Advances in Integrative Medicine 10 (2023) 122–130

Contents lists available at ScienceDirect

Advances in Integrative Medicine

journal homepage: www.elsevier.com/locate/aimed

Effect of Hatha Yoga and meditation on academic stress in medical students—Clinical trial☆

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ARTICLE INFO

Keywords:
Psychological Stress
Medical Students
Yoga
Meditation
Cortisol

ABSTRACT

Introduction: Medical students use to deal with high levels of academic stress. Hatha yoga and meditation have been found to reduce variables associated with stress. Objective: To evaluate and compare the effect of Hatha yoga and meditation on academic stress in medical students at a private university in Cali, Colombia.
Methods and materials: A total of 27 volunteers were randomly divided into two groups: Hatha Yoga(n = 13) and Meditation (n = 14). Each group undertook two weekly one-hour sessions for 14 weeks. Study outcomes were salivary cortisol and perceived academic stress. Anthropometric and clinical variables were additionally measured as characteristics of the study sample. Perceived academic stress was estimated via a structured questionnaire. An intra-group analysis was also conducted to compare the outcomes at baseline and post-intervention within the groups.
Results: At baseline demographic, clinical, and anthropometric variables were similar between the intervention groups. After the intervention, no differences were found between meditation and hatha yoga groups in salivary cortisol measures (time 0, 15, 30, 45 min, and mean of the 4 measures) and perceived academic stress. However, the intra-group analysis showed that only in Hatha yoga there was a significant increase in cortisol measures and improvement in the scale of perceived academic stress.
Conclusion: Based on the intra-group but not between-group comparisons of outcomes at baseline and post-intervention, Hatha yoga reduced perceived academic stress despite a concomitant significant increase in cortisol levels. Further trials with larger samples of medical students should be conducted to contrast our findings.

1. Introduction

Stress is defined as a process in which an individual faces environmental demands that exceed his or her resources [1]. It is also defined as a transactional phenomenon between an individual and the environment, that triggers a cognitive evaluation of the event [2]. This can induce an adaptation with positive outcomes, such as making the individual better prepared to deal with adversities in the environment. It may also lead to negative outcomes when the individual cannot adequately control the situation [3].

Stress induced by the demands of the educational environment is known as academic stress [4]. The teaching-learning process, educational models, and knowledge are important aspects of academic life. These stages can be stressful for some people, especially in higher education, due to the big academic workload, the need for autonomy, changes in schedule, and the university environment [5]. Different

☆ Clinical trial registration number: clinicaltrials.gov ID 1051. 26.

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https://doi.org/10.1016/j.aimed.2023.09.001
Received 14 September 2022; Received in revised form 14 July 2023; Accepted 3 September 2023
Available online 11 September 2023

studies have found higher stress levels in students in health and medicine programs. This can be attributed to a higher academic workload, the additional time they must spend studying during the basic training phase, and hospital shifts, or clinical practices that they have to undertake in the advanced phase of their training [4,6]. This high level of stress among health and medicine students has been correlated with unhealthy lifestyles, pre-hypertension anxiety, depression, and suicidal tendencies [7,8]. These subjects are also likely to experience a decrease in academic performance and motivation, leading to thoughts of dropping out of school, and low optimism [9]. In some cases, it can even lead to a state of physical, emotional, and mental exhaustion, leading the student to develop burnout syndrome, due to the demands of their academic and clinical responsibilities [10].

The lack of students' preparedness to deal with the academic environment can lead to responses related to perceived stress. This is a stimulus that acts on the hypothalamus-pituitary-adrenal (HHA) axis and promotes pathological conditions and behavioral changes by affecting the homeostasis of the central nervous system (CNS), autonomic nervous system(ANS), endocrine and immune systems [1]. It occurs as a result of the activation of the HHA axis that leads to the synthesis of a corticotropin-releasing hormone (CRH) and an adrenocorticotropic hormone (ACTH). These hormones mediate the secretion of cortisol through the adrenal glands. This glucocorticoid under normal conditions regulates metabolic and anti-inflammatory functions, but when it is produced in a chronic and sustained way, it attenuates responses that in turn stimulate the so-called chronic low-grade inflammation and immunosuppression. This phenomenon is involved in the pathophysiology of chronic noncommunicable diseases and behavioral changes such as anxiety, depression, and suicidal tendencies [11].

Different authors have proposed mechanisms for stress management such as counseling, psychology, mindfulness, physical activity, and disciplines that combine physical activity with meditation, such as yoga [12,13]. Hatha yoga is a type of yoga that integrates physical skills, musculoskeletal conditioning, and increased heart rate and blood flow, with breathing control and meditation. According to several authors, it improves cognitive capacity, social skills, and self-image, and promotes resilience and emotional stability [14,15]. Meditation is also a strategy that helps people to have greater control over their minds and thoughts, to improve their relationship with themselves, and to live in the present, alleviating conditions such as depression and rumination (that appear when the mind remains stuck in the past situations), or anxiety due to a fear of future situations [16,17]. Some authors have attributed the decrease in stress by Hatha Yoga to its meditation component. [12] However, a simultaneous evaluation contrasting Hatha Yoga and a meditation intervention separately has not yet been conducted, especially in medical students. Therefore, we aimed to evaluate the effect of Hatha yoga and meditation on academic stress in medical students at a private university in Southwest Colombia.

