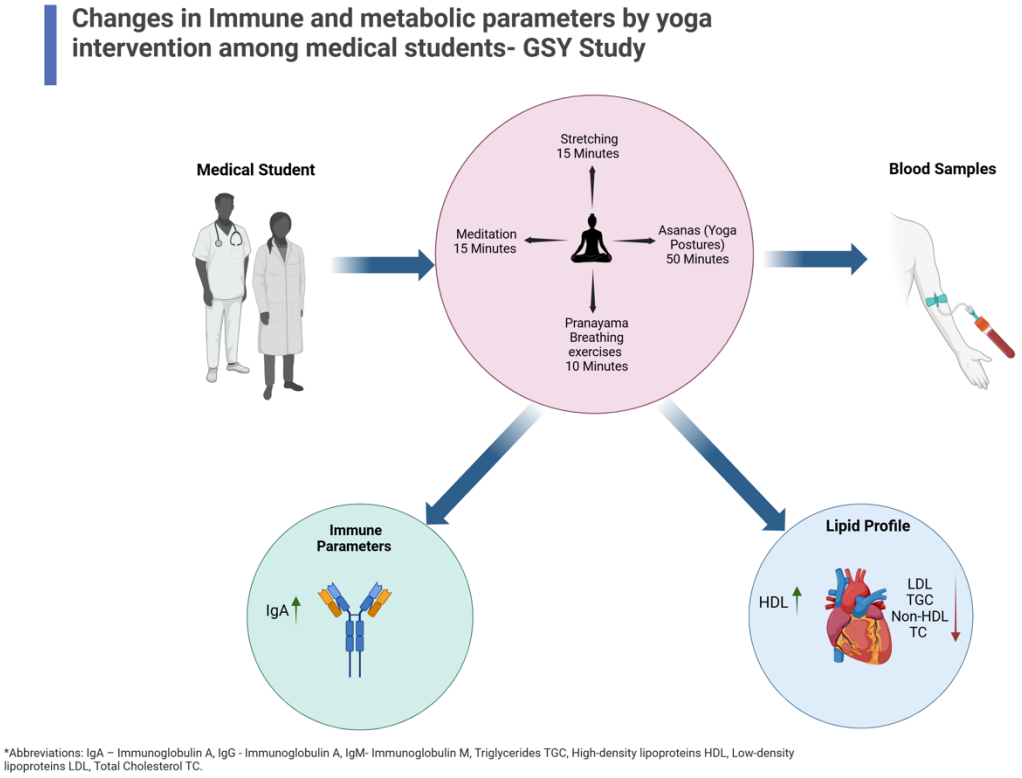

Figure 3 Visual abstract of chapter 3.
Note: Created by the author based on the findings of the accompanying manuscript.

3.5 Discussion

The finding of the current research shows that medical students who participated in the 10-week yoga intervention with GSY protocol(Chauhan et al., 2024) significantly improved their IgA levels and HDL levels among medical students is contributes uniqueness to the existing research. It is important to note that the post-intervention data was collected when the participants were close to their exam period, and all the reported outcome measures fell within the normal range. It has been proven that close to the exam period, students have high stress levels, hence the low IgA level(Engeland et al., 2016; Fritz, Stochl, Kievit, Van Harmelen, & Wilkinson, 2021). The results of the current study are important for the healthcare profession and university management for providing stress management techniques for medical students.

3.5.1 Immune Parameter

The average level of IgA significantly increased ($p<0.001$) after the 10 weeks of yoga intervention, showing that yoga plays an important role in improving the participants' immunity. Additionally, the strong effect size of ($r=-0.702$) between IgA level and yoga intervention presented a consistent effect of yoga on immune health. This result is supported by the study among older adults, where the concentration of secretory immunoglobulin A and secretion were increased after the yoga intervention (Eda et al., 2018). A study on pregnant women also showed that 20 weeks of yoga intervention significantly reduced the cortisol hormone and improved the IgA level during pregnancy, which not only improved immunity and health but, in the long term in the intervention, also improved the birth outcome (P.-J. Chen et al., 2017). The IgG level of the participants significantly ($p<0.01$) decreased (-0.80±1.04 g/l) after the yoga intervention. The effect size (cohen's $d = 0.771$) shows the moderate effect of yoga on the IgG. The Reduction in IgG should be interpreted cautiously as every value remained in the normal range. IgG level is part of long-term immune memory; this result can be interpreted as the short-term yoga intervention may not impact long-term immune memory. The observed finding of IgG might not represent to clinical significance of weakening of immune protection. The IgM level of the participants in the current study shows a significant ($p=0.001$) increase after the yoga intervention; however, a very small effect size (cohen's $d= 0.002$) demonstrates that this change may show low practical implication despite a significant increase. This result can be supported by the systematic review which states that no significant effect has been shown by the yoga intervention on IgM and IgG antibody (Ahlquist, 2023). However, there is a need to have further investigation on long-term yoga intervention on IgG and IgM levels for a better understanding.

3.5.2 Metabolic Parameter

The results of the current study showed varied results on the effect of 10 weeks of yoga intervention on metabolic parameters. HbA1C and HbA1C_IFCC levels show a increase but not significantly ($p = 0.360$ and $p=0.293$) after the yoga intervention. However, the very small negative effect size (cohen's d -0.157 and d -0.180) can be interpreted as the practical implication of short-term yoga intervention

having minimum impact on glycaemic control in the study population. In contrast, findings from the study conducted on the T2DM patient with three-month yoga intervention practice twice a week show a significant improvement in the glycaemic outcome with a significant decrease in HbA1C level (Misra et al., 2021). This variation in findings highlights the potential influence of frequency as well as duration of yoga intervention on the impact of metabolic health. Additionally, the sociodemographic characteristics of the participant with baseline glycaemic status may also impact the findings. There was no significant increase in the value of FBG after the yoga intervention, with a small to moderate effect size (r = 0.278) between the FBG level and yoga intervention, which indicates that the intervention did not strongly influence FBG levels in these medical students. On the other hand, evidence shows that there was a significant reduction in the FBG level after the yoga intervention (Dhali, Chatterjee, Das, & Cruz, 2023; Gupta et al., 2020; Saberipour, Gheibizadeh, Maraghi, & Moradi, 2020; Yuniartika, Sudaryanto, Muhlisin, Hudiyawati, & Pribadi, 2021). However, the small to moderate effect size shown in the current study concluded that there is an association between yoga and FBG levels. However, the short duration of the intervention, post-sampling in the exam period, may have influenced the non-significant changes in the current study. In terms of lipid profile, the current study shows the varied effects of 10 weeks of yoga intervention on the lipid profile of the participants. The level of cholesterol and triglycerides decreased after the intervention. However, this decrease was not statistically significant (p=0.0554 and p=0.298). The positive effect size (cohen's d 0.101) indicates the potential benefit of yoga intervention to improve cholesterol levels. There was a small negative effect size (r = -0.176) that shows the practical implication of this finding that short-term yoga intervention may not produce reliable changes in triglyceride levels in this population. These results can be supported by the previous literature, which showed that long-term yoga intervention showed a pronounced improvement in cholesterol levels and triglycerides. However, the consistency and duration of practice with participant baseline character may also influence the findings (Ghazvineh, Daneshvar, Basirat, & Daneshzad, 2022; RAJA; Shantakumari & Sequeira, 2013). Participants' LDL levels did not significantly increase p=0.14. The negative effect size (d -0.251) suggests that although there is a change in LDL level after the intervention, this change is not impactful in a practical clinical context. The level of HDL significantly increases after the yoga intervention (p=0.005) with a negative effect size (d = -0.474). This can be interpreted as a moderate improvement in HDL level through yoga intervention. These noteworthy findings show the positive shift of lipid metabolism among participants through yoga intervention since the elevated HDL level is also associated with reducing the risk of cardiovascular disease (S. S. Khan & Fonarow, 2022). The level of non-HDL decreased (p = 0.214) with a moderate effect size (d = 0.214) after the yoga intervention in the current findings. This moderate effect size shows the potential influence of long-term yoga intervention on lipid metabolism. The level of non-HDL slightly decreased but not significantly with a moderate effect size, which indicates the potential clinical relevance of the effect of yoga on the non-HDL level; however, further study is needed to confirm these results with long-term effects. These positive finding by a current study on lipid

metabolism is supported by meta-analysis (Isath et al., 2023), which states that yoga has a potential yet positive impact on lipid profile, Hba1c, and blood pressure; it plays a crucial role in contributing to the primary prevention of cardiovascular disease. However, there was no significant change found in LDL and non-HDL levels may reduce the potential benefits of an increase in HDL levels on overall cardiovascular risk in medical students. Given the short duration of intervention and post-data collection during the exam period, we cannot assess the long-term effect of the current intervention on lipid profile. The improvement in metabolic markers in our study is supported by previous research where it is demonstrated the positive effect of regular exercise on metabolic parameters (Seghatoleslami, Beik, Sadeghi, & Pavlovic, 2024). Additionally, studies demonstrate that regular exercise has significant benefits for well-being and mood (Taheri, Farzian, Esmaeili, & Shabani, 2020). However current study findings only focus on metabolic and immune markers.

Medical students are more at risk of developing habits that increase the incidence of chronic diseases such as diabetes, cardiovascular disease etc, due to high levels of stress (Mukhopadhay et al., 2021). One of these unhealthy habits is a high level of sedentary behavior among medical students (Janampa-Apaza et al., 2021). As mentioned, the post-sampling was collected during the exam period, this time is often associated with high sedentary behaviors due to high stress; this unexpected finding of some lipid parameters, IgG and IgM level after yoga intervention suggests that the other factors, for example, academic stress, lack of physical activity may counteract the effect of yoga intervention due to high-stress periods.

This study's interpretation necessitates acknowledging several methodological limitations. The use of convenience sampling without an a priori power analysis resulted in a small sample size (n=35), which limits statistical power and the generalizability of findings. While multiple comparisons were not adjusted using Bonferroni correction due to concerns about Type II errors in the exploratory context, this increases the risk of Type I errors, though effect sizes were considered for practical significance. Critically, the single-group, pre-post design lacks a control group, making it difficult to isolate the intervention's effects from potential confounders like external physical activity, diet, sleep patterns, or natural variations, particularly as post-data collection occurred during a high-stress exam period known to affect physiological outcomes. Furthermore, the absence of validated psychological instruments complicates the direct assessment of mental health changes. The finding from the systematic review reported a positive effect of yoga on decreasing depressive symptoms among the population with mental disorders (Brinsley et al., 2021). This study reported a dose-response relationship between the frequency of yoga intervention per week and improvement in depressive symptoms (Brinsley et al., 2021). This gap should be addressed in future studies by including the mental health outcome data collected before and after the yoga intervention among medical students, Many studies support that yoga intervention can decrease the level of stress and anxiety among students and improve their emotional well-being (Chauhan et al., 2024; Elstad et al., 2020; Lemay, Hoolahan, & Buchanan, 2019; Lucas Mullins;

Martínez-Calderon et al., 2023; Pradhan & Pramanik, 2024; Tripathi, Kumari, & Ganpat, 2018). Collectively, these factors, especially the lack of controls and potential confounding from the exam period timing, significantly impact the study's internal validity and weaken causal attributions. It is suggested to interpret the result with caution by considering above mentioned gaps.

3.6 Future Recommendation

Future studies should focus on the higher frequency of yoga intervention per week and its long-term impact on the clinical outcomes including mental health outcome of medical students. There is evidence that states that the frequency of yoga can improve the effect of yoga on health outcomes (Ayala, Wallson, & Birdee, 2018). Exploring frequent yoga practice with long-term yoga practice could provide deeper knowledge of the sustained yoga effect on medical students' immune, metabolic, and psychological health. Additionally, a well-structured randomized control trial is needed to reduce the bias and better understand the effect of yoga on different parameters, particularly in high-stress situations.

3.7 Conclusion

The current study shows the significant impact of 10 weeks of yoga intervention on immune and metabolic parameters among medical students. Regular yoga practice for 10 weeks improved medical IgA level, blood glucose control, and favourable lipid parameters. The current finding underscores the meaningful impact of yoga intervention can positively influence certain aspects of medical students' physiological health.

Sub-study 4- Impact of yoga intervention on mental health and overall well-being among medical students: GSY study[3]

4.1 Introduction

In the last decade, more attention has been given to the mental health issues faced by medical students (Jafari, Loghmani, & Montazeri, 2012). It has been proven by many studies that at the beginning of their education, medical students face the same level of stress as non-medical students (Dyrbye et al., 2005; Moffat, McConnachie, Ross, & Morrison, 2004). Still, evidence shows that medical student's mental health deteriorates throughout medical education and training (Goebert et al., 2009; Lomis, Carpenter, & Miller, 2009; Niemz, Griffiths, & Banyard, 2005). Medical students face a continuously demanding environment that often leads to poor sleep, high levels of stress, low levels of quality of life,

[3] Chauhan, S., Najaf, S. S., Gergely, L., Kinga, K. A., Karsai, I., & Prémusz, V. (2025). Impact of 10 Weeks of Yoga Intervention on Mental Health and Overall Well-Being Among Medical Students: GSY Study. *Sports*, *13*(4), 114.

and emotional dysregulation among medical students (Safhi et al., 2020; Weurlander et al., 2019). A recent systematic review by Shafiee et al. (2024) states that approximately half of medical students suffer from some type of sleep disturbance, anxiety, and depression problems (Shafiee et al., 2024).

