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EVALUATION OF THE POTENTIAL THERAPEUTIC BENEFITS OF BIOFEEDBACK TRAINING AS A METHOD FOR REDUCING STRESS AND IMPROVING THE MENTAL HEALTH OF INTERNATIONAL MEDICAL STUDENTS

THESIS SUMMARY

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CONTENTS

List of Abbreviations

5-HT – serotonin
ACTH – adrenocorticotropic hormone
ANCOVA – analysis of covariance
BAI – Beck Anxiety Inventory
BDI – Beck Depression Inventory
BNST – bed nucleus of the stria terminalis
cAMP – cyclic adenosine monophosphate
COMT – catechol-O-methyltransferase
CRH – corticotropin-releasing hormone
CRH-BP – CRH-binding protein
CRH-R – corticotropin-releasing hormone receptor
GABA – gamma-aminobutyric acid
HAM-D – Hamilton Depression Rating Scale
HF – high frequency
HPA – hypothalamic-pituitary-adrenal axis
HRV – heart rate variability
LF – low frequency
PSS – Perceived Stress Scale
PVN – paraventricular nucleus
SAM – sympathoadrenal medullary axis
SAS – Zung Self-Rating Anxiety Scale
ULF – ultra low frequency
VLF – very low frequency
BF – biofeedback
ANS – autonomic nervous system
EEG – electroencephalography
ECG – electrocardiography
PNS – parasympathetic nervous system
PTSD – post-traumatic stress disorder
WHO – World Health Organization
SNS – sympathetic nervous system
PPG – photoplethysmography
CNS – central nervous system

I. INTRODUCTION

Stress is a complex, integrative neuroendocrine and autonomic response of the body to external or internal stimuli that disrupt homeostatic equilibrium and require an adaptive reaction. This response involves coordinated activation of the central nervous system, the hypothalamic-pituitary-adrenal axis, and the sympathoadrenal medullary system, leading to systemic metabolic, hemodynamic, and immunological changes. During short-term exposure, these mechanisms support the restoration of functional balance. However, prolonged or repeated stress stimulation may result in a state of chronic neuroendocrine dysregulation associated with an increased risk of anxiety and depressive disorders, cardiovascular disease, and immune dysfunction.

Among medical students, and particularly among those studying abroad, the prevalence of chronic stress is especially high because of elevated academic expectations, cultural differences, adaptation to a new environment, separation from friends and family, and potential financial difficulties. These stressors can seriously affect mental health and academic performance, leading to the so-called burnout syndrome, as well as physical and mental exhaustion.

Biological feedback, also referred to as biofeedback (BF), is a psychosomatic technique through which individuals learn to regulate a range of physiological processes effectively in order to improve physical, mental, emotional, and spiritual health. Similar to physical therapy, BF training for autonomic regulation requires active patient participation and systematic practice between sessions.

Through BF, information about numerous physiological functions, such as heart rhythm, muscle tension, and brain-wave activity, can be obtained in real time. With the aid of specific techniques, trainees can develop the ability to consciously modulate these parameters. By promoting self-regulation and relaxation, BF can reduce stress and improve mental health.

Different types of BF, including electromyography (EMG), electroencephalography (EEG), and heart rate variability (HRV) assessment, have demonstrated effectiveness, particularly in stress management. Integrating BF into stress-management programs for medical students may offer a non-pharmacological approach to improving their psychological well-being.

The therapeutic potential of BF for reducing stress among international medical students remains insufficiently studied. The current literature focuses primarily on students studying in their home countries or on clinical populations, leaving a gap in understanding the specific needs and responses of international students. They face distinct challenges, including language barriers, social isolation, and differences between educational systems, which may affect their stress levels and the effectiveness of BF interventions.

The present study aims to address this gap by evaluating the efficacy of BF training in reducing stress and improving mental health among international medical students. This will make it possible to assess changes in stress levels and in the psychological profile following a BF training program.

Understanding the impact of BF on this population is important for several reasons. First, the findings could support the development of targeted institutional mental-health support programs in academic settings, adapted to the specific needs of international students. Second, the study could provide a better understanding of the broad applicability of BF as a stress-management tool across different populations. Finally, improving the mental health and well-being of international medical students may enhance their academic performance and professional development, contributing to a more competent and resilient healthcare workforce.

In summary, stress control and the improvement of mental health among international medical students constitute a current scientific and clinical issue. Existing studies emphasize the high levels of stress faced by this population and the potential of BF training as a therapeutic program. The study aims to fill the gap in the literature by investigating the efficacy of BF in reducing stress and improving mental health among international medical students, while also contributing to the development of effective, affordable interventions that can be readily integrated into everyday life to improve mental health in the academic environment.

II. AIM, OBJECTIVES, AND HYPOTHESES OF THE STUDY

The aim of the present study is to evaluate the therapeutic potential of BF training as a method for reducing stress and improving the mental health of international medical students.