2. Methods and materials

2.1. Study design

This study is a clinical trial that involved a control group with active treatment that performed meditation, and an intervention group that practiced Hatha yoga. The clinical trial registration number: clinicaltrials.gov ID 1051.

2.2. Participants

The subjects selected were undergraduate medical students between the second and fourth semesters who met the following inclusion criteria: attending medical school in the first or second semester of 2019, with more than or equal to 18 credits earned; sedentary, not taking medication or hallucinogenic substances. Subjects excluded were those with a previous diagnosis of chronic non communicable disease, those undergoing pharmacological treatments, physical disabilities, athletes, or those who do some type of regular physical activity. The sample size was calculated considering the total medical student population in the first four semesters of the program, a normal distribution of the variable cortisol in saliva and stress (with an expected post-intervention difference between groups of 30%, a power of 80%, an alpha of 0.05), and a standard deviation of 0.29 [18]. A dropout rate of 30% was expected, for which 40 participants were initially recruited. All participants gave their consent by signing the consent form. The study protocol was approved by the Research Ethics Committee of the Faculty of Health Sciences of the Pontificia Universidad Javeriana Cali.

2.3. Recruitment

This study used a non-probabilistic sample since the target population had to be university medical students and the study was advertised in one only University (Pontificia Universidad Javeriana Cali). The participants were contacted through an open call and an informative meeting in which the objectives of the study, methodology, and inclusion and exclusion criteria were explained. Initially, 52 volunteers were enrolled, of which 40 met the inclusion criteria. Each participant was provided with information and recommendations on healthy eating, avoiding alcohol consumption (according to the parameters of the National Institutes of Health (NIH) of the U.S. Department of Health and Human Services), non-consumption of tobacco, energy drinks, and/or stimulants, or psychoactive drugs during the trial.

Participants were assigned to the Hatha yoga group ($n = 20$) and the meditation group ($n = 20$) randomly through a system of ballots. During the study, 10 participants were withdrawn due to non-attendance, and 3 were excluded because the saliva sample (post-intervention) was not satisfactory for cortisol measurements. That left 13 participants in the Hatha yoga group and 14 in the meditation group. (Fig. 1).

2.4. Measurements

At the beginning and the end of the study, the following measurements were taken: saliva cortisol; anthropometric measurements for weight, height, waist and hip circumference, skin folds, and systolic and diastolic blood pressure. A questionnaire was also used to evaluate psychosocial risk factors [19], based on a dynamic model for psychosocial factors to determine chronic stress [20].

2.5. Level of cortisol in saliva

Saliva samples for measuring cortisol were collected in 1.5 ml tubes (Salivette™) before eating, in a resting state, and within an hour of waking up. The cortisol curve was performed for 45 min, and four samples were taken at 15-minute intervals [20]. The day before the saliva samples were taken, recommendations were given such as: To avoid the consumption of alcoholic beverages for 24 h, not eating, brushing teeth, or consuming caffeinated products two hours before or during the collection. Additionally, participants were instructed on how to take the saliva sample. Once the samples were received, they were centrifuged at 1000 g for 2 min and stored at − 80 °C. The concentration of cortisol in saliva was determined by an enzymatic test (Cortisol ELISA Kit, Diasource KAPDB290) following the manufacturer's instructions. Samples were measured in duplicate and measurements with a coefficient variation $\geq$ 10% were repeated.

2.6. Body mass index and blood pressure

Height was measured using a height rod in centimeters (cm) and weight was determined on a graduated scale in kilogram (kg). Body mass index (BMI) was calculated with these two measurements with the formula: BMI= weight (kg)/Height (cm) ^{2}. Blood pressure was measured by a single operator using a Welch Allyn 7670–01 wall-mounted aneroid