The amount of stress on a medical student is an especially concerning issue as it not only disturbs their professional development and academic performance but also affects their mental and physical health, which can lead to long-term chronic disease (K.-Y. Chen et al., 2013; Coentre & Góis, 2018; Eisenberg, Hunt, Speer, & Zivin, 2011; Romani & Ashkar, 2014). Chronic stress and symptoms of anxiety can disturb cognitive function, lead to burnout, and hinder decision-making, which collectively leads to the quality of care these students will provide to patients (De Hert, 2020). Evidence shows the top four reasons for high levels of stress were time pressure, excessive study material, examinations, and the thought of leaving behind in work (Yusoff, 2012). However, it has been proven that medical students and doctors are at very high risk of mental health problems and chronic health issues but still increased attention has been given to the well-being and mental health of doctors and health professionals, but not much attention has been given to medical students (Yusoff, 2012).

Studies show that educational institutes face challenges in providing stress management techniques (Chauhan et al., 2024). This data is concerning, as the year of study is the foundational year of medical student's education, which is crucial for building up the coping strategies and resilience they will require during their professional lives (Steiner-Hofbauer & Holzinger, 2020). Additionally, these students suffer more mental health challenges as they are confronted with topics like grief and death, which leads to depression cases in medical students (Dyrbye et al., 2005). Data from German studies demonstrate that quality of life, which is related to health, is reduced, and higher levels of depression are found in medical students (Jurkat, Höfer, Richter, Cramer, & Vetter, 2011). A study conducted by Saravanan and Wilks (Saravanan & Wilks, 2014) shows that there is a direct and significant relationship between depression and stress. The prevalence of sedentary behavior among students is higher (Lavie, Ozemek, Carbone, Katzmarzyk, & Blair, 2019). Medical students show higher levels of sedentary behavior, leading to sleepiness (Hosteng, Reichter, Simmering, & Carr, 2019). It has been proven that a higher level of sedentary behavior is a risk factor for chronic diseases such as diabetes, cardiovascular, etc (Janampa-Apaza et al., 2021). This evidence shows that high stressful environment negatively influences emotional discomfort and the overall well-being of medical students (Dyrbye, Thomas, & Shanafelt, 2006; Fazia et al., 2023) . Yoga and meditation interventions are known to improve mental health by reducing the symptoms of depression and anxiety. Research shows that yoga and meditation interventions positively impact individuals' overall well-being (Descilo et al., 2010; Elder, Nidich, Moriarty, & Nidich, 2014; Thordardottir, Gudmundsdottir, Zoëga, Valdimarsdottir, & Gudmundsdottir, 2014; Woodyard, 2011). Yoga comes from the Sanskrit word “yuj,” which means to join or to unite. Regular yoga and meditation practice promotes endurance, flexibility, and strength and cultivates calmness and well-being (Chauhan et al., 2024; Woodyard, 2011). Yoga is known as mind-body

medicine, and it involves an individual's mental, physical, and spiritual components to improve overall health and well-being (Atkinson & Permuth-Levine, 2009). It is proven that 10 weeks of yoga and meditation practice can decrease stress-related illness (C. Smith, Hancock, Blake-Mortimer, & Eckert, 2007). However, it has been proven that stress contributes to the cause of heart disease, stroke, cancer, diabetes, as well as many other chronic diseases (C. Smith et al., 2007).

However, the existing studies explore the prevalence of chronic stress and its expected consequences, but limited evidence is available where it has examined the structured interventions that target the reduction in stress and overall improvement in the health and well-being of medical students.

Understanding that medical students are continuously under chronic stress, the present study employs a rigorous methodological approach to decrease the level of publication bias. The current chapter's primary aim is to explore the effect of a 10-week yoga intervention on mental health outcomes (stress, anxiety, and depression), quality of life, emotional regulation, and quality of sleep of medical students. The findings would assist medical students and educational institutions in enhancing stress management techniques and influence overall well-being.

4.2 Methods

The study was reviewed and approved by the Regional Research Ethics Committee as the Institutional Review Board Record number 9117- PTE 2022 University of Pécs. In the present study, participants were medical faculty students currently enrolled at the University of Pécs, Hungary, who were selected based on non-random convenient sampling, as the students registered for the yoga course voluntarily. Due to the nature of the selection criteria, an active control group was not included for ethical reasons. Students registered for the course voluntarily, intending to decrease their level of stress. It would not be ethically feasible to randomly assign the students to the group that would not receive yoga intervention. The study was conducted between February 15 to May 15, 2023. The current study is the first phase of the main study, which builds upon the previously conducted pilot study (Chauhan et al., 2024) using the same yoga protocol for 10 weeks. The duration of the intervention was selected based on evidence from numerous studies that show that physical activity training lasting between 8 weeks to 12 weeks is effective for desirable health outcomes (Ahadi, Tabatabaee, Rajabpour, Ghadamgahi, & POURYOSEF, 2013; Andysz et al., 2014; Haraldstad et al., 2017; Klare et al., 2015).

4.2.1 Study Population

In total, 212 medical students participated with a mean age of 21.36 ± 2.20 years in the current study. 89.2% were female, and 10.8% were male out of total participants. Students registered for the course with the name "Indian Yoga" were encouraged to take part in the research, and the research team took their written and verbal consent. To ensure the safety of the participants, we build inclusion and exclusion criteria to select the participants to take part in the yoga intervention (Table 11). However, all participants who showed interest in the research met the inclusion criteria. Notably, there were no

exclusions or dropouts.

Table 11. Criteria for selecting participants in the current study

Criteria	Explanation
Inclusion Criteria	Medical students enrolled in the University of Pécs, Hungary were eligible to participate in the study.
Exclusion Criteria	Following are the exclusion criteria 1.Participants with chronic health pain condition 2.Participants with physical injuries 3.Participants with severe sclerosis 4.Participants with congenital skeletal abnormalities 5.Participants with severe arthritis 6. Participants with musculoskeletal abnormalities

4.2.2 Intervention

The current study's yoga intervention took place between 15 February and 15 May 2023. It was a 10-week (90-minute-per-week) program specifically designed to reduce participants' stress levels. The program's structure was based on the "Goodbye Stress with Yoga Protocol" (GSY) (Chauhan et al., 2024), developed collaboratively by a medical researcher and a certified yoga teacher from India.

In the intervention, each session includes components mentioned following:

1. Stretching and warm-up - The yoga intervention session began with a complete body warm-up stretching with breathing technique for 10 minutes; this prepares an individual for further yoga exercise.

2. Asanas (Yogic Posture)—This includes a 50-minute session of practicing yoga postures, which include standing, sitting, supine, and prone postures. (In between the sessions, participants practice Shavasana (Corpse Pose), a relaxation pose that helps them absorb the session's benefits.)

3. Pranayama (Breathing Exercise): This includes a 15-minute breathing exercise that helps relax and improve lung capacity.

4. Meditation – The detailed meditation session is specifically designed to reduce stress levels.

Instruction—Before participating, all the students were instructed not to drink or eat for two hours before the session. The intervention was held on the premises of the University of Pécs, in the RG room of the Faculty of Humanities. The place and mat were cleaned and sanitized before practice to ensure

the safety of the participants. The intervention was provided by a certified yoga teacher with a minimum of seven years of experience.

4.2.3 Outcome Reported

In the present study, we have used mental health outcomes and quality of life scores as the primary objective of the study. The mental health includes the depression, anxiety, and stress scores of the participants before and after the intervention. However, the quality of life consists of an overall score of quality of life and the sub-score of physical health, psychological health, social relationships, and environment before and after the yoga intervention. Additionally, the secondary outcome was quality of sleep and emotional regulation, which includes a score of nonacceptance of emotional responses, impulse control difficulties, difficulty in goal engaging in goal-directed behaviors, lack of emotional awareness, limited access to emotional regulation strategies, and lack of emotional clarity of the participants.

4.2.4 Measures

Self-reported questionnaires were used to collect the data from the participants. All questionnaires were provided in paper-and-pencil form, and data were calculated before and after the 10-week yoga intervention. Before collecting the data, all questionnaire details were explained thoroughly to participants to reduce recall bias.

4.2.4.1 Assessment of Mental Health

We used Depression Anxiety Stress Scale DASS (Norton, 2007) in the English version. In total, this validated questionnaire includes 21 questions in the three sets of questions built up to analyze the emotional state of anxiety, depression, and stress levels of the individual. These three sets include seven questions each for these subsections with an overall score ranging from “normal to extremely severe.”[31]

4.2.4.2 Assessment of Quality of Sleep

We used the Pittsburgh Sleep Quality Index PSQI (C. Smyth, 1999) in the English version. It is a self-assessment questionnaire that records sleep disturbance and sleep quality. It has, in total, 19 questions that produce seven component scores. These seven components are 1. sleep quality, 2. sleep duration, 3. sleep latency, 4. sleep disturbances, habitual sleep efficiency, daytime dysfunction, and use of sleep medication. Combined scores from all these seven components generate a global sleep quality score[32].

4.2.4.3 Assessment of Quality of Life

We used the World Health Organization Quality of Life BREF WHOQoL BREF (Group, 1998) tool in the English version. Participants reported their perception of quality of life over the last two weeks,

which reduced the possibility of recall bias. The tool is self-administered, contains 26 questions, and is presented in four domains: 1. Psychological 2. Physical Health 3. Social Relationships, and 4. Environment.

4.2.4.4 Assessment of Emotional Regulation

We used the Difficulties in Emotion Regulation Scale (DERS) (Grant, Salsman, & Berking, 2018). This instrument has 36 self-reported questions that report how respondents relate to their emotions, specifically in six categories: non-acceptance of emotional responses, difficulty engaging in goal-directed behavior, impulse control difficulties, lack of emotional awareness, limited access to emotion regulation strategies, and lack of emotional clarity (Haraldstad et al., 2017).

4.2.5 Statistical Analyses

We used SPSS 26.0 software (SPSS Inc., Chicago, ILUSA) to conduct statistical analyses. The distribution of the data was tested using the Kolmogorov-Smirnov test. Based on the distribution of the data, a paired sample T-test was conducted to compare the mean of continuous variables. The association between continuous variables was tested using Pearson's correlation. Based on the significance of these correlations, we conducted a stepwise linear regression analysis to define the association of changes in stress level, anxiety level, and depression level with psychosocial factors. Statistical significance for the overall linear regression model was assessed by using the F-test and p-value. The collinearity diagnostics shows that factors have variance inflation factor (VIF) and tolerance value. If VIF is below 10 and tolerance values are above 0.1, it indicates no multicollinearity concerns, which shows the selected model is well-conditioned. Data was demonstrated in mean and SD for the continuous variable as well as percentage and frequency for the categorical variable. The $p<0.05$ is considered significant in each case.

4.3 Results

Table 12: Characteristics of Study Populations

Characteristics (N=212)	Frequency (%)
Anthropometrics	
Height (cm)	167 (150- 190)
Weight (kg)	64.29 ± 13.08
Mean age (years)	21.36 ± 2.20
Gender (%)	
Female	189 (89.2)
Male	23 (10.8)
Residence	
Capital	190 (89.6)
City	9 (4.2)
County	6 (2.8)
Village	7 (3.3)
Marital	
1 Married	2 (0.9)
2 Single but living with a partner	43 (20.3)
3 Single and not living with a partner	167 (78.8)
Financial Education	
Scholarship	77 (36.3)
Self-funding	135 (63.7)
Major (%)	
1st year	66 (31.1)
2nd year	73 (34.4)
3rd year	70 (33.0)
4th year	3 (1.4)

* cm- centimeter, kg-kilogram, kg/m2-kilogram/square meter.

Table 12 demonstrates the characteristics of the included participants. In total, 212 medical students participated in the current study, averaging 21.36 ± 2.20 years; 89.2 % were female, and 10.8% were male.

4.3.1 Change in Reported Outcome

Based on Depression Anxiety Stress Scale 21 (Norton, 2007) (Table 13.), all parameters of mental health showed significant improvements after the yoga intervention among participants. The mean score of depression significantly decreased ($p < 0.001$) from before the intervention 13.04 ± 8.90 to after intervention 5.26 ± 4.39 with a mean difference of -8.00 ± 6.96. Anxiety score significantly decreased ($p < 0.001$) from pre-intervention 14.13 ± 8.22 to post-intervention 5.22 ± 4.33, with a mean difference of -8.90 ± 6.40. Similarly, stress score also significantly reduced ($p < 0.001$) from pre 14.20 ± 8.06 to

post-intervention 5.67 ± 4.39, with mean difference -8.52 ± 6.35.

According to the World Health Organisation Quality of life-BREF (Group, 1998) (Table 13.), significant improvement has been reported by participants in all quality-of-life domains. The overall quality of life score increased from pre-intervention 5.59 ± 1.30 to post-intervention 8.33 ± 0.89 with a mean difference of 2.74 ± 1.53. Significant improvement were also noted in psychological health score (16.19 ± 1.89 to 20.88 ± 2.07, mean difference = 4.69 ± 2.85, $p < 0.001$), physical health score (23.06 ± 2.42 to 24.54 ± 1.94, mean difference = 1.47 ± 3.03, $p < 0.001$), social relations score (9.15 ± 1.72 to 12.33 ± 1.48, mean difference = 3.18 ± 1.94, $p < 0.001$), and environmental conditions score (29.13 ± 3.52 to 32.06 ± 3.40, mean difference = 2.92 ± 4.18, $p < 0.001$).

Based on the Pittsburgh Sleep Quality Index (C. Smyth, 1999) (Table 13.), participants reported significant improvement ($p < 0.001$) in quality of sleep after the yoga intervention, which is demonstrated as a global PSQI score before the intervention 128.23 ± 24.19 to after the intervention 115.04 ± 15.16 with a mean difference of -13.19 ± 28.29.