To achieve this aim, the following objectives were defined:

- To investigate the levels of stress, anxiety, and depression among international medical students by administering highly informative and established psychometric tests for the assessment of stress levels (Perceived Stress Scale, PSS), anxiety (Zung Self-Rating Anxiety Scale, SAS, and Beck Anxiety Inventory, BAI), and depression (Beck Depression Inventory, BDI);
- To conduct a BF training program in a group of students with high levels of stress, anxiety, or depression;
- To assess the effect of BF training on the students' psychophysiological state and stress reactivity (before and after a stress test) by measuring:
  - heart rate variability recorded using a PPG sensor;
  - cortisol and IgA levels in saliva samples;
- To investigate the correlation between psychological state and the body's psychophysiological reactivity;
- To investigate the relationship between salivary cortisol and IgA levels during the stress response, states of stress and anxiety, and changes in physiological parameters.

The following working hypotheses were defined:

- BF training reduces the levels of stress, anxiety, and depression among international medical students at MU-Varna.

- There is a positive correlation between the students' psychological state (e.g., levels of stress and anxiety) and the body's psychophysiological reactivity (e.g., changes in heart rate variability).
- Changes in salivary cortisol and IgA concentrations during a stress response are associated with changes in the levels of stress, anxiety, and depression, as well as with alterations in certain physiological parameters, such as heart rate variability.

III. MATERIALS AND METHODS

1. Study Design

The present study was conducted using a controlled longitudinal design. Two groups of participants were used:

- a training group (included in a BF training program);
- a control group (not participating in the training program but undergoing the corresponding psychometric and psychophysiological assessments).

The study tracks the effects of BF training on:

- levels of perceived stress;
- anxiety;
- depressive symptoms;
- psychophysiological reactivity;
- biochemical markers of the stress response in international medical students.

Allocation to the groups was performed after an initial screening of psychological status using the following standardized questionnaires:

- Perceived Stress Scale (PSS) — for assessing perceived stress;
- Zung Self-Rating Anxiety Scale (SAS) — for self-assessment of anxiety;
- Beck Anxiety Inventory (BAI) — for assessing anxiety symptoms;
- Beck Depression Inventory (BDI) — for assessing depressive symptoms.

The study covered a period of 11 weeks, during which the students in the training group attended a soundproof laboratory:

- twice per week;
- with each session lasting 30 minutes;
- under controlled conditions limiting the influence of external stimuli.

BF training was aimed at developing self-regulation skills through real-time monitoring of physiological parameters. During the sessions, participants were trained to recognize and modulate physiological responses associated with the stress response and autonomic regulation.

In addition, students in the training group received instructions for independent practice outside the laboratory setting. They were encouraged to apply the acquired techniques:

- early in the morning, immediately after waking;
- late in the evening, before going to bed;
- while preparing for examinations;
- before anticipated stressful situations.

The psychophysiological assessment included recording parameters reflecting autonomic regulation and reactivity to stress, including:

- heart rate variability;
- respiratory rate;
- other physiological parameters recorded using the BF equipment.

To assess the biochemical response to stress, saliva samples were used to determine:

- cortisol;
- secretory IgA.

The samples were collected:

- in the morning after waking;
- immediately before the stress test;
- 10 minutes after the stress test;
- 30 minutes after the stress test.

The laboratory stress test was conducted under controlled conditions in a laboratory room of the Department of Physiology and Pathophysiology. Its purpose was to induce a standardized stress response that would allow comparison of the two groups in terms of psychophysiological and biochemical reactivity. The resulting data were used for:

- assessing the effect of biofeedback training;
- comparing the training and control groups;
- tracking changes over time;
- analyzing the relationships between psychological parameters, physiological regulation, and stress biomarkers.

2. Study Participants

A total of 47 international medical students enrolled at Medical University 'Prof. Dr. Paraskev Stoyanov' – Varna participated in the study. The group allocation was as follows:

- training group — 24 participants;
- control group — 23 participants.

All participants were students in the international Master's degree program in Medicine, in which instruction is conducted in English.

2.1. Inclusion Criteria

Students were included in the study if they met the following conditions:

- enrolled in the international English-language Medicine program at MU-Varna;
- voluntarily expressed willingness to participate;
- completed the preliminary questionnaires assessing stress, anxiety, and depressive symptoms;
- signed informed consent;
- provided basic demographic data;
- met the criteria for allocation to the respective group according to the PSS, SAS, BAI, and BDI results.

2.2. Exclusion Criteria

- students with acute illnesses at the start of the program or students with chronic diseases;
- individuals whose health status did not permit participation in the laboratory measurements or the stress test.

3. Methodology of BF Training

For the purposes of the present study, heart rate variability (HRV) biofeedback was used, based on monitoring and control of heart rate and heart-rhythm coherence. The method aims to enhance the participants' capacity for effective emotional and psychophysiological self-regulation by improving cardiac coherence and ANS balance.

Cardiac coherence is defined as the optimal and synchronized state of heart rhythms, reflecting harmonious interaction between the sympathetic and parasympathetic divisions of the ANS. This type of BF is characterized by clear visual and auditory feedback, enabling participants to observe directly the effects of the techniques applied on their heart rate and variability.