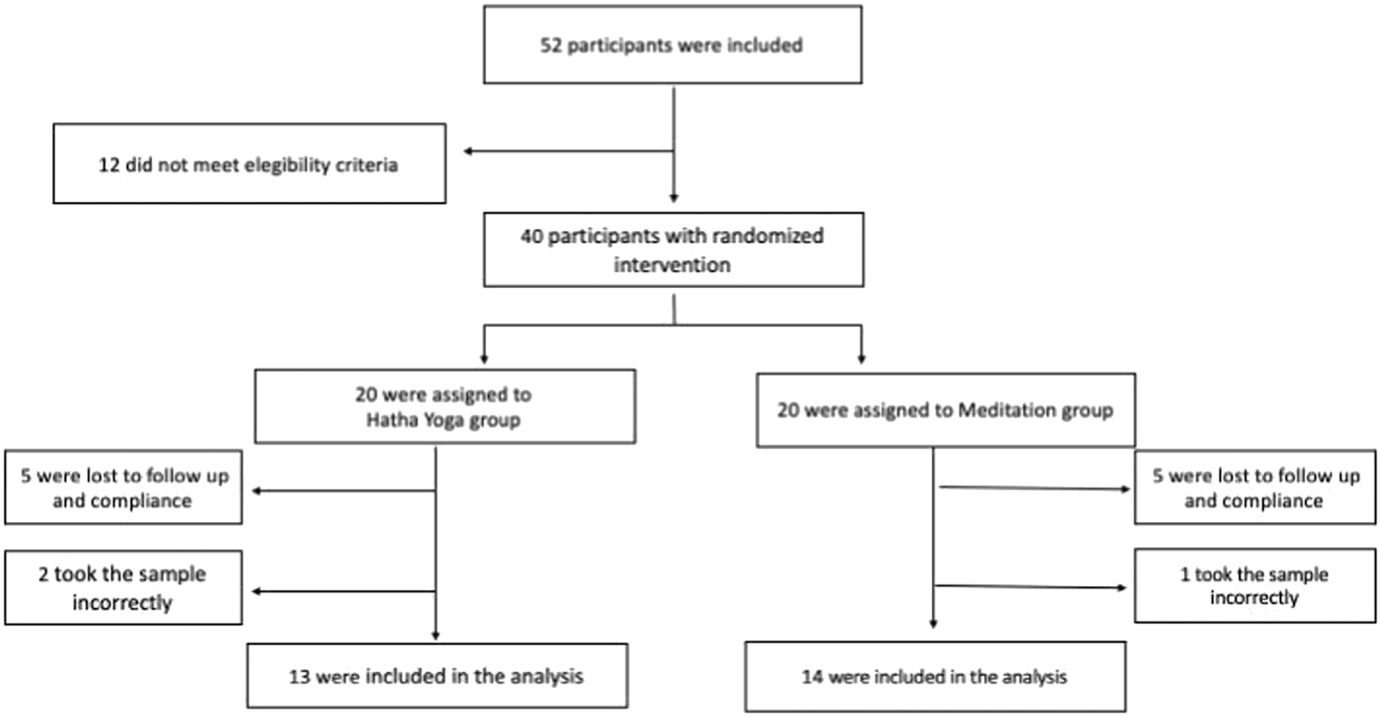

Fig. 1. Flowchart with information on the progress of the participants in the clinical trial.

blood pressure monitor and a Littman stethoscope. The diastolic blood pressure (DBP) and systolic blood pressure (SBP) were registered in millimeters of mercury (mmHg).

2.7. Chronic stress test

Academic stress was analyzed using a questionnaire for the evaluation of psychosocial risk factors from the Ministry of Social Protection in Colombia, in 2010 [19], based on the dynamic model of psychosocial factors [21]. This test consists of 31 statements corresponding to symptoms that reveal the presence of stress reactions. They are grouped into four different dimensions, and categorized into physiological, social, intellectual, academic, and psycho-emotional levels, as well as a series of socio-demographic data. Each item was evaluated on a Likert scale, which reported the frequency with which stressful situations occurred during daily academic life. The response options were; always, almost always, sometimes, or never. Each of these response options acquired a different value which depended on the question and the area of adjustment being evaluated. Lastly, the values obtained made it possible to classify the level of stress into categories (5: very high; 4: high; 3: medium; 2: low; and 1: very low). It is important to bear in mind that level 3 is the questionnaire's midpoint. Therefore, values below 3 are not considered stress, since the stimulus is low or very low, which does not generate pathological changes or consequences for the individual. Values above 3 are considered as stress or distress.

2.8. Intervention

The interventions in the Hatha yoga and meditation groups consisted of one-hour sessions, twice a week for 14 weeks for each intervention. Before and at the end of this follow-up (maximum of three days after the last session), the blood and saliva samples were taken, blood pressure and anthropometric measurements index were measured, and the academic stress questionnaire was filled out.

2.8.1. Intervention Hatha Yoga group

Subjects included in this group were appointed in non-class schedule, to sessions of 60 min on Mondays and Thursdays during 14 weeks, led by an instructor certified in Hatha Yoga. Each session started with a moment of relaxation and concentration through breathing to enable the participants could forget external distractions. Participants then continued with warm-up exercises, which included joint movements, stretching, and conscious breathing. Once the warm-up was completed, the postures or asanas were performed (standing, forward and backward bending, twisting, inverted, and balancing postures), sustained for several seconds or minutes. During the execution of these asanas, emphasis was placed on the correct alignment of the body, full attention to the breath, physical sensation, and connection with the present. The asana practice included controlled breathing, or pranayama, to calm the mind, balance energy and increase breathing capacity. In the end, there was a phase of conscious relaxation of the body and mind in a supine position on the mat, also called Savasana.

2.8.2. Intervention meditation group

Subjects included in this group were also appointed at non-class hours to 60-minute meditation sessions on Mondays and Thursdays for 14 weeks. Each one-hour session was led by an expert in meditation and mindfulness techniques. Participants started with an exercise of reflection and writing about their family, academic, social, etc. context to identify problem situations, situations to be grateful for, to change, to improve, and vision of the future, past and present according to the topic to be focused on in each class. After completing this individual and personal exercise, a practice was guided so that the participants could concentrate their attention on breathing, observing their thoughts without judging them, including bodily sensations, such as touch, body temperature, or the hearing of environmental sounds, as well as sensations associated with the imagination or visualization of colors, shapes, images, etc. After the sessions, the participants took some time to reflect on the experience and the physical, mental, and emotional sensations.