Based on Difficulties in Emotion Regulation Scale (Grant et al., 2018) (DERS) (Table 13.) Participants reported significant improvement in all the emotional regulation domains. Nonacceptance of emotional response reduced from 18.16 ± 3.84 to 11.79 ± 3.69 (mean difference = -6.36 ± 5.02, $p < 0.001$), difficulty in engaging in goal-directed behaviour reduced from 13.47 ± 2.90 to 10.89 ± 2.90 (mean difference = -2.58 ± 3.46, $p < 0.001$), impulse control difficulties improved 16.77 ± 3.50 to 11.82 ± 3.10 (mean difference = -4.95 ± 4.09, $p < 0.001$), lack of emotional awareness score increased 16.17 ± 3.73 to 17.33 ± 4.39 (mean difference = -1.16 ± 5.11, $p = 0.001$), limited access to emotion regulation strategies improved 19.53 ± 4.26 to 16.62 ± 3.54 (mean difference = -2.90 ± 4.65, $p < 0.001$) and lack of emotional clarity score decreased 14.99 ± 3.14 to 10.49 ± 1.93 (mean difference = -4.50 ± 3.31, $p = 0.001$).

Table 13: Mean and Standard deviation of Mental Health and Well-being after 10 weeks of yoga intervention in participants

N=212	Pre	Pre	Post	Post	Change	Change	Significance
Variables	**Mean**	**SD**	**Mean**	**SD**	**Mean diff.**	**SD**	**Two-Sided p**
Mental Health							
Depression	13.04	8.90	5.26	4.39	-8.00	6.96	<0.001
Anxiety	14.13	8.22	5.22	4.33	-8.90	6.40	<0.001
Stress	14.20	8.06	5.67	4.39	-8.52	6.35	<0.001
Quality of Life							
Overall Quality of Life	5.59	1.30	8.33	0.89	2.74	1.53	<0.001
Physical Health	23.06	2.42	24.54	1.94	1.47	3.03	<0.001
Psychological Health	16.19	1.89	20.88	2.07	4.69	2.85	<0.001
Social Relation	9.15	1.72	12.33	1.48	3.18	1.94	<0.001
Environment	29.13	3.52	32.06	3.40	2.92	4.18	<0.001
Quality of Sleep (PSQI)							
Global quality of sleep score (PSQI)	128.23	24.19	115.04	15.16	-13.19	28.29	<0.001
Emotional Regulation (DERS)							
Nonacceptance of emotional responses	18.16	3.84	11.79	3.69	-6.36	5.02	<0.001
Difficulty engaging in goal-directed behaviour	13.47	2.90	10.89	2.90	-2.58	3.46	<0.001
Impulse control difficulties	16.77	3.50	11.82	3.10	-4.95	4.09	<0.001
Lack of emotional awareness	16.17	3.73	17.33	4.39	1.16	5.11	0.001
Limited access to emotion regulation strategies	19.53	4.26	16.62	3.54	-2.90	4.65	<0.001
Lack of emotional clarity	14.99	3.14	10.49	1.93	-4.50	3.31	0.001

Note: *Significant at P <0.005

4.3.2 Stepwise linear regression analysis

Stepwise linear regression analysis of change in stress, depression, and anxiety has been demonstrated and summarised in Tables 14-16. An increase in depression ($\beta = 0.626$, $p < .001$, Adjusted $R^2 = 0.392$, tolerance 0.615, VIF 1.623) and anxiety ($\beta = 0.608$, $p < .001$, Adjusted $R^2 = 0.366$, tolerance 0.601, VIF 1.663) showed a strong positive relation to higher stress levels. while environmental condition improvement is associated with decreased stress levels ($\beta = -0.302$, $p < .001$, Adjusted $R^2 = 0.087$, tolerance 0.941, VIF 1.063) (Table 14). Table 15 shows the change in depression is primarily and positively associated with stress ($\beta = 0.626$, $p < .001$, Adjusted $R^2 = 0.392$, tolerance 0.622, VIF 1.608)

and anxiety (β = 0.619, p < .001, Adjusted R^2 = 0.380, tolerance 0.577, VIF 1.734). Additionally, a small contribution of physical health of activity (β = -0.262, p < .001, Adjusted R^2 = 0.064, tolerance 0.909, VIF 1.101) is also associated with a decrease in depression. Table 16 shows the change in anxiety is strongly associated with depression (β = 0.619, p < .001, Adjusted R^2 = 0.383, tolerance 0.562, VIF 1.780) and stress (β = 0.608, p < .001, Adjusted R^2 = 0.369, tolerance 0.561, VIF 1.782) levels. However, improvement in environmental conditions (β = -0.237, p < .001, Adjusted R^2 = 0.052, tolerance 0.894, VIF 1.118) and physical health (β = -0.280, p < .001, Adjusted R^2 = 0.070, tolerance 0.905, VIF 1.105) leads to a reduction in anxiety. The fitness of the model showed statistical significance for change in stress (p<0.001), change in depression (p<0.001), and change in anxiety (p<0.001). The collinearity diagnostics showed that all the predictors had VIF below 10 and tolerance values above 0.1, this indicates that multicollinearity is not a concern, and the model used is well conditioned.

Table 14: Stepwise regression investigating which factors contribute most to Change in Stress.

Empty Cell	**B**	**SE**	***β***	**P-value**	**Adjusted R^2**
Change Anxiety	0.602	0.054	0.608	<0.001	0.366
Change Depression	0.571	0.049	0.626	<0.001	0.392
Change in Environmental Condition	-0.459	0.100	-0.302	<0.001	0.087

*B = Unstandardized coefficient; SE = Standard error; β = Standardized coefficient; P-value indicates statistical significance; Adjusted R^2 reflects explained variance in the outcome variable by each predictor. significant (p < 0.001).

Table 15: Stepwise regression investigating which factors contribute most to the Change in Depression.

Empty Cell	**B**	**SE**	***β***	**P-value**	**Adjusted R^2**
Change Stress	0.687	0.059	0.626	<0.001	0.392
Change Anxiety	0.673	0.059	0.619	<0.001	0.380
Change in Physical Health	-0.601	0.153	-0.262	<0.001	0.064

*B = Unstandardized coefficient; SE = Standard error; β = Standardized coefficient; P-value indicates statistical significance; Adjusted R^2 reflects explained variance in the outcome variable by each predictor. significant (p < 0.001).

Table 16: Stepwise regression investigating which factors contribute most to Change in Anxiety.

Empty Cell	**B**	**SE**	***β***	**P-value**	**Adjusted R^2**
Change Depression	-4.349	0.528	0.619	<0.001	0.383
Change Stress	0.613	0.055	0.608	<0.001	0.369
Change in Physical Health	-0.591	0.140	-0.280	<0.001	0.070

Change in Environmental Condition	-0.363	0.103	-0.237	<0.001	0.052

*B = Unstandardized coefficient; SE = Standard error; β = Standardized coefficient; P-value indicates statistical significance; Adjusted R^2 reflects explained variance in the outcome variable by each predictor. significant ($p < 0.001$).

4.4 Discussion

We explore the 10-week yoga intervention's impact on mental health and well-being using the experimental pre-post-test intervention method. Our findings showed that a 10-week yoga intervention has a significant decrease in stress ($p < .001$), anxiety ($p < .001$), and depression ($p < .001$), which leads to improvement in the mental health of medical students. Among the participants, there more than male participants, this observation is supported by the systematic review that yoga practice is greatest among female participants, another study showed that male participants reported heterosexual self-presentation as a barrier to not adopting yoga practice (Park, Braun, & Siegel, 2015), However, we would like to convey the message that yoga is not for a particular gender, and current findings support the benefits of yoga not depending on the gender of the participants (Motzkus & Jarry, 2024). In the present study, participants felt improvement in their emotional regulation, quality of sleep, and quality of life, leading to overall improvement in well-being after 10 weeks of yoga intervention. This highlights that yoga intervention can potentially improve mental health and well-being (Woodyard, 2011). Additionally, current findings present an interrelated relationship between mental health outcomes and psychosocial factors that show an essential role in influencing changes in mental health outcomes. The finding supports the idea that practicing meditation and yoga can improve quality of life and mental health, resulting in a healthy body and mind (Grant et al., 2018; Norton, 2007). The results show that 10 weeks of yoga intervention significantly enhances the medical students' mental health. Additionally, it reveals that reducing depression and anxiety is a strong predictor of decreasing stress levels, and improvements in physical health and environmental conditions contribute to reducing anxiety and stress levels. This shows that yoga is a holistic intervention that not only improves mental health and well-being but also creates a calming environment that results in stress relief. The focus of yoga intervention on breathing exercises, posture with conscious breathing, and meditation relaxation techniques aligns with previous research, which demonstrates that yoga's integrative and holistic approach leads to exploring not only physical activity but the whole journey toward reduction of stress, improvement in emotional well-being, and balanced lifestyle (Park et al., 2015).

The finding supports the idea that practicing meditation and yoga can improve quality of life and mental health, resulting in a healthy body and mind (Dhawan, Chopra, Jain, & Yadav, 2015; Kavitha et al., 2023). The evidence supports yoga's holistic practice and promotes better sleep quality by decreasing stress and calming the mind before bedtime (Dhawan et al., 2015; Kavitha et al., 2023; Motzkus & Jarry, 2024). In the current study, yoga intervention was provided in the evening after 7:00 PM, and intervention was ended by breathing and meditation techniques. Practicing in the evening time of the day can also improve the current findings as it leads to calming the mind before bedtime. The study participants reported the benefits of the yoga intervention, as reflected by the significant PSQI score,

which demonstrates better sleep duration with sleep efficacy. Controlled breathing and physical yoga poses encourage relaxation (Field, 2016; Sovik, 2000), and yoga practice helps improve parasympathetic activation and modulation of the autonomic nervous system and favors restful sleep (Patra & Telles, 2010).

Participants responded to significant improvement in quality of life after the yoga intervention using WHOQOL-BREF scores. This tool also shows that the overall quality of life, well-being, and life satisfaction significantly improved after the yoga intervention. Quality of life measured in the current study includes the sub-score of improvement in physical health, environmental condition, psychological health, and social relationships, all significantly improved after the yoga intervention. This is supported by the systematic review, which states that yoga is associated with a significant decrease in negative thoughts and an increase in quality of life (Yin et al., 2024). Medical student experiences intense emotions and an absence of emotional regulation that affect their performance, motivation, and overall well-being (Chambers, Gullone, & Allen, 2009; Doulougeri, Panagopoulou, & Montgomery, 2016). In the current research, medical students showed improvements in the DERS scale, which includes the sub-score of emotional awareness, regulations, emotional clarity, and impulse control; these findings highlight that yoga interventions improved participants' ability to respond to emotion and manage emotion effectively. This is supported by findings that show that yoga has a potential effect on emotional regulation and beneficially healthier mental health outcomes(Menezes et al., 2015).

The strength of the current study is that it shows that the sample size provides sufficient value to detect the impact of yoga on meaningful changes in mental health, quality of life, quality of sleep, and emotional regulation among medical students. However, several limitations should be addressed in future studies. There was no control group, so adding a current group can help decrease the expected bias and give more clarity that the outcome reported is due to yoga practice or another factor contributing. Future research should focus on rigorous methodological randomized control trials with long-term intervention among medical students to minimize potential bias, as seen in the current study.

4.5 Limitations of the current study

In the current chapter, a major limitation is the absence of a control group, which limits the chance to establish causality, and it can also determine if the findings are solely attributable to the 10 weeks of yoga intervention. However, the current finding shows a significant improvement in mental health, sleep quality, quality of life, and emotional regulation. Additionally, potential confounding factors were not explicitly controlled in the study, such as lifestyle factors and dietary habits that can influence the findings. Another key limitation is a lack of long-term follow-up, which prevents the observation of the benefits over time. It is recommended to interpret the findings with caution. The predominance of female participants in the current study and non-random convenient sampling may limit the generalizability of the findings, it is recommended to interpret the findings with caution. Furthermore, Future studies should focus on long-term intervention with randomized control trials to improve the

robustness of the findings.

4.6 Conclusion

The current chapter shows that yoga intervention used in GSY protocol can significantly improve medical students' mental health and overall well-being. The findings highlight that yoga is a holistic tool to enhance the quality of sleep, quality of life, emotional regulation, and mental health outcomes. The observed improvement in the outcomes suggests that yoga intervention can be an effective practice for overall health promotion among medical students to navigate the challenges faced by students, such as emotional and mental health challenges during their training. By including yoga and meditation practice in the daily lives of medical students, educational institutes can provide a more supportive environment that focuses on students' overall health. However, the present study does not have a control group, which limits the ability of the findings to show that all observed outcomes are solely due to yoga intervention. Future studies should focus on long-term intervention with randomized control trials.

Sub-study 5- Effect of 10 weeks of yoga intervention on Evening and Morning salivary cortisol of medical students: Randomised control trial[4]

5.1 Introduction

The cortisol hormone, also called as "stress hormone," plays a vital role in the physiological response of the body to stress (Holsboer & Ising, 2010). It is a steroid hormone released and synthesized by the adrenal cortex. During stressful situations, the hypothalamic-pituitary-adrenal (HPA) axis is activated, and in response to this, the cortisol hormone is released (Tang et al., 2021). The cortisol hormone follows a natural daily cycle, also called the diurnal rhythm. It is higher in the morning to enhance energy and alertness, and it is lower in the evening for relaxation and sleep (Foster, 2020; Niu et al., 2011). This cortisol cycle regulates the stress response, metabolism, and immune function and maintains overall homeostasis (Jefferies, 1991; Niu et al., 2011). Individuals must have a stable cortisol cycle, which helps in proper physiological stability and energy distribution (J. M. Smyth et al., 1997).