BF training was conducted using emWave® Pro Plus, a stationary system developed by the HeartMath® Institute for scientifically grounded measurement and training in heart rate variability and physiological coherence. The system uses an optical PPG sensor that can be placed either on the ear (ear sensor) or on the finger (finger sensor). It measures the intervals between heartbeats (RR intervals), on the basis of which HRV parameters are calculated. The sensors are highly sensitive and transmit data in real time, allowing accurate monitoring of heart rhythm and its variations.

The emWave Pro Plus software includes two modules:

- emWave Pro, which provides real-time visualization of heart rhythm, coherence, HRV graphs, and physiological coherence indicators;
- HRV Assessment, which enables five-minute HRV analyses to be performed in accordance with the standards of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996).

4. Methods for Assessing Psychological Parameters

Four standardized and widely validated psychometric instruments were used to assess the participants' psychological state, administered in a translated form adapted for English-speaking students. Each test has demonstrated validity and reliability for investigating symptoms of stress, anxiety, and depression in academic and clinical contexts.

The Perceived Stress Scale (PSS) is a self-report scale measuring the subjective perception of stress during the preceding month (Cohen et al., 1983). The Zung Self-Rating Anxiety Scale (SAS) measures the frequency of anxiety symptoms, reflecting both somatic and cognitive aspects (Zung, 1971). The Beck Anxiety Inventory (BAI) is a specifically targeted instrument for assessing the intensity of anxiety symptoms, including physiological manifestations such as palpitations, sweating, tension, and others (Beck et al., 1988). The Beck Depression Inventory (BDI-II) is a gold standard for screening depressive symptoms and assesses cognitive, behavioral, and affective aspects of depression (Beck et al., 1961).

All participants completed the above-mentioned tests at three clearly defined time points:

- Stage 1 – November (preliminary selection): A screening questionnaire survey was conducted to identify students with moderate to high levels of psychological distress, anxiety, and depression.
- Stage 2 – February (baseline measurement): Participants were surveyed immediately before the beginning of the BF training program in order to establish baseline levels.
- Stage 3 – May (after completion of the program): Participants were surveyed again at the end of the 11-week intervention period to evaluate the effectiveness of the BF program.

5. Measurement of Salivary Cortisol and Secretory Immunoglobulin A (sIgA) Levels

The physiological response to BF training was assessed by measuring salivary cortisol and secretory immunoglobulin A (sIgA) levels, which are recognized biomarkers of stress, immune function, and psychophysiological reactivity. Saliva samples were collected at two key points in the study:

- Before the start of the training program (baseline assessment);
- After completion of the training program (final assessment).

Within the study, the Maastricht Acute Stress Test (MAST) was used to provoke acute psychophysiological stress. It is a laboratory protocol combining cold pain, mentally demanding tasks, social evaluation, and unpredictability. The test was validated by Smeets et al. (2012) and is considered a reliable method for inducing strong and reproducible activation of the HPA axis.

In both cases, samples were collected at four different time points during the day on which the psychological stress test (MAST) was performed:

- Immediately after waking (at rest);
- Immediately before the MAST;
- 10 minutes after completion of the MAST;
- 30 minutes after completion of the MAST.

This approach makes it possible to capture both the baseline level and the body's dynamic response to the acute stress stimulus. Salivary cortisol was determined by competitive electrochemiluminescence immunoassay (ECLIA) on an automated Cobas 6000 analyzer after prior centrifugation of the samples. The sIgA concentration was measured using an enzyme-linked immunosorbent colorimetric assay (ELISA; LDN Immunoassays and Services) after centrifugation and serial dilution of the supernatant to a final dilution of 1:1000. The sIgA results were calculated using a four-parameter calibration curve.

6. Statistical Methods of Analysis

The collected empirical data were analyzed using STATA version 18, Jamovi version 2.4.8, and GraphPad Prism version 5.0, which provide a broad range of options for descriptive, comparative, multivariate, and longitudinal statistical processing. The choice of methodology was tailored to the type of data, the hypotheses, and the study design, which included repeated measurements over time, a control group, and a broad range of physiological and psychometric parameters. Descriptive, comparative, analysis-of-variance, longitudinal, multivariate, factor, correlation, and regression analyses were performed. The selected statistical methods allowed a comprehensive and integrated approach to data analysis, combining descriptive, comparative, longitudinal, and multivariate approaches for valid interpretation of the therapeutic effects of BF training on the participants’ mental and physiological state. All tests were interpreted as statistically significant at a significance level of $p \leq 0.05$.

IV. RESULTS AND DISCUSSION

1. Descriptive Analysis

The participants included in the program were divided into two groups—the training group and the control group—and various demographic variables were analyzed. The data are presented using descriptive statistics (mean, median, standard deviation, minimum, and maximum) and frequency analysis (absolute and relative frequency).

1.1. Sex

Of the total number of participants (N=47), 14.9% were men and 85.1% were women (Table 1).

Sex	Group	Number	% of total	Cumulative %
Male	Training	3	6.4 %	6.4 %
	Control	4	8.5 %	14.9 %
Female	Training	21	44.7 %	59.6 %
	Control	19	40.4 %	100.0 %

Table 1. *Distribution of participants by sex and group*

The analysis of sex among the participants revealed a clearly uneven distribution. The predominance of women is typical of many medical specialties and should be taken into account when interpreting the results, particularly in view of known sex differences in stress reactivity and psychophysiological parameters (Verma et al., 2011; Kristoffersson et al., 2018).