The duration of the 14-week intervention was adjusted at the duration of the medical students' academic semester (18 weeks), so that we could have the subjects during their most active academic periods, since at the end of the semester, the participants enter a vacation period, which mimics their availability to continue the intervention and change

academic stress levels. In addition, as previously reported, yoga and meditation practices contribute to stress management, in a short to medium time and frequency [22,23].

3. Data analysis

Demographic, clinical, and anthropometric characteristics of the study sample were described as median and its interquartile range since there was a mixture of these variables with normal and non-normal distribution. The median is a valid central trend measure either for skewed or normally distributed variables because, in a normal distribution, the median and mean are comparable. Comparisons of the demographic, clinical, and anthropometric characteristics between the control and Hatha Yoga groups were carried out by using the Mann-Whitney U test.

The outcome variables of the study were salivary cortisol levels and perceived academic stress levels which were continuous and categorical variables, respectively, and thus their statistical approach was different. We analyzed cortisol levels by different approaches for pre and post-intervention measurements. One approach was simply using individual cortisol measurements at each time of sampling (0, 15, 30, and 45 min). We also calculated areas under the curve between measurements as follows (24): AUC 1 [(cortisol 15 min + cortisol 0 min/2)x 0.25 h], AUC 2[(cortisol 30 min+ Cortisol 15 min/2) x 0.25 h], AUC 3 [(cortisol 45 min+ Cortisol 30 min/2) x 0.25 h], and AUC from the ground (AUCG) as AUC1 +AUC2 +AUC3. Similarly, the mean of the four measurements was estimated. The above parameters of cortisol levels were described as mean and standard error, and differences between the intervention groups were estimated by using ANOVA (non-adjusted difference), and ANCOVA to adjust post-intervention comparisons for baseline values of these parameters since baseline might influence differences. Meanwhile, perceived academic stress levels were described as the number and percentage of cases with different scores on the scale: very low (1), low (2), middle (3), high (4), and very high (5). Differences between the intervention groups at baseline and post-intervention were estimated via the χ^2 test. We additionally explored intra-groups differences in the outcome variables between baseline and post-intervention stages. For this, we used paired sample methods. The paired sample t-test was used in the case of continuous cortisol-related variables. McNemar's Chi-squared test was used to compare pre and post-intervention scores/categories of perceived academic stress within meditation and Hatha Yoga groups. All analyses were carried out using STATA software 14. 0.

4. Results

4.1. Characteristics of the study population

61.5% of the subjects were women aged between 18 and 25 years with a mean age of 19.2 ± 2.0. All the subjects belonged to the first four semesters of the medical program, the training phase in basic medical sciences. No differences were found between the groups regarding age, sex, or semester (Table 1). Table 1 also shows the clinical and anthropometric characteristics of intervention groups before and after the intervention. There were no significant differences in these variables between the intervention groups at baseline and after the intervention, with the only exception of post-intervention values of diastolic blood pressure, which were significantly higher in the Hatha yoga group than in the meditation group.

4.2. Comparison of the outcome variables between groups

By chance, the saliva cortisol levels at baseline were significantly higher in the meditation in comparison with the Hatha yoga group, particularly at 15 min and 30 min measurements (Table 2) despite the randomization. The same trend for higher values was observed for changes between measurements estimated as AUC. The mean of the four salivary cortisol measurements was similarly significantly higher in the meditation group (Table 2). The perceived stress was comparable in both groups either at baseline or at the post-intervention stage (Table 3).

4.3. Intra-group comparisons of the outcome variables

When differences between baseline and post-intervention values for salivary cortisol were explored within the groups, the Hatha Yoga, but not the meditation group, showed a significant increase in the individual mean of the four measurements (Fig. 2). Perceived stress showed a trend to improve in specific categories of low stress and high stress within the Hatha yoga group (P = 0.012) (Fig. 3). No significant difference between baseline and post-intervention perception of stress was observed with the meditation group.

5. Discussion

The objective of this study was to evaluate the effect of Hatha yoga and meditation on academic stress in medical students. In the Hatha yoga but not in the meditation group, the perceived academic stress significantly decreased between baseline and post-intervention stages,

Table 1
Characteristics of the subjects by groups of intervention.