It has been shown in many studies that medical students encounter a high level of stress because of many reasons, such as academic pressure and irregular sleep which all lead to a high level of cortisol (Rachmawati, Farid Wafi, & Resmi Melani, 2017; Shah, Hasan, Malik, & Sreeramareddy, 2010; Weekes et al., 2006). Elevated cortisol levels and a chronic stress environment increase the risk of chronic

[4]Manuscript under review

disease among medical students (Jones & Gwenin, 2021). Exposure to high levels of cortisol for a prolonged time can contribute to cardiovascular disease, immune dysfunction, metabolic disorders, hormonal imbalance, and mental health disorders, all of which are non-communicable disease burdens (Kivimäki, Bartolomucci, & Kawachi, 2023; Knezevic, Nenic, Milanovic, & Knezevic, 2023; Krishnaveni & Srinivasan, 2019; Ortiz, Kluwe, Lazarus, Teruel, & Joseph, 2022; Upadhyay, 2022).

The study showed that medical students suffer from poor quality sleep (Azad et al., 2015), However, one of the causes of poor quality sleep is by high levels of cortisol, as it can disturb the sleep-wake cycle, reduce the level of deep sleep and it can also delay sleep onset (Nicolaides, Vgontzas, Kritikou, & Chrousos, 2020; Zisapel, 2007). These symptoms of poor-quality sleep due to high levels of cortisol can contribute to fatigue and mental health disorders, and it also affect overall quality of life (Henry, 2019). Evidence shows that medical students and experts should have good cognitive function, memory performance, abilities of decision making, and these all factors are affected by chronic stress, which can directly influence clinical and academic performance for students (Ericsson, 2004; Rosiek et al., 2016). Above it is explained how elevated levels of cortisol leads to many chronic diseases, and medical students are at a high level of risk of chronic stress-related health disease (Tran et al., 2017). The existing education system struggles to implement stress management techniques as medical students have already packed schedules (Bustamam et al., 2020; Chauhan et al., 2024). Therefore, it is very crucial to introduce strategies for stress management which is not only time-efficient and low-resource but also non-exhausting.

Yoga is proven to be one of the stress management techniques, its accessible approach, time efficiency, and very low resource(Dhungana, Khatiwoda, Gurung, Pedišić, & de Courten, 2021) required make it highly suitable for medical students. It has been scientifically proven that Yoga is an effective mind-body practice, including posture exercise, breathing and relaxation techniques, and meditation, which helps regulate stress and its health outcomes(Brems, 2020). A systematic review showed that yoga practice can stabilize the salivary cortisol rhythm(Ryan, Booth, Spathis, Mollart, & Clow, 2016), although there is very limited evidence in the case of medical students(Moreno, Becerra, Ortega, Suarez-Ortegón, & Moreno, 2023). Given that these students are the future of health care, their quality of life and well-being will also impact patients' health care quality. Considering the demanding schedule and high-stress environment, it is crucial to investigate whether practicing yoga can stabilize cortisol levels.

The primary aim of the current study was to investigate the effect of 10 weeks of yoga intervention on evening and morning salivary cortisol. Additionally, it also investigates the association between cortisol hormone, sleep quality, and overall quality of life after 10 weeks of yoga practice among medical students. By assessing this investigation, this study aims to provide clinical guidelines for integrating

yoga practice as a practical and sustainable strategy for reducing stress and improving quality of life in this high-risk population.

5.2 Methods

5.2.1 Trial design

The current study is a single-centered, two-arm simple randomisation control trial (RCT) with an equal allocation of participants to an experimental group, and a passive control group was pre-registered at Clinical Trial.gov (NCT06661603). The Current RCT was consistent with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Moher et al., 2010) (Figure 4). The passive control group was advised not to engage in any other structured physical activity and to follow the usual daily routine.

5.2.2 Participants and procedures

Medical students from all the years of study were invited to the seminar at the Medical School, University of Pécs, Hungary, where the principal investigator of the current study presented a research plan to the medical students. Participation in the research was voluntary, and it was based on informed consent after getting information about the aim of the research and data management. Inclusion criteria were students with an active student status in the Medical School of the University of Pecs, participants who had no previous experience with yoga, and participants who were not involved in any other structured physical activity program during the duration of the study were eligible to participate. Participants suffering from chronic injury or chronic disease were excluded from the study. Participants allocated to the yoga group were provided once a week, for a 10-week yoga session with 90 minutes duration of each session from February 2024 to May 2024. The intervention group was registered for the Physical Education course with the name “Indian Yoga” for the whole semester. Both female and male participants in the current study were from diverse international backgrounds so the session was provided in English. Participants from the control group were given information about the data collection method and that they should follow their routine for the duration of the current study.

To minimize the bias, participants from the control group were advised to report any significant change they may have made during the duration of the study, especially in the case of physical activity habits, although there were no participants who reported any change in their habits during the duration of the study. Sociodemographic data, including age, height, weight, relationship status, and financial support, questionnaire data, morning saliva sample, and evening saliva sample were gathered before the intervention from both groups.

5.2.3 Randomization

In the current study, participants were allocated in a 1:1 ratio to the intervention group and control group using a computer-generated simple random sequence number. The generated numbers were printed out on paper and later put inside of envelope. To ensure accuracy in randomization, all papers and envelop looked the same, and envelopes were sealed before being given to the participants. After all the participants provided informed consent and their eligibility was confirmed, envelopes were opened by the principal investigator. Based on the identification number inside the envelope, participants were allocated to the control group and yoga group. Due to the nature of the intervention used in the current study, double blinding was not possible, this study used single-blinding methods. The data collectors, statisticians, and laboratory experts were blinded to the participants' group allocation and assignment, ensuring the outcome assessment remained free from potential bias.

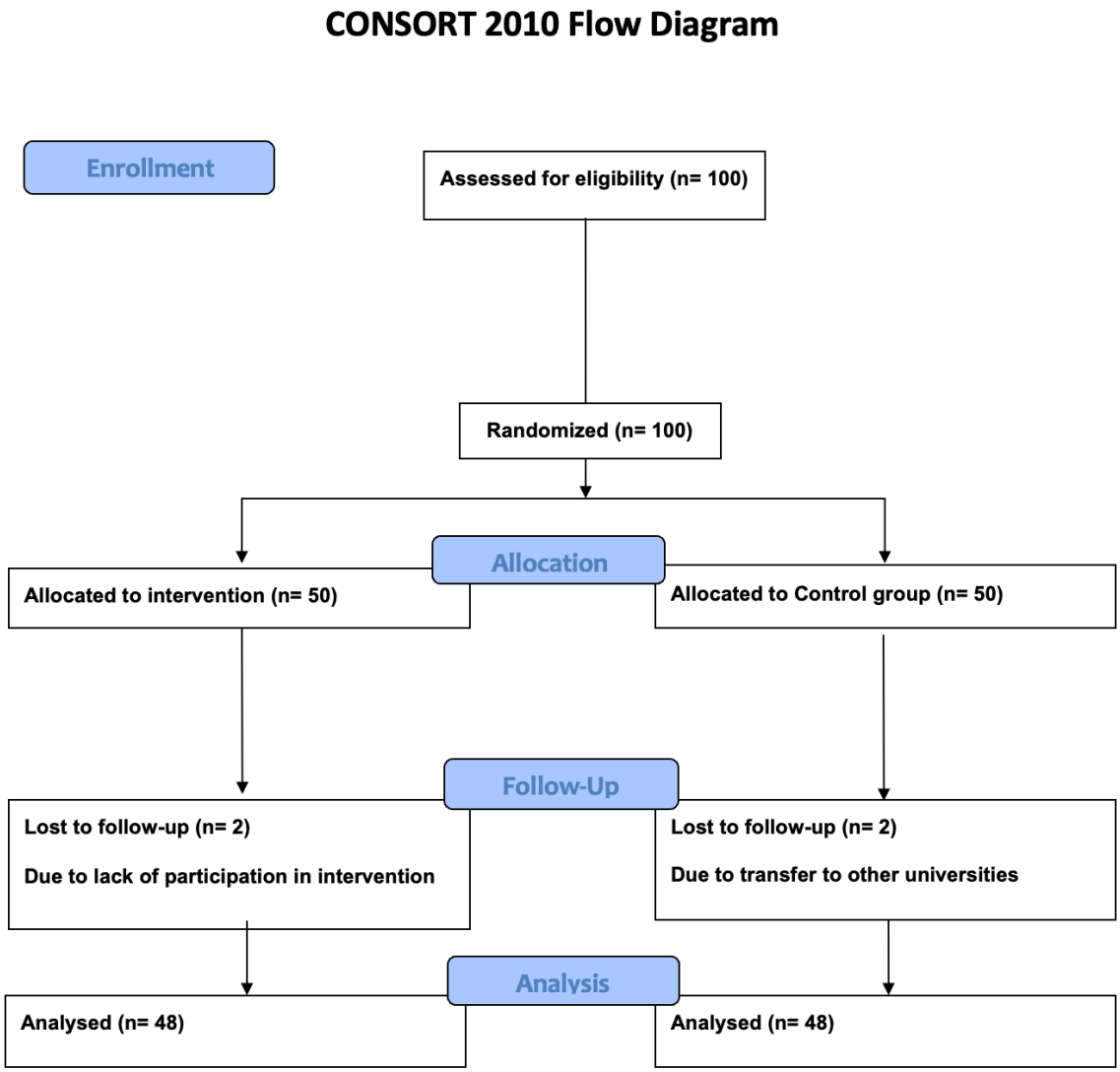

Figure 4: CONSORT Flow Chart

5.2.4 Intervention

A detailed 20-minute in-person information session was provided to the participants allocated to the yoga group about the goal, topic, data collection procedure, and structure of the intervention. They were informed about the timing of the yoga intervention course. After all the participants agreed to all the detailed information provided by verbal and written consent, the pre-data collection intervention began. The intervention was given, once a week, a 90-minute session for 10 weeks to Intervention participants. Each yoga session followed specific yoga protocols named "GSY Goodbye Stress with Yoga Protocol" (Table 1), which were developed by a team of yoga instructors, medical researchers, and experienced yogis from India. Every yoga session was provided by a certified yoga teacher with a minimum of 9 years of teaching experience. Yoga practice includes stretching, asanas (yogic postures: supine, sitting, standing, and prone), pranayama (breathing techniques), and meditation. All the yoga lecture was

closely monitored to ensure accuracy in the intervention delivered, and that every component of the protocol was covered in every class. The yoga group was instructed to practice breathing exercises and meditation thrice a week for 15 minutes empty stomach, at home. Reminders were given weekly to the individual participant to ensure they adhered to their home breathing practice.

Table 17: Yoga Protocol used in the current research, specifically for balancing cortisol hormone among medical students: GSY Study

Warm up and Stretching (Head to toe) and Balancing 15 Minutes		
Name	**Practice Frequency**	**Benefits**
Neck: Neck Rotations, Side-to-Side Neck Stretch	**3 times**	Relieves tension and improves neck flexibility.
Wrist and Shoulders: Shoulder Rotations, Arm Swings, Shoulder Shrugs		Increases shoulder mobility and reduces stiffness.
Hand & Fingers: Finger Bending, Wrist Rotations		Enhances dexterity and reduces hand fatigue.
Knee: Knee Rotations, Leg Extensions		Improves knee joint flexibility and strength.
Toe & Ankle: Ankle Rotations, Toe Bending, Toe Stretching		Enhances ankle flexibility and promotes foot health.
Vrikshasana (Tree Pose)	2 times each side	Enhances concentration & stability
Asana (Standing,Sitting,Supine and Prone Posture) 50 Minutes		
Pose Name	**Practice Frequency**	**Benefits**
Surya Namaskar (Sun Salutation)	3 rounds	Improves circulation & warms up the body
Utkatasana (Chair Pose)	3 times	Strengthens legs & enhances stability
Malasana (Garland Pose)	3 times	Opens hips & improves digestion
Tadasana (Mountain Pose)	3 times	Enhances posture & balance
Trikonasana (Triangle Pose)	2 times each side	Improves flexibility & spinal alignment
Konasana (Angle Pose)	2 times each side	Enhances lateral flexibility & core strength
Natarajasana (Dancer's Pose)	2 times each side	Improves balance & focus
Pavanamuktasana (Wind Releasing Pose)	3 times	Relieves bloating & promotes digestion
Setu Bandhasana (Bridge Pose)	3 times	Reduces stress & enhances circulation

Chakrasana (Wheel Pose)	2 times	Increases spinal flexibility & energy flow
Bhujangasana (Cobra Pose)	3 times	Reduces adrenal overactivity & strengthens spine
Dhanurasana (Bow Pose)	3 times	Stimulates digestion & strengthens back muscles
Navasana (Boat Pose)	3 times	Strengthens core & reduces abdominal fat
Balasana (Child's Pose)	3 times	Induces relaxation & relieves tension
Vajrasana (Thunderbolt Pose)	1 time, hold longer	Aids digestion & stabilizes the mind
Vrikshasana (Tree Pose)	2 times each side	Enhances concentration & stability
Paschimottanasana (Seated Forward Bend)	3 times	Stretches hamstrings & calms the nervous system
Janu Sirsasana (Head-to-Knee Pose)	3 times	Improves flexibility & reduces stress
Ardha Matsyendrasana (Half Spinal Twist)	2 times each side	Enhances spinal mobility & detoxification
Shashankasana (Hare Pose)	3 times	Activates the relaxation response
Viparita Karani (Legs-Up-The-Wall Pose)	1 time, hold longer	Improves blood flow & reduces cortisol
Shavasana (Corpse Pose)	1 time, final relaxation	Promotes deep relaxation & nervous system balance
Pranayama (Breathing Techniques) – 10 Minutes		
Anulom Vilom (Alternate Nostril Breathing)	15 breaths	Balances sympathetic & parasympathetic activity
Bhramari (Bee Breath)	10 rounds	Lowers heart rate & induces relaxation
Breathing Meditation & Relaxation – 15 Minutes		
Guided Breath Awareness	5 minutes	Deep breathing with instructor cues
3:1 Breathing (Three Inhales, One Exhale)	10 rounds	Enhances oxygenation & reduces stress response
OM Chanting Meditation	5 minutes	Vibrational healing & mental clarity
Silent Observation with OM Music	5 minutes	Mindfulness & emotional balance

5.2.5 Outcome Measurement

In the current RCT, two types of measurement are used in data collection, subjective measurement and objective measurement. Subjective measurement was done by using validated questionnaires to assess both groups' participants' mental health, quality of life, and sleep quality before and after 10 weeks of intervention. However objective measurement was conducted by collecting saliva samples to assess the cortisol hormone rhythm. Outcome measures were assessed at baseline (before the intervention began) and after the 10-week intervention period.