1.2. Age

The participants' age profile reflects the young age typical of the student population studied, with some variability between the two groups (Table 2).

Age	Group	N	Mean	Median	SD	Minimum	Maximum
	Training	24	21.88	22.00	1.676	19	25
	Control	23	24.35	24	3.588	20	31

Table 2. *Age characteristics of participants by group*

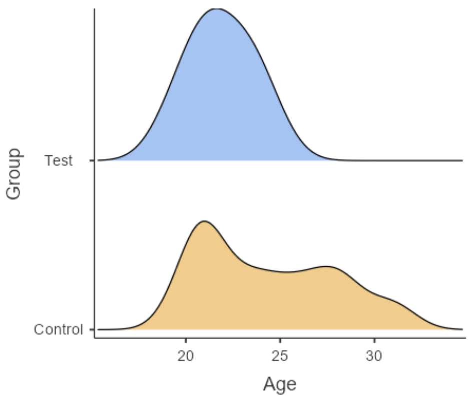

Figure 1. *Graphical presentation of the age structure in the two study groups*

The data distribution was practically symmetrical (skewness = 0.033), while kurtosis was slightly platykurtic (kurtosis = –0.86), suggesting a normal distribution and permitting the use of parametric statistical tests in the subsequent analysis (Fig. 1).

1.3. Year of Study

Year of study	Group	Number	% of total
1	Training	4	8.5 %
	Control	0	0.0 %
2	Training	8	17.0 %
	Control	3	6.4 %
3	Training	5	10.6 %
	Control	15	31.9 %
4	Training	0	0.0 %
	Control	5	10.6 %
5	Training	6	12.8 %
	Control	0	0.0 %
6	Training	1	2.1 %
	Control	0	0.0 %

Table 3. *Distribution of participants by year of study and group*

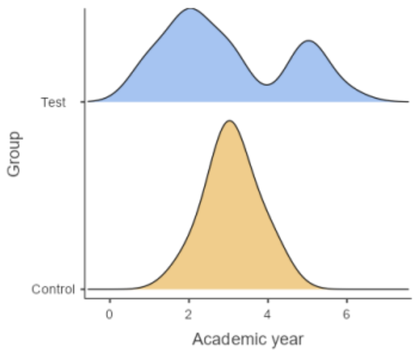

Figure 2. *Distribution of participants by year of study in the training and control groups*

There was heterogeneity among the participants with regard to academic year (Table 3). Third-year students constituted the largest proportion (42.5%), followed by second-year students

(23.4%) and fifth-year students (12.8%). First-, fourth-, and sixth-year students were represented to a substantially lesser extent (Fig. 2). The greater representation of third-year students is important for interpreting the results because this period coincides with the transition from the preclinical to the clinical stage of medical education. Adaptation to clinical disciplines, contact with patients, and new practical and professional requirements may constitute an additional source of academic and psycho-emotional burden. The data show a concentration of participants in the middle stages of training, when academic workload and stress often peak, which is highly relevant to the context of the study (Al-khlaiwi et al., 2023).

1.4. Weight and Height

	Group	N	Mean	Median	SD	Minimum	Maximum
Weight	Training	24	68.13	66.50	15.434	40	100
	Control	23	71.52	68	15.863	45	115
Height	Training	24	166.50	167.50	7.902	152	180
	Control	23	168.35	168	10.342	150	188

Table 4. *Anthropometric characteristics of participants in the two groups*

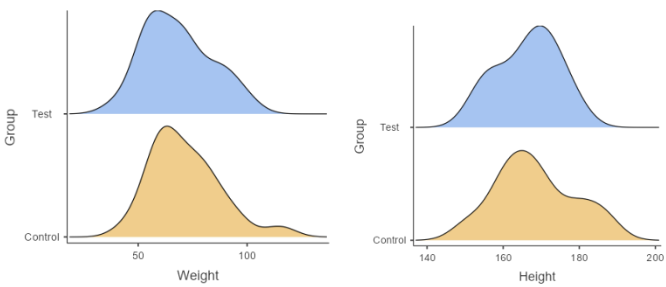

Figure 3. *Comparative analysis of anthropometric parameters between the training and control groups*

The mean weight in both the training and control groups was within healthy ranges, although with some variability (Table 4). No substantial differences between the groups were found for either height or body weight (Fig. 3). This provides a sound basis for comparability in physiological measurements such as heart rate variability and cortisol levels (Pulopulos et al., 2018).