	Baseline			Post-Intervention		
	Meditation	Yoga	P Value	Meditation	Yoga	P value
Age (years)	18(18–19)	19(18–21.5)	0.402			
Sex (Male /Female (n))	5/9	5/8	0.999			
SBP mmHg	120(114–120)	120(119.5–125)	0.905	120(110–120.5)	120(118.7–121.2)	0.943
DBP mmHg	80(65–81)	80(79.5–84)	0.550	80(70–85)	85(80–85)	0.038
BMI (Kg/m^2)	24.4(21.1–29.2)	24.9(20.8–29.4)	0.830	23.3(20.9–29.4)	23.1(20.3–28.2)	0.840
WC (cm)	79.5(67.5–89.7)	77.5(66.2–90.5)	0.905	77(67.6–92.8)	79.8(66.2–90.5)	0.999
AC (cm)	85.5(78–96.2)	86.5(73.7–97.7)	0.905	85.5(75.6–99.2)	89.4(73.9–99)	0.687
HC (cm)	104.5(94–110.1)	102.5(90.7–112.2)	0.793	102.7(94.4–110.3)	102(86–108.7)	0.762
Tricipital skinfold (cm)	14(9–17.5)	12(9.5–17)	0.905	14(12–15)	12(11–14)	0.960
Bicipital skinfold (cm)	9(6–11.5)	9(5.5–13)	0.943	9(6–11)	12(11–14)	0.999
Subscapular skinfold (cm)	13(10.75–18.5)	13(11–17.5)	0.488	15.5(11.5–20)	15(11–19)	0.650
Abdominal skinfold (cm)	15(11.5–16.5)	15(11–17)	0.430	12.5(9.75–15.5)	11(8.5–15)	0.125
Suprailiac skinfold (cm)	18(11.5–21.5)	19(14–21)	0.999	15.5(12.5–22)	16(11–19.5)	0.448
Thigh skinfold (cm)	15(10.5–15)	15(10.5–15)	0.220	15(13.75–17)	15(13.5–16)	0.418
Body fat%	12.8(10.1–18.1)	12.6(10.8–17.3)	0.830	13.9(11.8–15.9)	13.7(12.1–16)	0.840

*Values are median(interquartile range). Comparisons between groups by Mann-Whitney U test.
SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: Body mass index.

Table 2
Outcome variable of Salivary cortisol [μg/dL]* and its changes throughout the study by intervention groups.

	Baseline			Post-Intervention			
	Meditation (n = 13)	Yoga (n = 14)	P Value* *	Meditation	Yoga	P value* * (unadjusted)	P Value* ** (adjusted for baseline values)
Cortisol 0 min	20.2 ± 3.4	21.1 ± 3.2	0.837	33.1 ± 7.1	41.3 ± 9.0	0.484	0.512
Cortisol 15 min	48.8 ± 5.6	30.9 ± 4.9	0.024	49.4 ± 8.7	51.1 ± 6.7	0.876	0.625
Cortisol 30 min	61.7 ± 7.6	37.7 ± 6.0	0.020	64.7 ± 8.5	61.2 ± 6.2	0.743	0.621
Cortisol 45 min	61.1 ± 7.7	42.7 ± 7.1	0.077	65.4 ± 7.9	65.7 ± 5.1	0.972	0.593
Changes between measurements							
AUC 1	0.86 ± 0.08	0.65 ± 0.09	0.108	1.03 ± 0.17	1.15 ± 0.19	0.638	0.341
AUC 2	1.38 ± 0.16	0.85 ± 0.13	0.017	1.42 ± 0.21	1.40 ± 0.15	0.933	0.607
AUC 3	1.54 ± 0.18	1.0 ± 0.67	0.036	1.62 ± 0.20	1.58 ± 0.13	0.871	0.575
AUC G	3.79 ± 0.40	2.51 ± 0.36	0.028	4.08 ± 0.55	4.15 ± 0.46	0.930	0.470
Mean of the four measurements	48.2 ± 4.9	33.1 ± 4.8	0.038	53.1 ± 6.9	54.8 ± 6.2	0.855	0.422

*Values are mean ± standard error. * * Statistical significance by using ANOVA. * ** Statistical significance by using ANCOVA

Table 3
Academic stress* at baseline and post-intervention.

	Baseline			Post-Intervention		
	Meditation (n = 13)	Yoga (n = 14)	P Value	Meditation (n = 13)	Yoga* * (n = 13)	P value
Very low (1)	4(30.8)	2(14.3)	0.387	4(30.8)	5(35.7)	0.607
Low (2)	4(30.8)	6(42.9)		5(38.5)	5(35.7))	
Middle (3)	2(15.4)	1(7.1)		2(15.4)	1(7.1)	
High (4)	3(23.1)	4(28.6)		1(7.7)	2(14.3)	
Very high (5)	0	1(7.1)		1(7.7)	13 (92.9)	

*Values are n(%). Comparisons between groups by χ^2 test. * **One missing value in follow-up

despite a concomitant significant increase in cortisol levels during this intervention. Intra-groups but not inter-groups comparisons enabled the detection of the above effects.

5.1. Hatha and salivary cortisol levels

The levels of saliva cortisol increased in the Hatha yoga intervention. It is important to highlight that the pre-intervention measurements were performed at the beginning of the academic semester when students were less exposed to academic stressors. However, the post-intervention analyses were performed at the end of the semester, when the students are exposed simultaneously to several stressors such as evaluations, submission of papers, and reports. These factors can enhance the activity of the HHA axis, leading to an increase in cortisol levels. This is supported by previous studies such as that of Pérez-Lancho et al. [24], that measured in a group of adolescents cortisol in saliva twice a day (when waking up and before sleep), at the beginning and the end of the semester. They found an increase in cortisol at the end of the semester at