5.2.5.1 Measurement of Saliva Sample

Saliva samples were collected from participants at two time points: the evening before the first yoga session (7:00 p.m.–8:30 p.m.) and the following morning immediately upon awakening (7:00 a.m.–8:30 a.m.). The precise collection time was recorded for all participants. This same procedure was repeated after 10 weeks of yoga intervention. Approximately 1- 1.5 mL of unstimulated saliva was collected from participants by using passive drool in sterile Eppendorf tubes (Prémusz et al., 2022; Salimetrics & SalivaBio, 2011). Morning samples were collected at home by participants before brushing, eating, or drinking, with research assistants providing proper education on immediate collection upon waking and accurate time recording. Evening samples were collected with the assistance of a research assistant. After collection of the sample, it was stored in the refrigerator at 2-8 degrees Celsius, and within 6 hours samples were centrifuged at 3000 rpm for 5 minutes. The supernatant aspiration was removed after the centrifugation, and all the samples were stored at -20 °C (Michels et al., 2012; Prémusz et al., 2022).

5.2.5.2 Laboratory measurement

Cortisol concentrations in the prepared saliva samples were determined using an Enzyme-Linked Immunosorbent Assay (ELISA). The assay was performed according to the manufacturer's instructions with the NovaTec Immundiagnostica GmbH (Dietzenbach, Germany) Cortisol Saliva ELISA kit (DSNOV20). Samples were assayed in duplicate, with 20 µl per well. The intra-assay coefficient of variation was below 10%, and the inter-assay coefficient of variation was below 8.3%. Measured cortisol concentrations, expressed in ng/ml.

5.2.5.3 Measurement of subjective assessment

Sociodemographic data, including age, height, weight, relationship status, and financial support, from both groups. Depression Anxiety Stress Scale (DASS-21) (Norton, 2007), the World Health Organization Quality of Life Scale (WHOQOL-BREF)(Group, 1998), and the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds III, Monk, Berman, & Kupfer, 1989) were used in the validated English version of the current study to analyze the level of depression, anxiety, and stress, as well as quality of life and quality of sleep of the participants.

Depression Anxiety Stress Scale (DASS-21) was used to assess the variations in stress, depression, and anxiety levels experienced by students, pre- and post-implementation of yoga intervention. This questionnaire consists of 21 questions, which include 3 sets of self-reported instruments which is used to assess the emotional state of depression, anxiety, and stress. DASS 21 uses a dimensional approach to evaluate psychological disorders, rather than relying on categorical classifications. The subscales of DASS-21 (Depression, Anxiety, and Stress), scores were derived from the total of seven relevant questions and range from normal to extremely severe.

World Health Organization Quality of Life BREF (WHOQOL-BREF) tool was used to analyze the individual perception of quality of life in recent days before and after the yoga intervention is done. This tool consists of 26 questions which are self-administered, it includes questions on the individual perceptions of the quality of life and health over the past two weeks. This questionnaire consists of four sections, the first one is on Physical health, the second on Psychological, the third on Social relationships, and lastly on the Environment. Scoring is done based on a 1 to 5 Likert scale where 1 represents “Disagree” and 5 represents “Completely agree.

Pittsburgh Sleep Quality Index (PSQI) was used to assess the quality of sleep, it is a validated and self-reported questionnaire that evaluates the quality of sleep over the past month. This questionnaire includes 19 items, which generate seven component scores. These seven component scores indicate sleep quality, sleep duration, sleep latency, habitual sleep efficiency, sleep disturbances, daytime dysfunction, and use of sleep medication. All component scores collectively represent the global PSQI score, the lower the score the better the quality of sleep.

5.2.6 Sample Size

The target sample size of 100 participants, with 50 allocated to the yoga intervention group and 50 to the control group, was primarily determined by the practical limitations inherent in this research project, including available research personnel time and the feasible timeframe for participant recruitment and data collection within the academic schedule. This sample size was considered a pragmatic and achievable goal within the study's constraints, allowing for an initial exploration of potential differences between the groups on the outcome variables.

5.2.7 Ethical Approval

The study was carried out following the Declaration of Helsinki. The study was reviewed and approved by the Regional Research Ethics Committee as the Institutional Review Board Record number 9117-PTE 2022, University of Pecs, Hungary.

5.2.8 Statistical methods

In the current study, statistical analyses were performed using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to check the distribution of the data. Based on the normality test, a paired sample T-test was used to compare the means within the intervention group and control group. Independent samples T-tests were also performed to compare differences between the intervention and control groups. Cohen's *d* was used to calculate the effect size for these within-group and between-group changes. Analysis of Covariance (ANCOVA) was employed to compare differences between groups' post-intervention scores, controlling for baseline scores of the respective outcome variables. This method was chosen to account for significant differences between the groups at baseline for several outcome variables, providing a more accurate assessment of the intervention's effect. The effect size for the between-group changes from ANCOVA was evaluated using partial eta squared ($\eta p2$). A significance level was set at $p<0.05$ for all the tests used.

5.3 Results

In the current RCT, 100 medical students participated, out of which 50 students were randomly allocated to the yoga group and 50 students were allocated to the control group. Both groups were included in the final analysis for the primary and secondary outcomes. Table 18 demonstrates the sociodemographic data of the current participants. There were no significant differences were observed in terms of age (21.82 ± 2.48 years vs. 22.72 ± 3.41 years, p = 0.135), weight (63 ± 12.35 kg vs. 63.52 ± 8.98 kg, p = 0.810) and height (167.06 ± 8.92 cm vs. 169.38 ± 8.64 cm, p = 0.190) between the groups. No significant difference was found in the gender distribution ($\chi 2$ test, p = 0.656) (Intervention group: 37 females, 13 males, Control group: 35 females, 15 males).

Table 18. Baseline Characteristics of Study Population

Characteristics (N=100)	Frequency (%)		Test statistic
	Intervention (50)	Control (50)	
Anthropometrics			
Height (cm)	167.06±8.92	169.38±8.64	0.190
Weight (kg)	63±12.35	63.52±8.98	0.810
Mean age (years)	21.82±2.48	22.72±3.41	0.135
Gender (%)			p=0.656
Female	37 (74)	35 (70)	
Male	13 (26)	15 (30)	
Residence			0.884
Capital	3 (6)	4 (8)	
City	26 (52)	24 (48)	
County	21 (42)	22 (44)	
Relationship			0.509
Single but living with partner	16 (32)	13 (26)	
Single not living with partner	34 (68)	37 (74)	
Financial Education			0.230
Scholarship	22 (44)	28 (56)	
Self-funding	28 (56)	22 (44)	
Major (%)			0.63
1st year	18 (36)	26 (52)	
2nd year	18 (36)	8 (16)	
3rd year	14 (28)	14 (28)	
4th year	0 (0)	2 (4)	

*Independent *t*-tests for continuous data, chi-square for categorical data. None of the tests were statistically significant.

Table 19 shows the pre- and post-intervention scores for both groups and changes within the group achieved after 10 weeks of duration in morning salivary cortisol, evening salivary cortisol, DASS 21 (depression, anxiety stress) score, WHOQOL-BREF (scores of physical health, psychological health, social relations, environmental) and Global PSQI (Pittsburgh sleep quality index) score.

Morning salivary cortisol significantly decreased in the intervention group ($p = 0.027$) but significantly increased in the control group ($p = 0.021$). Evening cortisol showed a non-significant reduction in the intervention group ($p = 0.060$) and a non-significant increase in the control group ($p = 0.081$). (Figure 5).

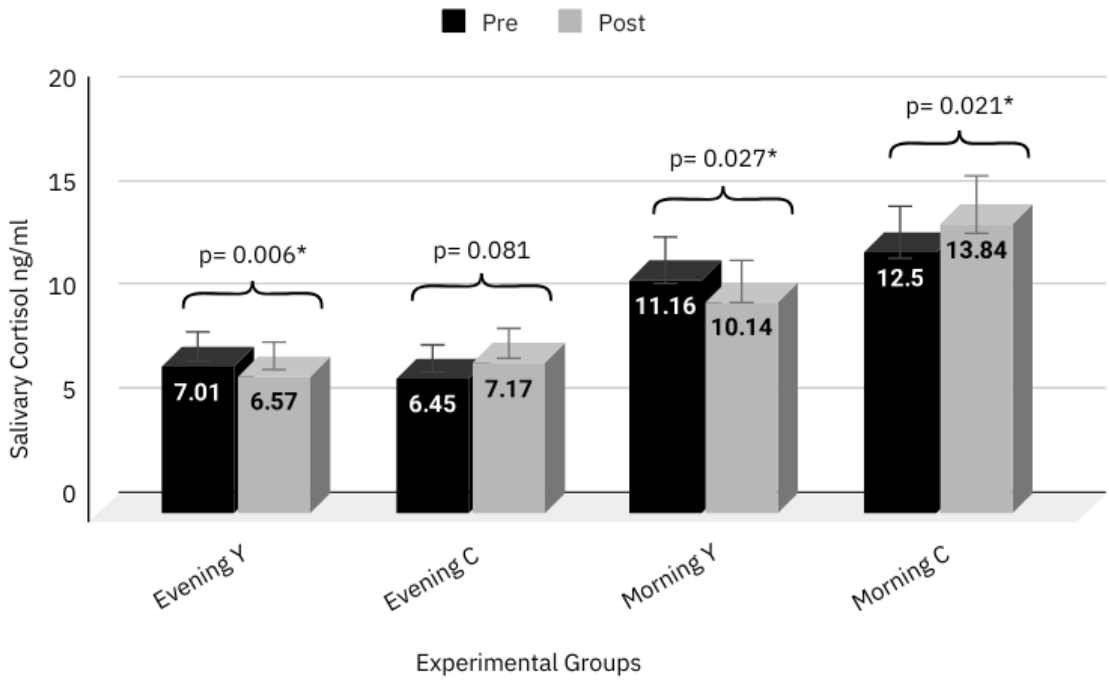

Figure 5. Comparison of the Mean of Morning and Evening salivary cortisol levels within the Intervention group and the control group.

*Note: Created by the author based on the findings of the accompanying manuscript.: Y: Yoga intervention group, C: Control group, Asterisk (*) indicates statistical significance ($p<0.05$).

The DASS 21 revealed significant improvements ($p < 0.001$) in depression, anxiety ($p = 0.007$), and stress ($p = 0.061$) scores in the intervention group. Conversely, the control group showed significant increases ($p < 0.001$ for all) in depression, anxiety, and stress scores.

For the WHOQOL-BREF, the intervention group experienced a significant increase in physical health score ($p = 0.024$), while the control group showed no significant change ($p = 0.151$). Psychological health and environmental scores did not significantly change in the intervention group ($p = 0.40$ and $p = 0.230$, respectively). In the control group, psychological health also showed no significant change ($p = 0.243$), but there were significant decreases in social relations ($p < 0.001$) and environmental scores ($p = 0.024$). The overall quality of life significantly increased in the intervention group ($p < 0.001$) and significantly decreased in the control group ($p < 0.001$).

The Pittsburgh Sleep Quality Index (PSQI) global score significantly decreased (indicating better sleep) in the intervention group (p = 0.019) and significantly increased (indicating worsened sleep) in the control group (p = 0.021).