1.5. Additional Information

	Option	Number (N=47)	Proportion	p-value
Religion	Religious	31	0.660	0.040*
	Atheist	16	0.340	0.040*
Ethnicity	Caucasian	14	0.298	0.008*
	Asian	18	0.383	0.144
	Other	15	0.319	0.019*
Smoking	<1 pack/day	6	0.128	< .001*
	One/> packs/day	1	0.021	< .001*
	No	40	0.851	< .001*
Alcohol consumption	Light	18	0.383	0.144
	Moderate	1	0.021	< .001*
	None	28	0.596	0.243
Financial status	Good	14	0.298	0.008*
	Satisfactory	29	0.617	0.144
	Poor	4	0.085	< .001*
Family support	Financial	8	0.170	< .001*
	Financial and emotional	37	0.787	< .001*
	None	2	0.043	< .001*
Presence of chronic diseases	Yes	11	0.234	< .001*
	No	36	0.766	< .001*
Mental health conditions	In the family	14	0.298	0.008*
	Second-degree relatives	5	0.106	< .001*
	No	28	0.596	0.243
Use of medications/supplements	Yes	20	0.426	0.382
	No	27	0.574	0.382
Hours of study/day	<2 hours	11	0.234	< .001*
	2–4 hours	25	0.532	0.771

	4–6 hours	11	0.234	< .001*
Hours of sleep/day	4–6 hours	10	0.213	< .001*
	6–8 hours	31	0.660	0.040*
	>8 hours	6	0.128	< .001*
Vigorous exercise (times per week)	1	23	0.489	1.000
	2	6	0.128	< .001*
	3	12	0.255	0.001*
	4	3	0.064	< .001*
	5	3	0.064	< .001*
Moderate exercise (times per week)	1	7	0.149	< .001*
	2	11	0.234	< .001*
	3	12	0.255	0.001*
	4	10	0.213	< .001*
	5	4	0.085	< .001*
	7	3	0.064	< .001*
Light exercise (times per week)	1	10	0.213	< .001*
	2	4	0.085	< .001*
	3	10	0.213	< .001*
	4	9	0.191	< .001*
	5	5	0.106	< .001*
	6	3	0.064	< .001*
	7	6	0.128	< .001*

Table 5. *Descriptive characteristics of the sample according to additional demographic and behavioral variables*

To assess the distribution of the principal sociodemographic and behavioral characteristics in the sample, a one-sample binomial test was applied to dichotomous variables, with a null hypothesis of an equal-probability distribution between the two categories (H_0: p=0.5). For variables with more than two categories, interpretation should be based on tests of multinomial distribution. The results revealed a number of statistically significant deviations that outline important aspects of the profile of the participants studied (Table 5).

With regard to the additional sociodemographic characteristics, the sample displayed a relatively clearly expressed religious identity and ethnocultural diversity. These features suggest a heterogeneous social environment that should be considered when interpreting psychometric and physiological parameters, because cultural and value-related factors may influence the perception of stress and adaptive resources.

The participants' behavioral profile indicates a comparatively favorable healthy lifestyle. Smoking and regular alcohol use did not emerge as dominant behavioral patterns in the sample, providing a better basis for interpreting physiological measurements without a pronounced influence from some common behavioral confounders. In this context, the study population may be regarded as relatively healthy in terms of daily habits.

The socioeconomic and family context also indicated a relatively stable profile. Participants predominantly reported satisfactory to good financial circumstances and a clear presence of family support. This is important because a supportive family environment may buffer stress and influence an individual's capacity to cope (Hostinar and Gunnar, 2015; An et al., 2024).

Health status further supports the impression of a relatively low somatic and psychiatric burden. At the same time, a family history of mental health conditions in some participants suggests that certain individuals may have increased vulnerability to stressful influences, which is relevant when discussing individual differences in response to the program (van Sprang et al., 2020).

Analysis of the daily routine showed a relatively structured pattern of study and recovery, characterized predominantly by moderate-intensity studying and sleep duration close to that recommended for this age group. This creates conditions for more adequate recovery and more stable ANS functioning.

The physical-activity data indicated a predominance of moderate forms of exercise over more intensive regimens. Such a pattern is characteristic of young people with high academic engagement and may be regarded as a potentially favorable factor in terms of stress regulation and overall psychophysiological status (Childs and de Wit, 2014).

In summary, the demographic profile of the study participants revealed a predominantly female, academically active, young population with relatively homogeneous anthropometric characteristics. This profile is typical of highly motivated medical students and should be interpreted in the context of increased academic pressure and the need for resilience to stress.

2. Comparative Analysis

2.1. Demographic and Behavioral Variables

A comparative analysis was performed between participants in the training and control groups with respect to major demographic and behavioral characteristics. The aim was to determine whether the two groups differed significantly in any key factors that could influence the physiological and psychological outcomes.

A χ^2 test of independence was used to examine the association between group membership (training or control) and the following nominal variables: sex, religious affiliation, ethnicity, smoking, alcohol consumption, presence of mental or chronic diseases, parental support, and use of medications/supplements. Statistical significance was set at $p < 0.05$ (Table 6).