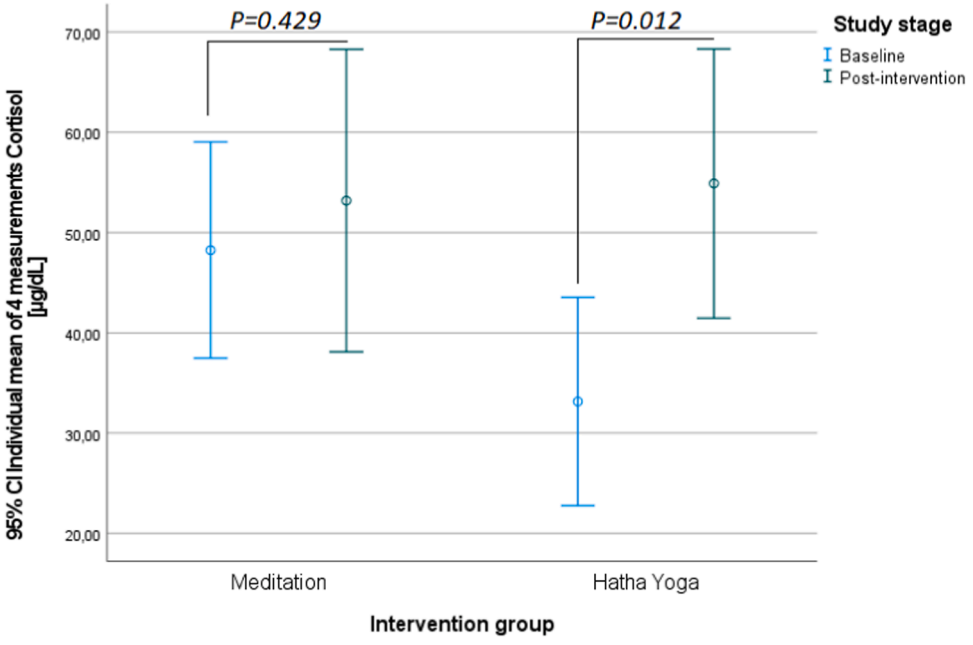

Fig. 2. Intra-groups comparison of baseline and post-intervention individual mean of the four measurements of salivary cortisol levels. Statistical significance was estimated via student t test.

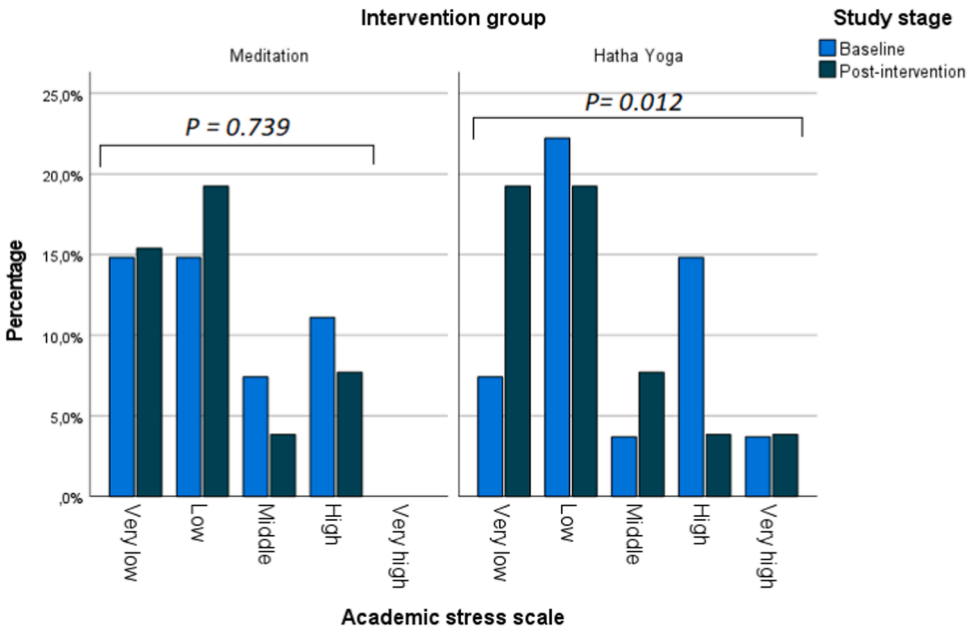

Fig. 3. Intra-groups comparison of baseline and post-intervention academic stress scale. Statistical significance as estimated via McNemar's Chi-squared.

both times of the day; with higher levels in female teenagers. This was attributed to the final exams of courses. Ringeisen et al. [25], found an increase in saliva cortisol in students, minutes before taking an oral exam, associated with higher levels of anxiety.

In addition, the significant difference in cortisol in the Hatha yoga group could be explained because this type of yoga involves high physical activity, integrating physical skills, musculoskeletal conditioning, breathing control, and meditation [14]. Taking into account that we included a sample of sedentary students, it is important to consider that for these individuals starting a physical activity might be a source of additional stress that can increase cortisol by the stimulation of the HHA axis [26]. Intense aerobic exercise consistently increases cortisol in saliva, while strength exercise has shown variable results on cortisol, depending on the intensity and frequency [27]. The students in the Hatha yoga group had an exposition of two hours/week for 14 weeks. It is possible that if the practice of Hatta yoga had been longer, the participants could reach the homeostasis that chronic exercise generates.