Table 19. Mean (± SD) Pre- and Post-Intervention Scores and Within-Group Changes

Variable	N Y= 48 C=48	Pre	Post	Mean Diff	Intra pValue
Cortisol					
Morning (ng/ml)	Y	11.16 ± 4.71	10.14 ± 5.05	1.01 ± 3.08	0.027
	C	12.50 ± 3.76	13.84 ± 4.37	-1.34 ± 3.89	0.021
Evening (ng/ml)	Y	7.01 ± 2.66	6.57 ± 3.15	0.43 ± 1.56	0.060
	C	6.45 ± 2.31	7.17 ± 2.91	-0.72 ± 2.79	0.081
DASS 21					
Depression	Y	16.48 ±9.67	11.71±7.00	4.77±8.138	<0.001
	C	11.06±2.59	15.15±3.73	-4.08±4.45	<0.001
Anxiety	Y	14.88±7.42	12.29±7.70	6.29±0.003	0.007
	C	11.92±2.48	16.63±2.95	-4.70±3.66	<0.001
Stress	Y	16.67±8.88	13.63±9.92	3.04±10.98	0.061
	C	11.44±3.18	14.96±4.03	-3.52±4.66	<0.001
WHOQOL Bref					
Overall_QOL	Y	6.06±1.31	8±1.52	-1.93±1.65	<0.001
	C	4.81±1.14	3.37±1.28	1.43±1.32	<0.001
Physical Health	Y	21.41±3.94	22.87±4.18	4.32±0.012	0.024
	C	26.11±14.03	22.45±13.39	3.66±17.39	0.151
Psychological	Y	19.00±4.33	20.52±4.08	-1.52±4.98	0.040
	C	17.89±5.13	17.11±4.18	0.77±4.51	0.243
Social Relations	Y	11.04±2.65	11.58±2.34	-0.54±2.60	0.156
	C	12.54±1.57	9.79±1.92	2.75±2.24	<0.001
Environmental	Y	30.66±4.86	29.87±4.88	0.79±4.50	0.230
	C	27.65±11.10	24.77±10.55	2.88±8.54	0.024
PSQI					
Global PSQI score	Y	64.50±35.32	53.49±35.62	11.01±31.31	0.019
	C	49.21±23.59	55.14±22.44	-5.93±17.16	0.021

*Abbreviations: N, number of participants: Y, Yoga group; C, Control group; DASS-21, Depression Anxiety Stress Scale-21; WHOQOL-BREF, World Health Organization Quality of Life-BREF; QOL, Quality of Life; PSQI, Pittsburgh Sleep Quality Index; ng/ml, nanograms per millilitre; *p*, probability value; ±, standard deviation.

Table 20. shows the comparison between the groups of all outcome variables, including morning salivary cortisol, evening salivary cortisol, DASS 21 (depression, anxiety stress) score, WHOQOL-

BREF (scores of physical health, psychological health, social relations, environmental) and Global PSQI (Pittsburgh sleep quality index) scores represented by intergroup significance (p-value), the mean difference with 95% confidence interval values, and Cohen's d effect sizes values. The between-group comparison result showed significant benefits of intervention across multiple outcomes variables such as morning salivary cortisol level (mean difference -2.35 ng/ml, 95% CI -3.78, -0.93, $p < 0.001$, $d = -0.671$), evening cortisol level (mean difference -1.15 ng/ml, 95% CI -2.07, -0.23, $p = 0.014$, $d = -0.516$), depression score (mean difference -8.85, 95% CI -11.52, -6.18, $p < 0.001$, = -1.35), stress score (mean difference -6.56, 95% CI -9.98, -3.14, $p < 0.001$, $d = -0.778$), anxiety score (mean difference -7.29, 95% CI -9.37, -5.20, $p < 0.001$, $d = -1.406$), physical health (mean difference 5.12, 95% CI -0.010, 10.26, $p = 0.053$, $d = 0.404$), psychological health score (mean difference 2.29, 95% CI 0.36, 4.22, $p = 0.02$, $d = 0.482$), social relation score (mean difference 3.29, 95% CI 2.30, 4.27, $p < 0.001$, $d = 1.355$), overall quality of life score (mean difference 3.37, 95% CI 2.76, 3.98, $p < .001$, $d = 2.252$). However, there were no significant changes observed in environmental scores (mean difference 2.08, 95% CI -.678, 4.85, $p = 0.137$, $d = 0.303$) between the groups.

Table 20. Comparison of mean change score between Yoga groups and Control groups

Variable	Mean Difference (Yoga - Control)	95% CI	Intergroup significance p value	Cohen's d
Change Morning Cortisol	-2.35 ng/ml	-3.78, -0.93	<0.001	-0.671
Change Evening Cortisol	-1.15 ng/ml	-2.07, -0.23	0.014	-0.516
Change Depression	-8.85	-11.52, -6.18	<0.001	-1.35
Change Stress	-6.56	-9.98, -3.14	<0.001	-0.778
Change Anxiety	-7.29	-9.37, -5.20	<0.001	-1.406
Change Overall QOL WHOQOL Bref	3.37	2.76,3.98	<0.001	2.252
Change Physical Health WHOQOL Bref	5.12	-0.010, 10.26	0.053	0.404
Change Psycho Health WHOQOL Bref	2.29	0.36, 4.22	0.02	0.482
Change Social Health WHOQOL Bref	3.29	2.30, 4.27	<0.001	1.355
Change Environmental Health WHOQOL Bref	2.08	-.678, 4.85	0.137	0.303
Change PSQI Score	-16.94	-27.18, -6.71	0.001	0.671

*Abbreviation CI, confidence interval; QOL, quality of life; WHOQOL-BREF, World Health Organization Quality of Life-BREF; PSQI, Pittsburgh Sleep Quality Index; ng/ml, nanograms per millilitre; *p*, probability value. Note: Change scores were calculated by subtracting pre-intervention scores from post-intervention scores.

Table 5 represents ANCOVA results, controlling for baseline scores, revealed significant between-group differences in several post-intervention outcomes. Morning salivary cortisol was significantly lower in the yoga group compared to the control group ($F = 14.357$, $p < 0.001$, $\eta p2 = 0.134$). Evening salivary cortisol was also significantly lower in the yoga group ($F = 5.307$, $p = 0.023$, $\eta p2 = 0.054$). The yoga group showed significantly lower post-intervention scores for depression ($F = 26.202$, $p < 0.001$, $\eta p2 = 0.220$), anxiety ($F = 38.043$, $p < 0.001$, $\eta p2 = 0.290$), and stress ($F = 3.872$, $p = 0.052$, $\eta p2 = 0.040$). Global PSQI scores were significantly lower in the yoga group, indicating improved sleep quality ($F = 5.580$, $p = 0.020$, $\eta p2 = 0.057$). Significant differences were also observed between the groups in psychological health ($F = 15.038$, $p < 0.001$, $\eta p2 = 0.139$), social relation scores ($F = 29.133$, $p < 0.001$, $\eta p2 = 0.239$), and environmental health scores ($F = 6.187$, $p = 0.015$, $\eta p2 = 0.062$), favoring the yoga group. However, there was no significant difference between the groups in physical health scores ($F = 0.467$, $p = 0.485$, $\eta p2 = 0.005$).

Table 21. ANCOVA Results for Post-Intervention Outcomes, Controlling for Baseline Variable.

Outcome Variable	Groups	N	Yoga	Control	Between Group Difference (ANCOVA
Morning Salivary Cortisol					
	Mean SD Baseline	48	11.16 ± 4.71	12.50 ± 3.76	
	Mean SD post	48	10.14 ± 5.05	13.84 ± 4.37	F =14.357 p<0.001, ηp2= 0.134
Evening Salivary cortisol					
	Mean SD Baseline	48	7.01 ± 2.66	6.45 ± 2.31	
	Mean SD post	48	6.57 ± 3.15	7.17 ± 2.91	F= 5.307, p=0.023, ηp2= 0.054
Depression Score					
	Mean SD Baseline	48	16.48 ± 9.67	11.06 ± 2.59	
	Mean SD post	48	11.71 ± 7.00	15.15 ± 3.73	F= 26.202, p<0.001, ηp2= 0.220
Anxiety Score					
	Mean SD Baseline	48	14.88 ± 7.42	11.92 ± 2.48	

	Mean SD post	48	12.29 ± 7.70	16.63 ± 2.95	F= 38.043, p<0.001, ηp2= 0.290
Stress Score					
	Mean SD Baseline	48	16.67 ± 8.88	11.44 ± 3.18	
	Mean SD post	48	13.63 ± 9.92	14.96 ± 4.03	F= 3.872, p=0.052, ηp2= 0.040
Global PSQI Score					
	Mean SD Baseline	48	64. 50 ± 35.32	49.21 ± 23.59	
	Mean SD post	48	53.49 ± 35.62	55.14 ± 22.44	F= 5.580, p=0.020, ηp2= 0.057
Physical Health Score					
	Mean SD Baseline	48	21.41 ± 3.94	26.11 ± 14.03	
	Mean SD post	48	22.87 ± 4.18	22.45 ± 13.39	F= 0.467, p=0.485, ηp2= 0.005
Psychological Health Score					
	Mean SD Baseline	48	19.00 ± 4.33	17.89 ± 5.13	
	Mean SD post	48	20.52 ± 4.08	17.11 ± 4.18	F= 15.038, p<0.001, ηp2= 0.139
Social Relation Score					
	Mean SD Baseline	48	11.04 ± 2.65	12.54 ± 1.57	
	Mean SD post	48	11.58 ± 2.34	9.79 ± 1.92	F= 29.133, p<0.001, ηp2= 0.239
Environmental Health Score					
	Mean SD Baseline	48	30.66 ± 4.86	27.65 ± 11.10	
	Mean SD post	48	29.87 ± 4.88	24.77 ± 10.55	F= 6.187, p=0.015, ηp2= 0.062

*Abbreviations: N: Number, M: Mean, SD: Standard Deviation, F: F-statistic, p: p-value indicates statistical significance, ηp2: Partial Eta Squared, significant ($p < 0.005$).

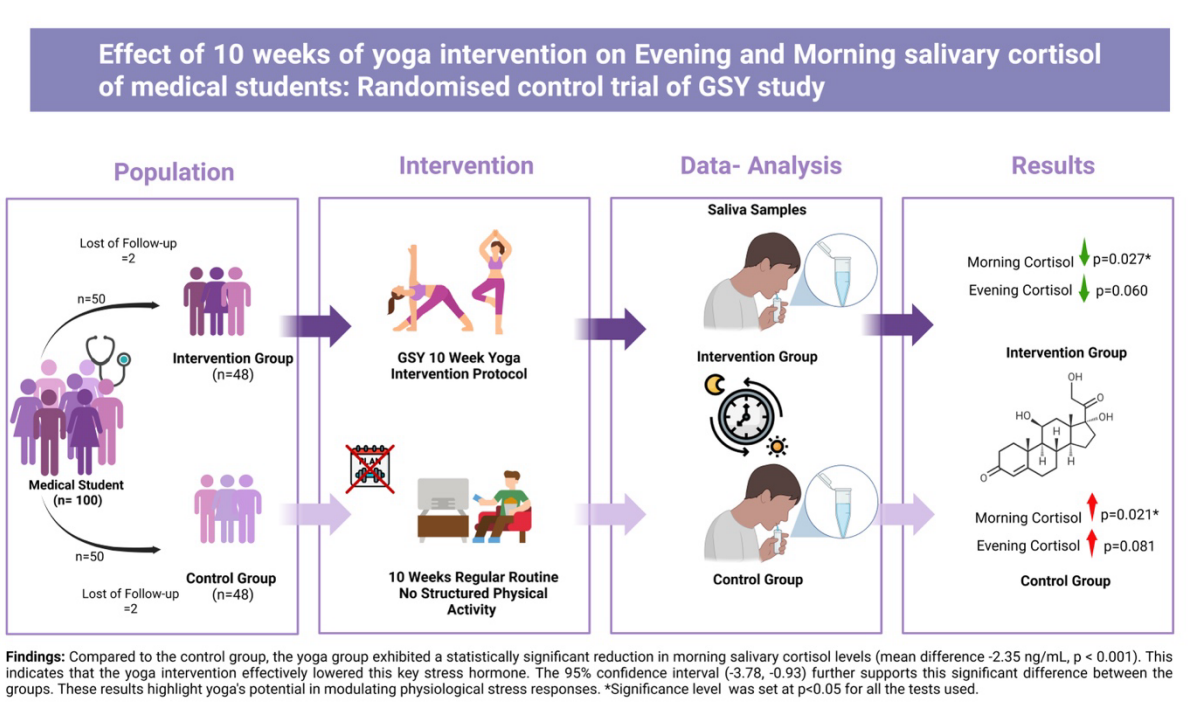

Figure 6. Visual abstract of Chapter 5.

Note: Created by the author based on the findings of the accompanying manuscript.

5.4 Discussion

The current RCT examined the effect of 10 weeks of yoga intervention on morning and evening salivary cortisol levels, depression, anxiety, stress, quality of life, and quality of sleep among medical students. The results demonstrate that participation in weekly 90-minute yoga sessions was associated with a decrease in morning salivary cortisol levels, improvement in quality of sleep, quality of life, and mental health outcomes. Comparison between groups showed that participants in the yoga group got significant benefits in multiple outcomes. The yoga group showed a significant reduction in morning salivary cortisol levels (mean difference -2.35 ng/ml, $p < 0.001$), evening salivary cortisol levels (mean difference -1.15 ng/ml, $p = 0.014$), depression score (mean difference -8.85, $p < 0.001$), anxiety score (mean difference -7.29, $p < 0.001$), and stress score (mean difference -6.56, $p < 0.001$) compared to the control group. Additionally, the intervention group demonstrated improvement in overall quality of life (mean difference 3.37, $p < .001$), physical health (mean difference 5.12, $p = 0.053$), psychological health (mean difference 2.29, $p = 0.02$), and social relation scores (mean difference 3.29, $p < 0.001$) compared to the control group. However, there was no significant change in environmental score (mean difference 2.08, $p = 0.137$) between the groups. While interpreting the result, it is important to note that the data collection post-intervention was conducted close to the academic exam period, this time is associated with a high stress level among students. Despite this stressful period, the yoga group showed significant improvements, while the control group either had no change or deterioration of the parameters, like

cortisol level. The finding showed that yoga interventions also served a preventive role in buffering the medical students against the effects of exam-related stress on physiological and psychological parameters.