№	Variable	χ^2 value	p-value
1.	Sex	0.22	0.638
2.	Religion	0.51	0.471
3.	Ethnicity	5.82	0.060
4.	Smoking	2.05	0.359
5.	Alcohol consumption	1.12	0.571
6.	Mental disorders	1.47	0.481
7.	Chronic diseases	2.70	0.101
8.	Use of medications/supplements	0.22	0.638
9.	Parental support	5.16	0.076
10.	Hours of study per day	0.20	0.905
11.	Hours of sleep per day	0.68	0.712

Table 6. *Chi-square analysis of demographic and behavioral variables*

The results showed that none of the variables examined reached the threshold for statistical significance, suggesting homogeneity between the groups with respect to the specified nominal variables. The absence of statistically significant differences between the groups in the demographic and behavioral characteristics examined is particularly important for the validity of the study. This suggests that the subsequently observed effects of the BF program can be attributed with greater confidence to the training program itself rather than to pre-existing differences between participants. In other words, the results of the subsequent analyses of physiological and psychological parameters will not be influenced by systematic differences in the baseline characteristics of the two groups.

Before performing the comparative analysis between the two groups with respect to the quantitative variables, the Shapiro-Wilk test was conducted to assess the normality of the distributions (Table 7).

	Group	Age	Weight	Height	Year of study
N	Training	24	24	24	24
	Control	23	23	23	23
Skewness	Training	0.0330	0.419	-0.258	0.513
	Control	0.465	0.927	0.291	-0.0133
Kurtosis	Training	-0.856	-0.536	-0.886	-1.08
	Control	-1.08	1.19	-0.536	0.161
Shapiro-Wilk W	Training	0.951	0.957	0.962	0.862
	Control	0.895	0.947	0.965	0.761
Shapiro-Wilk p	Training	0.284	0.381	0.479	0.004*
	Control	0.020*	0.257	0.568	< .001*

Table 7. *Results of the Shapiro-Wilk test for the quantitative variables in the two study groups*

The results showed that the variables age, body weight, and height did not deviate substantially from a normal distribution in either the training group (p=0.284, 0.381, and 0.479, respectively) or the control group (p=0.020, 0.257, and 0.568, respectively). Although the p-value for age in the control group (p=0.020) suggests some deviation from normality, the remaining parameters were within acceptable limits. We therefore accepted that the distribution was approximately normal, particularly given the symmetry (skewness < ±1) and flatness of the curve (kurtosis < ±1) for most variables.

In contrast, the variable 'year of study' showed a significant deviation from a normal distribution in both groups—p=0.004 (training) and p<0.001 (control). Therefore, a nonparametric test (Mann-Whitney U) was used to compare the groups on this parameter.

Based on these results, an independent-samples t-test was applied in the next stage of the analysis to the quantitative variables with approximately normal distributions (age, weight, and height), whereas the Mann-Whitney U test was used for ‘year of study’ (Table 8).

Variable	Statistical test	Value	df / U	p-value	Effect (Cohen’s d / Rbc)
Age	Student's t	-3.048	45	0.004*	-0.889
Weight	Student's t	-0.744	45	0.461	-0.217
Height	Student's t	-0.690	45	0.494	-0.201
Year of study	Mann-Whitney U	226	–	0.264	0.183

Table 8. *Comparison of the main demographic and anthropometric parameters between the training and control groups*

The independent-samples t-test results showed a statistically significant difference in age between the two groups—the participants in the training group were younger on average than those in the control group (p=0.004). The effect size calculated using Cohen’s coefficient (Cohen’s d = –0.889) indicated a large effect, meaning that the difference was not only statistically significant but also practically substantial. This is important to consider when interpreting the subsequent results, because age may act as a moderating variable for a range of psychophysiological and behavioral parameters (K. Li et al., 2025).

For the remaining quantitative variables—body weight (p=0.461) and height (p=0.494)—no statistically significant differences were found between the groups. The effect sizes were small, supporting the conclusion that the groups were relatively homogeneous with respect to these characteristics as well (Fig. 4).

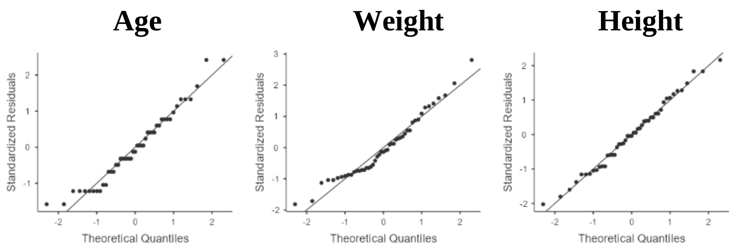

Figure 4. *Graphical assessment of normality for age, weight, and height*

With respect to the variable 'year of study,' the Mann-Whitney U test results (p = 0.264) likewise showed no significant difference between the two groups. Although the mean value was slightly lower in the training group (M = 2.96) than in the control group (M = 3.09), the difference did not reach statistical significance, and the rank-biserial correlation coefficient (0.183) indicated a small effect.

The two groups were comparable in terms of body characteristics and year of study, but the age difference between them requires attention and potentially the inclusion of age as a covariate in additional analytical models.

2.2. Laboratory Tests – Cortisol

The physiological stress response was assessed by measuring salivary cortisol at different time points—in the morning immediately after waking, before the MAST, and 10 and 30 minutes after the acute stress test—in February (before the training program) and May (after completion of the training program). Comparison between the training and control groups made it possible to track both baseline hormone levels and the degree of HPA-axis hyperactivation in response to induced stress (Table 9).