An important aspect to take into account is that although saliva cortisol levels significantly increased in the Hatha yoga, in both groups salivary cortisol means were within the normal range (0.77–0.93 μg/dL) [28]. Therefore, it might be possible that measuring cortisol in a single morning sample does not entirely capture all the physiological adaptations of stressed individuals, since chronic stress increases cortisol responses and stimulate sustained high levels of this hormone [29,30]. In this study, saliva samples were taken by the participants at their usual time of waking up and no scheduled time was instructed to take this sample. Differences in waking time might have influenced our findings on cortisol levels. It is not possible to determine whether there were attenuated responses to cortisol before the intervention, or if after the intervention, the increase could be related to better control of the hormone through the circadian rhythm. In addition, the non-academic context of the subjects was not controlled before the beginning of the study and during the study.

5.2. Meditation and salivary cortisol levels

The meditation control group showed a non-significant post-intervention elevation in saliva cortisol levels (P = 0.429) (Fig. 2). This result is similar to that reported by Sibinga et al. [31] in adolescents after 12 weeks of meditation during the school period. However, Sanada et al. [32], a meta-analysis that included meditation interventions in healthy adults, found that meditation was associated with lower salivary cortisol levels and that this association was related to population characteristics such as age, intervention intensity, hours, and number of mediation sessions. Another meta-analysis [22], that included clinical trials with active controls similar to our study, found that mindfulness-focused meditation was associated with lower blood cortisol levels. However, studies involving meditation of subjects under academic stress have reported discordant effects on cortisol levels. Some studies show cortisol reduction, as reported by Turakitwanakan et al. [33], in a group of medical students, after a 4-day meditation program. Similarly, a clinical trial conducted by Beerse et al. [34], in high school students, showed a reduction in saliva cortisol levels after five weeks of intervention with art-based meditation, although this same trend was observed in the control group. Bottaccioli AG et al. [17], reported in a group of psychology students that 30 h of meditation during four days decreased cortisol levels in saliva after exposure to stressful conditions. In contrast and agreeing with our finding, other authors have found no effect of meditation on cortisol levels, under stressful stimuli. Sousa G et al. [35] reported in Brazilian undergraduate and graduate students that meditation for three days did not reduce cortisol levels. Similarly, Myint et al. [16], after applying a two-hour meditation program for five weeks in university students, did not find changes in serum cortisol, anxiety, or depression. Variables such as exposure time, type of meditation, and type of population should be considered to measure the impact of meditation on cortisol.

It is also relevant to bear in mind that the non-significant difference between pre and post-intervention cortisol levels in the meditation group could be related to the significant baseline higher cortisol level this group had in comparison with the Hatha yoga group despite the randomization.

5.3. Effect of Hatha Yoga and meditation on academic stress

We analyzed academic stress using the questionnaire that classifies stress into 5 levels (very low, low, medium, high, and very high), a level higher than 3 (medium) indicates chronic academic stress, associated with physical and psychological symptoms as a consequence of a lack of resources to cope with it. In this study, we found after the interventions

few students with stress in the categories medium, high, and very high, with no significant differences between groups. However, when analyzing the intra-group differences, we found a significant reduction in upper levels of academic stress in the Hatha yoga group ($P = 0.012$). This finding may be associated with a decrease in psychosomatic symptoms. Hatha yoga combined with breathing, exercises (pranayama), cleansing processes (kriyas), attitudes of interval (mudras), neuromuscular blocks (bandhas), ethical practices (yamas), concentration (dharana), meditation (dhyana), had shown influence the mental health by relieving tension, perceived stress, anxiety, and improving emotional, cognitive and visceral functions [22,36,37]. The above may be related to the reduction of stress-related psychosomatic symptoms and perceived stress observed in our study, as it has been reported in studies that showed a reduction in perceived stress after yoga practice, related to factors such as increased well-being [38–40], and cognitive functions [41], decreased physical and physiological symptoms related to stress [41–43] and anxiety reduction [44,45].

García et al. [46], reported that in medical students the practice of Hatha yoga for 14 weeks, with a frequency of one hour twice a week, reduced academic stress with a positive effect on the perception of stress and improved peer relationships through creating a more positive mindset, relaxation, and tranquility. This study also found that Hatha yoga increased self-compassion and three positive dimensions (self--kindness, common humanity, and mindfulness), and decreased negative dimensions of self-judgment, isolation, and over-identification, although not significantly [47,48]. The self-compassion, derived from mindfulness, allows each individual to experience a kinder feeling towards themselves, which helps to improve their emotional abilities to cope with stressors and reduce the likelihood of experiencing depression and anxiety [49].

In our study, perceived stress decreased in interventions of Hatha yoga and meditation (with significant differences for the former), although awakening responses in postintervention salivary cortisol were within the normal range. This could mean that yoga improved the strategies for coping with stressful conditions and reduced negative factors that represent a risk to physical and mental health. As reported by Otto J. et al. [50], negative coping and low tolerance to distress, are transdiagnostic indicators of mental illness and an inability to resolve the difficulties of life.