The current study's key finding of a significant reduction in morning salivary cortisol levels is supported by prior research findings indicating that yoga practice can modulate cortisol secretion (Pascoe, Thompson, Jenkins, & Ski, 2017; Thirthalli et al., 2013). A higher level of morning cortisol is a biomarker of dysregulation in the HPA axis and chronic stress (Hellhammer, Wüst, & Kudielka, 2009). Evening cortisol also showed a greater reduction in the control group; however, it did not reach the statistical significance level. This finding, supported by the systematic review, states that long-term intervention can modulate cortisol(Campelo, de Araújo, Aristizabal, de Souza, & de Castilho, 2025), however, future studies with longer duration in higher-stress populations may help to clarify the yoga intervention effect on evening salivary cortisol levels. The significant reduction in mental health outcomes based on DASS-21(Norton, 2007) is in line with existing research supporting the positive effect of yoga intervention among medical students. An RCT by Telles et.al (Büssing et al., 2012) showed a significant reduction in DASS-21(Norton, 2007) scores after 8 weeks of mindfulness-based intervention among medical students from India. Another study found that 12 weeks of yoga intervention led to a significant decrease in depression and anxiety symptoms in nursing students(Pradhan & Pramanik, 2024). The positive changes demonstrated by yoga practice promote a healthier balance in the nervous system, enhance bodily awareness, and decrease physical stress levels, which are typical outcomes of mind-body activities(Sengupta, 2012; Streeter, Gerbarg, Saper, Ciraulo, & Brown, 2012).

Results from the current study showed improvement in the quality of life domain based on the WHOQOL-BREF(Group, 1998) score, specifically in physical health and psychological health in the yoga intervention group. This is supported by the study from Büssing et al (Büssing et al., 2012) demonstrated the positive effect of yoga practice compared to the control group in WHOQOL-BREF scores. Although the current study findings did not show any significant improvement in the environmental domain, However, it can be understood that the nature of the yoga intervention does not directly address factors in the environmental domain. The result showed that participants reported significant improvement in the social relations domain among the yoga group. This indicates indirect benefits of a yoga group practice, enhancing social connections, which is also noted in the study with yoga-based group sessions among university students (Cheshire, Richards, & Cartwright, 2022; Rakhshani, Maharana, Raghuram, Nagendra, & Venkatram, 2010; Riley & Park, 2015). The current finding demonstrates that the quality of sleep improved significantly in the intervention group based on the global PSQI score; these findings add to the earlier studies. Such as the study reported improved sleep quality after following a yoga-based mindfulness intervention among adolescents (Blake et al., 2016), similarly, a study by Khala showed significant improvement in quality of sleep after 8 weeks of

Kundalini yoga practice in participants suffering from chronic insomnia (Khalsa, 2004). It's possible that better sleep came from yoga calming their 'fight or flight' response, helping their sleep hormone levels, and quieting their racing minds, especially for people under a lot of pressure, like medical students. Generally, yoga intervention is proven to improve sleep quality in a diverse population (Chauhan et al., 2025; Halpern et al., 2014; W.-L. Wang, Chen, Pan, Yang, & Chan, 2020).

The current RCT has several strengths, including standardized yoga protocol, inclusion of both objective and subjective assessments, and strong participant retention. Additionally, the diverse international student population receiving yoga intervention in a common language, English, in the current study, enhances the generalizability of the findings. The structured yoga protocol used in the current study can influence both psychological and physiological pathways. The current findings also imply that the intervention played a part in the preventive strategy for enhancing resilience to stress. The observed improvements in psychological well-being, sleep quality, salivary cortisol, and subjective quality of life all point to yoga's promise as an accessible, flexible method of promoting medical students' overall health.

5.5 Limitation

A detailed analysis of the study's methodological foundations reveals notable limitations in this study. The single-center design, while potentially aiding internal validity through standardization of procedures, necessarily restricts the applicability of the findings to more diverse student populations and varied institutional contexts. Furthermore, the relatively short 10-week intervention period raises questions about whether it was long enough to change cortisol levels and psychological well-being in a lasting way. Additionally, the key issue is that post-intervention data collection was conducted close to the exam period(Fritz et al., 2021), which proved to be a high-stress period. It is suggested to interpret the findings of the current trial considering the above-mentioned limitations.

5.6 Conclusion

This randomized controlled trial demonstrates that a 10-week yoga intervention using a structured GSY protocol significantly reduces morning cortisol levels and improves psychological well-being, sleep quality, and quality of life in medical students. These findings highlight the potential of yoga as a practical and accessible stress-management strategy for this high-risk population. Implementing yoga programs within medical education could contribute to the overall well-being and resilience of future healthcare professionals. However, it is suggested as a future recommendation to explore the long-term effects of using the current yoga protocol among medical students.

Practical and Clinical Implications

The current dissertation includes five chapters and shows significant clinical future implications for preventing chronic disease, which can be caused by high levels of stressful environment, leading to mental health disorders, elevated cortisol hormone, and suppressed immune function. Chronic diseases caused by high levels of cortisol and suppressed IgA levels include cardiovascular disorders, diabetes, hormonal imbalances, mental health disorders, etc. Studies in the dissertation emphasize practicing yoga and meditation, following the Goodbye Stress with Yoga (GSY) protocol offers a holistic and effective intervention for managing stress, balancing cortisol hormone, improving mental health outcomes, and enhancing the immune function and overall health among students. Against stress-related illness, integrating the GSY protocol in medical education institutes can be used as a preventive measure against diseases that can be caused by chronic stress. This intervention can help medical students prioritize their well-being by not only improving their quality of life but also reducing long-term health risks.

Adopting the GSY protocol from a public health perspective has far-reaching implications. Evidence shows that health issues related to stress impose resource and financial burdens on healthcare systems. By implementing current interventions to manage stress effectively for medical students, educational institutes can give their contribution to provide healthier healthcare professionals. Additionally, guidelines can be created for the implementation of yoga protocol and yoga-based intervention with the collaboration of public health policymakers and educational institutions. This will not only ensure sustainability but also the accessibility of these interventions to all the students. The following are point-by-point elaborations for the steps that can be used for implementing current findings for preventing elevated levels of cortisol hormone and improving the IgA level. These steps can also be understood as steps for preventing chronic disease among medical students.

1. **Policy Development and Collaboration:** Collaboration of health care professionals, educational institutions, and public health organizations for creating guidelines that include yoga and meditation-based interventions, which are accessible to all medical students. This guideline may also include the formal adoption of the GSY protocol.
2. **Long-term intervention and follow-up program:** Building up the research project for monitoring the long-term effect of intervention on health outcomes in students. This can include measuring different objectives, for example, mental health outcomes and heart health outcomes, and tracking changes in hormonal levels, cortisol levels, and IgA levels.
3. **Promoting awareness of Behavioural changes**: Provide knowledge for students, faculty, and admiration staff about evidence that supports yoga intervention's effect on reducing chronic stress outcomes. Encouraging them to include this intervention into their daily routine as

practicing these interventions regularly has many health benefits. This can be achieved by advertising through seminars, workshops, and events.

Future Perspective

The current dissertation findings initiate and open numerous ideas for future research, specifically focusing on long-term intervention of yoga and meditation for the health consequences of chronic stress. Building up future studies, we are actively working on creating a project that focuses on existing gaps in current available studies, including RCTs with rigorous methodological designs and long-term follow-up. Following this vision, initially we have already taken the beginning steps by conducting a cross-sectional study of the effect of meditation practice among medical students to analyse the academic performance and self-efficacy. In this cross-sectional study, we are also examining the different frequencies of meditation practice and seek to analyse optimal intervention strategies for reducing stress and improving productivity among medical students.

Additionally, we also conducted a systematic review on the effect of yoga on anti-Müllerin hormone and androgen levels among women with PCOS. One of the risk factors of elevated cortisol hormone in women is also PCOS. The following provides a summary of the conducted systematic review. The findings of the study not only revealed the effect on these biomarkers after yoga intervention but also showed the existing gaps in the available research.

Summary of the systematic review of the Impact of yoga intervention on the Anti-Mullerian Hormone (AMH), androgen level, and metabolic parameters in women suffering from polycystic ovarian syndrome[5]

Six bibliographic databases were searched for relevant studies, including PubMed, Cochrane CENTRAL, Embase, Web of Science, Scopus, and Clinical Trials. A total of 275 citations were identified after running the search in 6 databases (PubMed = 21, Embase = 45, Cochrane Trial = 54, Scopus = 83, Web of Science = 66, Clinical Trials = 6). No relevant studies were identified through a Google search. Of these, 183 citations were left after removing the duplicates using the referencing manager EndNote. After title, abstract screening, and full text screening, in total of 5 studies were included in the current review according to the inclusion criteria. featuring 10 arms, which compare interventions of yoga, either in isolation or in conjunction with other interventions or a control group with usual care. The findings of this systematic review showed that yoga intervention has the potential to alter androgen, AMH, and insulin levels, although the evidence is limited to draw firm conclusions. In addition, the small number of participants included in the trials highlights the importance of more

[5] Manuscript under peer review. Editorial stage completed.

studies to generalize the results for all the women with different sociodemographic backgrounds. The important recommendation that can arise from this systematic review is the necessity for well-methodologically designed RCT trials to evaluate the impact of yoga in managing PCOS focusing on AMH level, androgen, and insulin level. (See Figure 6 for the Visual abstract).

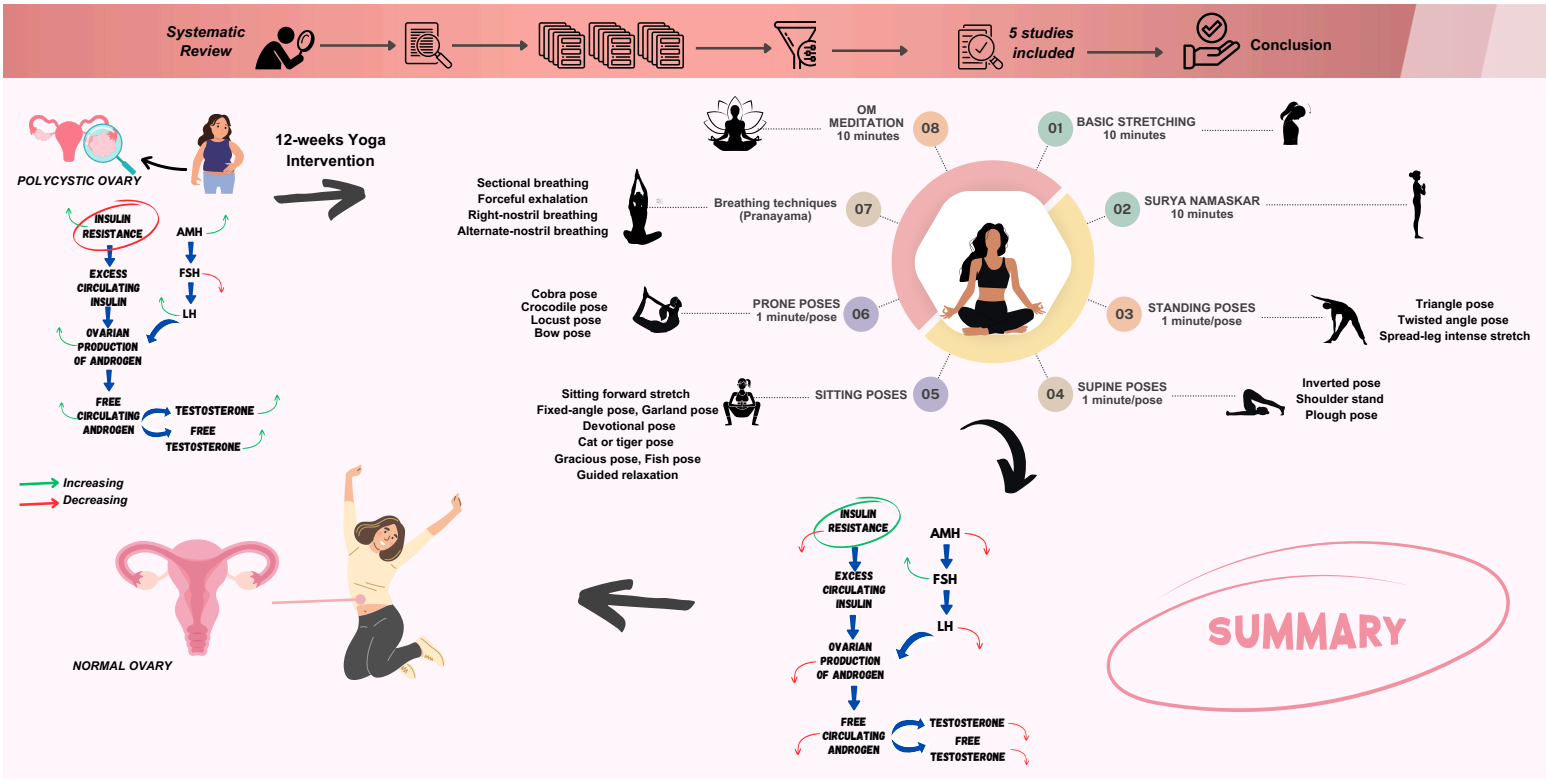

Figure 4. A visual abstract finding of the systematic review of the effect of yoga on AMH hormone and androgen levels.

Point by Point Future Research Agenda

1. Conducting randomized controlled trial with long term yoga intervention with follow up to explore long term outcome focuses on stress related health issues.
2. Building up 12 weeks of yoga intervention among student with PCOS condition to explaore the changes in AMH level, and androgen level, additionally addressing the gap which was identified in systematic review.
3. Completing the study with cross sectional design to analyse the academic performance and emotional regulation among medical students.

4. Exploring and implementing yoga meditation practices, to achieve the sustain benefits on psychological and physiological benefits.