Parameter	Group	Mean	Min.	Max.	Shapiro-Wilk (p)
Cortisol – February	Control	13.4	2.30	30.9	0.615
	Training	15.3	1.87	59.9	< 0.001*
Morning cortisol – May	Control	13.5	3.97	35.9	0.036*
	Training	13.6	1.65	38.6	0.076
Cortisol before test – May	Control	13.1	3.16	34.6	0.020*
	Training	18.2	6.12	33.3	0.862
Cortisol 10 min – May	Control	13.1	3.38	33.7	0.049*
	Training	20.6	3.68	53.2	0.139
Cortisol 30 min – May	Control	10.2	2.99	22.5	0.053
	Training	14.6	1.50	30.9	0.761

Table 9. *Descriptive statistics and normality test for cortisol parameters by group*

Higher mean cortisol values were found at all time points in the training group compared with the control group. The greatest difference between the two groups was observed 10 minutes after the stress test, when the mean value was 20.6 ng/mL in the training group versus 13.1 ng/mL in the control group.

In the morning measurements in May, cortisol values were similar in the two groups. Before the test, values were higher in the training group, whereas 30 minutes after the test, levels had decreased in both groups (Fig. 5).

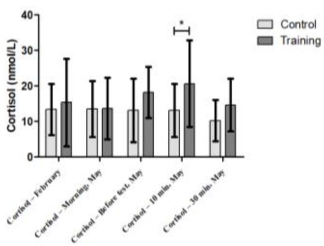

Figure 5. *Comparison of mean cortisol values between the control and training groups at the different measurement time points*

The results obtained are partially consistent with the available literature. The MAST is established as a reliable model for inducing acute physiological stress, and repeated administration has been reported to preserve cortisol reactivity without substantial evidence of habituation or sensitization (Quaedflieg et al., 2017; Shilton et al., 2017). At the same time, studies of BF more commonly report a reduction in salivary cortisol or no significant differences compared with control conditions (Brinkmann et al., 2020; Kotozaki et al., 2014). This suggests that, in the present sample, the program may have influenced the nature of the stress response rather than absolute cortisol levels. This interpretation is made in the context of individual differences in reactivity and the potential emergence of the expected effect over a longer period.

2.3. Laboratory Tests – IgA

Immune reactivity was assessed by measuring salivary immunoglobulin A (IgA) levels at the same time points—in the morning immediately after waking, before the MAST, and 10 and 30 minutes after the MAST—in February (before the training program) and May (after completion of the training program). The comparison between the two groups aimed to evaluate both baseline mucosal immunity and the dynamics of IgA in response to acute psychophysiological stress under conditions of prolonged BF exposure (Table 10).

Parameter	Group	Mean	Min.	Max.	Shapiro-Wilk (p)
IgA – February	Control	163.7	38.3000	588	< .001*
	Training	172.1	5.0300	1048	< .001*
IgA – morning, May	Control	103.2	14.8400	382	< .001*
	Training	130.3	0.0200	557	< .001*
IgA – before test, May	Control	62.2	0.7300	305	< .001*
	Training	46.9	0.0200	166	0.001*
IgA – 10 min after test, May	Control	50.6	12.5700	217	< .001*
	Training	44.0	0.0200	153	0.023*
IgA – 30 min after test, May	Control	43.9	14.6800	107	0.013*
	Training	47.7	0.0200	154	< .001*

Table 10. *Descriptive statistics and normality test for IgA by group*

The analysis showed that salivary IgA levels varied substantially between the two groups, with the control group demonstrating higher values at almost all time points, except for the morning and 30-minute measurements in May, where the training group showed a slight increase. The highest IgA values were observed in February before the start of the program, probably reflecting the absence of induced stress and/or ongoing immune activation regardless of group (163.7 μg/mL in the control group versus 172.1 μg/mL in the training group).

After completion of the BF program (in May), there was a tendency toward lower IgA in the morning measurements and before the stress test in both groups, with levels being lower in the training group (e.g., 46.9 μg/mL versus 62.2 μg/mL before the test). This could reflect reduced immune activity or an adaptive effect after the training period (Scheffer and Latini, 2020). Following the MAST, IgA levels decreased in both groups, which is an expected response to an acute stress challenge and has also been demonstrated in several other studies (Maes et al., 1997; Laurent et al., 2015; Paribello et al., 2024). Nevertheless, 30 minutes after the test, a slight upward trend was observed in the training group (47.7 μg/mL versus 43.9 μg/mL), which may be interpreted as more rapid recovery of immune function after stress among participants who completed the BF program (Fig. 6).