The increases found in cortisol after Hatta yoga and meditation with the decrease in perceived stress is a paradox that could have two explanations previously mentioned. The first potential explanation relates to the adaptation to major stressors at the end of the study. It should be considered that the hatha yoga and meditation practices helped some individuals to develop positive coping strategies, which may be a way of managing emotions better and helping reduce psychosomatic symptoms and perceived stress. This hypothesis is based on the transactional model of Lazarus and Folkman, which considers a stressful event the cognitive evaluation, that implies a transaction, or negotiation between the individual and the environment, after which the individual will respond in one way or another according to the resources that have to cope with the situation [2].

The second plausible explanation for the paradox of decreased perceived stress with an increase in salivary cortisol after interventions is the collection of saliva samples only at one part of the day (upon awakening). This did not enable us to observe how cortisol varied throughout the day and whether the intervention attenuated responses to cortisol during the day induced by chronic stress.

It is important to consider that psychological attributes related to stress may not be directly related to biological biomarkers such as cortisol. The trend in the reduction of symptoms and the perception of stress in the meditation group could suggest that the decrease in stress perception and symptoms is an adaptive phenomenon to stress (that is independent of physiological variables), which by improving the subject's perspective on the situation, translates into emotional self-regulation. In the particular case of mindfulness meditation, it has been documented that it initially induces an increase in salience activity, or the recognition of sensory and limbic stimuli, and then facilitates states of full mental task. These are mainly executive functions that decrease the excess of narrative self-reference or prospection in any situation. All these changes are related to a decrease in the perception of stress and facilitate the execution of tasks that are specific to the academic environment [51].

5.4. Limitations and strengths

Several limitations have to be acknowledged. First, we did not control the time of waking for the saliva sample in which cortisol was measured. This was intended to obtain the sample under the normal context of each individual. However, heterogeneity in times for saliva sampling might have influenced cortisol findings. Second, this study did not evaluate additional measurements of salivary cortisol during the day that might have captured a more detailed effect of the interventions. Our study also lacked additional evaluation of biomarkers related to chronic and subclinical inflammation and oxidative stress that might be potentially altered by stress. In particular, further research on Hatha yoga and meditation interventions may focus on metabolomics analyses to identify acute changes in intra and extra-cellular metabolites and pathways targeted by these interventions. In addition, although the sample size was calculated according to an estimated statistical power, we cannot discard a beta-type error in terms of not detecting stronger differences because of an insufficient sample size. We also cannot discard findings by chance due to multi-test, and therefore our results must be interpreted with caution, and should not be generalized to other academic populations.

To the best of our knowledge, this study might be the first analysis comparing the effects of Hatha yoga and meditation on academic stress, and the first with medical students. Moreover, our analysis seems to be the first report on Hatha yoga and academic stress in Latin-American individuals.

6. Conclusion

In this study, there was a significant decrease in perceived stress among students that practiced Hatha Yoga, and increases in saliva cortisol in this group might be influenced by measurements obtained only at waking time, and by the fact that waking time was different among the participants. Despite this increase in salivary cortisol, saliva cortisol mean levels did not exceed normal concentration in both groups. It was not possible to establish whether there were changes in the diurnal cortisol curve. On the other hand, the osteomuscular activation experienced in yoga practice, or the end-of-semester stage in which stressful stimuli increase, may activate the secretion of cortisol without increasing psychological stress, due to the gain in coping strategies.

Further research is required in a larger sample of individuals and that includes students from different university programs and with longer periods of Yoga or meditation practices. In addition, studies involving cortisol measurements should be conducted at different times of the day to establish the relationship with the diurnal curve and to analyze changes in attenuated responses.

Financial support

This work was supported by the Pontifical University Javeriana Cali research grant #1051.648.

Ethical statement

The authors declare that this study was carried out taking into account the ethical considerations of research with human subjects in accordance with the Declaration of Helsinsky. Likewise, this study protocol was approved by the Research Ethics Committee of the Faculty

of Health Sciences of the Pontificia University Javeriana, Cali, and all participants gave their consent by signing the consent form before initiating the procedures consigned in the methodology.

CRediT authorship contribution statement

Sandra Moreno: Formulation or evolution of overarching research goals and aims, Development and design of methodology, conducting a research and investigation process, preparation, creation and/or presentation of the published work,management and coordination responsibility for the research activity planning and execution, acquisition of the financial support for the project leading to this publication. Lina Becerra: Formulation or evolution of overarching research goals and aims, Development and design of methodology, conducting a research and investigation process, provision of study materials, reagents, materials, patients, laboratory samples, preparation, creation and/or presentation of the published work, acquisition of the financial support for the project leading to this publication. Guillermo Ortega: Development and design of methodology, verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs, conducting a research and investigation process, specifically performing the experiments, creation and/or presentation of the published work, acquisition of the financial support for the project leading to this publication. Milton Suarez: Data analyses, Management activities to annotate, scrub data and maintain research data, Preparation of the published work by those from the original research group, specifically critical review, commentary or revision. Freddy Moreno: Ideas, formulation or evolution of overarching research goals and aims, Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision, visualization/ data presentation, acquisition of the financial support for the project leading to this publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Any of the information can be obtained from the author on request.

Acknowledgments

We thank the Pontificia Universidad Javeriana Cali and its Research Office for all the support provided and the Medicine program of the same institution for allowing the execution of the research.

Conflicts of interest

The authors declare that there is no conflict of interest. 645.

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