Summary of novel findings

1. As per the author's knowledge this dissertation topic is the first study conducted on the effect of yoga intervention on cortisol hormone and immunoglobulin A. For this it was important to explore evidence to assess the different measurement tools in yoga research to assess the difference in cortisol hormone and IgA for understanding more about the methodological approach of the studies available. In the review in total 17 studies were included with a diverse population of 1007 participants. Most of the included studies used ELISA kits to measure salivary cortisol biomarkers, and most of the finding supports that the effect of yoga intervention leads to a reduction the salivary cortisol level in the participants. Salivary IgA level is measured in two included studies which showed significant improvement in the marker, although the small duration of intervention can be explained as temporary improvement in IgA level. Addressing the potential benefits of the yoga intervention to reduce stress biomarkers and improve in Immune market highlights the need for further studies with a longer duration of intervention to better understand the effects.
2. After reviewing the literature, we conducted a pilot study using the GSY protocol as an intervention for 10 weeks among medical students to analyze changes in levels of stress, anxiety, and depression. In the pilot survey only, validated questionnaires were used. The finding of this chapter not only showed that the current intervention significantly reduced the level of anxiety and depression ($p = 0.019$ and $p = 0.049$) but also provided efficacy and necessity of yoga intervention.
3. We conducted an experimental study using pre-post intervention to analyse the effect of a 10-week yoga intervention on the immune markers and metabolic parameters among medical students. Thirty-seven medical students with a mean age of 21.84±2.67 years and a BMI of 22.56±3.85 kg/m2 participated, of which 81.1% were female in a 10-week yoga intervention at the University of Pécs, Hungary. Standard laboratory procedures were followed during the collection of fasting blood samples from the participants. The results from pre- and post-blood marker parameters fell within the normal range. Regular yoga practice for 10 weeks improved medical IgA level, blood glucose control, and favorable lipid parameters. The current chapter findings underscore the meaningful impact of yoga intervention using GSY protocol as a

holistic practice that helps promote medical students' overall health Future studies should focus on the higher frequency of yoga intervention per week and its long-term impact on the clinical outcomes including mental health outcomes of medical students.

4. Evidence supports that yoga intervention can improve mental health outcomes and overall well-being(De Manincor et al., 2016). We conducted an experimental study focusing on the effect of 10 weeks of practice of GSY protocol among medical students. Data was collected before and after the intervention using validated questionnaires DASS-21, WHOQoL-BREF, PSQI, and DERS. The present chapter findings show a significant stress reduction ($p < 0.001$), depression ($p < 0.001$), and anxiety ($p < 0.001$) of participants, overall quality of life ($p < 0.001$), quality of sleep ($p < 0.001$) and emotional regulation ($p < 0.001$) significantly improved after the intervention. The observed improvement in the outcomes suggests that yoga intervention can be an effective practice for overall health promotion among medical students to navigate the challenges faced by students, such as emotional and mental health challenges during their training. By including yoga and meditation practice in the daily lives of medical students, educational institutes can provide a more supportive environment that focuses on students' overall health.
5. Medical students face high stress, which impacts cortisol levels, sleep, and quality of life. Yoga, a mind-body practice, may offer a sustainable stress-management strategy. We conducted a randomised controlled trial focusing on the effect of a 10-week yoga intervention on diurnal cortisol, sleep quality, and quality of life in medical students. A single-centre, two-arm randomized controlled trial (n=100, mean age 22.27 ± 3.00 years) was conducted. Medical students were randomized to a 10-week yoga intervention (90-minute sessions weekly) or a control group. Salivary cortisol (morning and evening), sleep quality (Pittsburgh Sleep Quality Index, PSQI), quality of life (WHOQoL-BREF), and psychological distress (DASS-21) were assessed pre- and post-intervention. The yoga group exhibited a significant -9.14% reduction in morning cortisol levels (mean difference -2.35 ng/mL, $p<0.001$, Cohen's $d = -0.671$), improved sleep quality (-17.07% change mean difference -11.01 on global PSQI score, $p=0.019$), and enhanced overall quality of life (+32.01% change mean difference 3.37 score, $p<0.001$, Cohen's $d = 2.252$) compared to the control group. Significant improvements were also observed in depression (-28.94% change mean difference -8.85 points, $p<0.001$, Cohen's $d = -1.35$), anxiety (-17.41% change mean difference -7.29 points, $p<0.001$, Cohen's $d = -1.406$), and stress (-18.24% change mean difference -6.56 points, $p=0.007$, Cohen's $d = -0.778$) scores in the yoga group. Yoga offers a promising, accessible, and effective strategy for managing stress and enhancing well-being in this high-risk population.

List of Publications

Published articles related to the thesis

Chauhan, S., Babu, A. M., Galgalo, D. A., Melczer, C., Prémusz, V., & Karsai, I. (2024). Effect of yoga in medical students to reduce the level of depression, anxiety, and stress: pilot study (Goodbye Stress with Yoga GSY). *BMC Complementary Medicine and Therapies*, *24*(1), 203.

Chauhan, S., Najaf, S. S., Gergely, L., Kinga, K. A., Karsai, I., & Prémusz, V. (2025). Impact of 10 Weeks of Yoga Intervention on Mental Health and Overall Well-Being Among Medical Students: GSY Study. *Sports*, *13*(4), 114.

Other published articles

Galgalo, D. A., Mokaya, P., Chauhan, S., Kiptulon, E. K., Wami, G. A., Várnagy, Á., & Prémusz, V. (2024). Utilization of maternal health care services among pastoralist communities in Marsabit County, Kenya: a cross-sectional survey. *Reproductive Health*, *21*(1), 126.

Prémusz, V., Lendvai-Emmert, D., Makai, A., Amrein, K., Chauhan, S., Bódis, J., ... & Várnagy, Á. (2022). Pre-Treatment Physical Activity Could Positively Influence Pregnancy Rates in IVF despite the Induced Oxidative Stress: A Cohort Study on Salivary 8-Hydroxy-2′-deoxyguanosine. *Antioxidants*, *11*(8), 1586.

Articles under review

Chauhan, S., Najaf, S.S., Nagy, Z., Al-Jawarneh1, M., Nagy, T., Miseta,A., Prémusz, V., & Karsai, I. Changes in immune and metabolic parameters by yoga intervention among medical students.

Chauhan, S., Muka, T., Najaf, S.S., Galgalo, D.A., Atmaca, L., Karsai, I., Prémusz, V. Impact of yoga intervention on the Anti-Mullerian Hormone (AMH), androgen level, and metabolic parameters in women suffering from polycystic ovarian syndrome – A systematic review.

Chauhan, S., Najaf, S.S., Babu, A. M., Atmaca, L., Galgalo, D. A., Prémusz, V., & Karsai, I. Effect of 10 weeks of yoga intervention on Evening and Morning salivary cortisol of medical students: Randomised controlled trial.

Chauhan, S., Najaf, S. S., Babu, A. M., Atmaca, L., Galgalo, D. A., Ács, P., Karsai, I., & Prémusz, V. (Year). Effect of 10 weeks of yoga intervention on evening and morning salivary cortisol of medical students: A randomized controlled trial.

List of Appendices

Appendix 1. Consent form

Declaration of Consent

Effects of practicing yoga and meditation on cortisol hormone rhythm and Immunoglobin A in among Medical Students : GSY (Good Bye Stress With Yoga)

……(name)

[ID:……..…], [TAJ/Health Insurance Number:……..] , [Date of Birth:…….] declare that I was fully informed, and I am giving my consent to participate in the GSY (Goodbye Stress with Yoga) Project. I consent to donate the agreed upon amount of blood samples and saliva samples for the purposes of this research study. I understand that I am free to withdraw from the study within the time limits outlined in the information sheet, without giving a reason for my withdrawal or to decline to answer any questions in the study without any consequences to my future treatment by the researcher.

I understood that there will not be financial compensation for the participation. I agree to follow the Intervention protocol, participate in the sessions.

I consent that my data can be processed anonymously and for scientific purposes

can be used. I declare that I received and understood the information sheet. I received satisfactory answers to my questions.

Pécs, 20. …… month …… day.

……

……

name (legible) and signature of the subject participating in the research

……....

……

name (legible) and signature of the person providing the information

…….

…….

Witness 1's name (legible) and signature

…….

…….

Witness 2's name (legible) and signature

Appendix 2. Yoga Protocol used in the current dissertation: GSY Study

Stretching (Head to toe) and Balancing 15 Minutes		
Name	**Practice Frequency**	**Benefits**
Neck: Neck Rotations, Side-to-Side Neck Stretch	**3 times**	Relieves tension and improves neck flexibility.
Wrist and Shoulders: Shoulder Rotations, Arm Swings, Shoulder Shrugs		Increases shoulder mobility and reduces stiffness.
Hand & Fingers: Finger Bending, Wrist Rotations		Enhances dexterity and reduces hand fatigue.
Knee: Knee Rotations, Leg Extensions		Improves knee joint flexibility and strength.
Toe & Ankle: Ankle Rotations, Toe Bending, Toe Stretching		Enhances ankle flexibility and promotes foot health.
Vrikshasana (Tree Pose)	2 times each side	Enhances concentration & stability
Asana (Standing,Sitting,Supine and Prone Posture) 50 Minutes		
Pose Name	**Practice Frequency**	**Benefits**
Surya Namaskar (Sun Salutation)	3 rounds	Improves circulation & warms up the body
Utkatasana (Chair Pose)	3 times	Strengthens legs & enhances stability
Malasana (Garland Pose)	3 times	Opens hips & improves digestion
Tadasana (Mountain Pose)	3 times	Enhances posture & balance
Trikonasana (Triangle Pose)	2 times each side	Improves flexibility & spinal alignment
Konasana (Angle Pose)	2 times each side	Enhances lateral flexibility & core strength
Natarajasana (Dancer's Pose)	2 times each side	Improves balance & focus
Pavanamuktasana (Wind Releasing Pose)	3 times	Relieves bloating & promotes digestion
Setu Bandhasana (Bridge Pose)	3 times	Reduces stress & enhances circulation
Chakrasana (Wheel Pose)	2 times	Increases spinal flexibility & energy flow
Bhujangasana (Cobra Pose)	3 times	Reduces adrenal overactivity & strengthens spine
Dhanurasana (Bow Pose)	3 times	Stimulates digestion & strengthens back muscles
Navasana (Boat Pose)	3 times	Strengthens core & reduces abdominal fat

Balasana (Child's Pose)	3 times	Induces relaxation & relieves tension
Vajrasana (Thunderbolt Pose)	1 time, hold longer	Aids digestion & stabilizes the mind
Balasana (Child's Pose)	3 times	Induces relaxation & relieves tension
Vajrasana (Thunderbolt Pose)	1 time, hold longer	Aids digestion & stabilizes the mind
Vrikshasana (Tree Pose)	2 times each side	Enhances concentration & stability
Paschimottanasana (Seated Forward Bend)	3 times	Stretches hamstrings & calms the nervous system
Janu Sirsasana (Head-to-Knee Pose)	3 times	Improves flexibility & reduces stress
Ardha Matsyendrasana (Half Spinal Twist)	2 times each side	Enhances spinal mobility & detoxification
Shashankasana (Hare Pose)	3 times	Activates the relaxation response
Viparita Karani (Legs-Up-The-Wall Pose)	1 time, hold longer	Improves blood flow & reduces cortisol
Shavasana (Corpse Pose)	1 time, final relaxation	Promotes deep relaxation & nervous system balance
Pranayama (Breathing Techniques) – 10 Minutes		
Anulom Vilom (Alternate Nostril Breathing)	15 breaths	Balances sympathetic & parasympathetic activity
Bhramari (Bee Breath)	10 rounds	Lowers heart rate & induces relaxation
3:1 Breathing (Three Inhales, One Exhale)	10 rounds	Enhances oxygenation & reduces stress response
Breathing Meditation & Relaxation – 15 Minutes		
Guided Breath Awareness	5 minutes	Deep breathing with instructor cues
OM Chanting Meditation	5 minutes	Vibrational healing & mental clarity
Silent Observation with OM Music	5 minutes	Mindfulness & emotional balance

Data Availability Statement

The dataset, the questionnaires and the informed consent form are available from the author upon reasonable request.

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Appendix 3.

Submission of the doctoral dissertation and declaration of the originality of the dissertation

Submission of the doctoral dissertation and declaration of the originality of the dissertation

The undersigned,
Name: Shalini Chauhan
Maiden name: Giano Chauhan
Mother's maiden name: Giano Chauhan
Place and time of birth: Bilaspur, Himachal Pradesh, 06/01/1995

on this day submitted my doctoral dissertation entitled:

Effects of practicing yoga and meditation on cortisol hormone rhythm and immunoglobulin a among medical students at the University of Pécs: A Mixed Method Study

to the PR-7 Sport and Health Science Programme

of the Doctoral School of Health Sciences, Faculty of Health Sciences, University of Pécs.

Names of the supervisor(s): Dr István Karsai

Names of the Co-supervisor(s): Dr Viktória Prémusz

At the same time, I declare that
- I have not submitted my doctoral dissertation to any other Doctoral School (neither in this country nor abroad),
- my application for degree earning has not been rejected in the past two years,
- in the past two years I have not had unsuccessful doctoral procedures,
- my doctoral degree has not been withdrawn in the past five years,
- my dissertation is independent work, I have not presented others' intellectual work as mine, the references are definite and full, on preparation of the dissertation I have not used false or falsified data.

Furthermore, I declare that I contribute to the request of DOI identification of my doctoral dissertation.

Dated: 16/May/2025

signed by Candidate

Supervisor

Co-supervisor