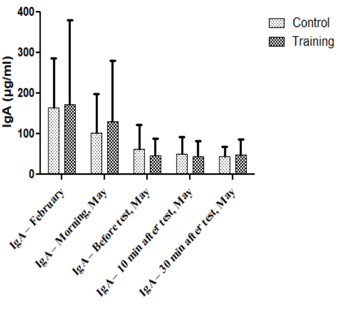

Figure 6. *Comparison of mean cortisol values between the control and training groups at the different measurement time points*

Although statistical significance was absent, certain trends were observed. For example, before the MAST in May, the mean IgA level was higher in the control group (62.2 µg/mL) than in the training group (46.9 µg/mL), and the effect measured by Cohen's d (0.297), although small to moderate, suggests potential biological relevance. A similar difference was also observed 10 minutes after the test, with the control group again showing higher values (50.6 versus 44.0 µg/mL).

The direction of the post-test changes is also of interest. Whereas cortisol exhibited an acute peak followed by recovery, IgA showed no clearly expressed dynamics, which may reflect its slower immunological reactivity to acute stressors. This is consistent with some previous studies showing that IgA is more strongly influenced by chronic stress and that its levels vary over the longer term rather than as sharply as cortisol (Takatsuji et al., 2008; Fan et al., 2009).

The low effect sizes (Cohen's d=0.016 and rank-biserial correlation=0.101) further confirm that the program did not lead to substantial changes in the immune response measured by IgA. However, more pronounced results may be observed with a longer follow-up period or a different type of intervention, as described in other studies (Díaz-Silveira et al., 2020; MacLaughlin et al., 2011; Morgan et al., 2014).

3. Analyses of Variance

Analysis of variance (ANOVA) was applied to assess changes in the parameters studied and to compare the training and control groups. This approach was selected because it allows simultaneous examination of the effects of time, group membership, and their interaction, which is particularly important in training studies with repeated measurements (Muhammad, 2023). In this way, it is possible to determine not only overall differences between groups and between measurement time points, but also whether the changes observed over time are specifically associated with the training program applied.

The same analytical approach was used for both the psychometric and laboratory parameters in order to achieve a unified and comparable statistical assessment of the program's effect.

3.1. Psychometric Parameters

3.1.1. Difference between Groups (Training vs. Control)

The levels of perceived stress (PSS), anxiety (SAS and BAI), and depressive symptoms (BDI) were assessed among participants in the training and control groups at two time points: before the start of the program (February) and after its completion (May) (Table 11).

Parameter	Time	Group	N	Mean	SD	Shapiro-Wilk p
PSS	February	Control	23	13.70	7.34	0.061
		Training	24	24.38	4.62	0.049*
	May	Control	23	15.43	8.56	0.535
		Training	24	15.83	5.33	0.713
SAS	February	Control	23	31.48	8.56	0.007*
		Training	24	47.83	9.81	0.750
	May	Control	23	32.48	9.88	0.003*
		Training	24	34.33	8.62	0.009*
BAI	February	Control	23	10.00	10.86	< .001*
		Training	24	27.17	9.65	0.090
	May	Control	23	10.91	11.11	< .001*
		Training	24	13.38	9.31	0.005*
BDI	February	Control	23	6.04	7.22	< .001*
		Training	24	23.75	8.92	0.066
	May	Control	23	8.09	9.16	< .001*
		Training	24	11.75	8.67	0.018*

Table 11. *Distribution and normality of PSS, SAS, BAI, and BDI in the control and training groups*

For the purposes of the analysis, an independent-samples t-test was applied and, where violation of the homogeneity-of-variance assumption was suspected, the Mann-Whitney U test was also used. The corresponding effect size (Cohen’s d and rank-biserial correlation) was calculated for each analysis.

The February results showed clearly expressed differences between the two groups across all psychometric parameters studied. The training group, which subsequently completed the BF training program, demonstrated significantly higher levels of perceived stress, anxiety, and depression than the control group (Table 12).

Scale	Time	t(df)	p (t-test)	Cohen’s d	p (U test)	Rank bis. corr.
PSS	February	–5.993 (45)	< 0.001*	–1.75	< 0.001*	0.7844
	May	–0.192 (45)	0.848	–0.056	0.717	0.0634
SAS	February	–6.079 (45)	< 0.001*	–1.77	< 0.001*	0.8207
	May	–0.687 (45)	0.496	–0.200	0.322	0.1703
BAI	February	–5.734 (45)	< 0.001*	–1.67	< 0.001*	0.7880
	May	–0.825 (45)	0.414	–0.240	0.114	0.2699
BDI	February	–7.462 (45)	< 0.001*	–2.18	< 0.001*	0.8768
	May	–1.408 (45)	0.166	–0.410	0.051	0.3333

Table 12. *Comparison of psychometric parameters between the training and control groups (t-test and U test)*

A highly significant difference was observed on the Perceived Stress Scale (PSS) (t(45) = –5.993, p<0.001), accompanied by a very large effect (Cohen’s d = –1.75), indicating a higher level of subjectively experienced stress in the training group (Fig. 7). Similar results were recorded on both anxiety scales: SAS (t(45) = –6.079, p<0.001, d = –1.77) and BAI (t(45) = –5.734, p<0.001, d = –1.67). The most pronounced difference was found for depression assessed using the BDI, where the training group achieved significantly higher scores (t(45) = –7.462, p<0.001) with a very large effect (Cohen’s d = –2.18). These results support the hypothesis that participants in the training group began the program with more pronounced stress.

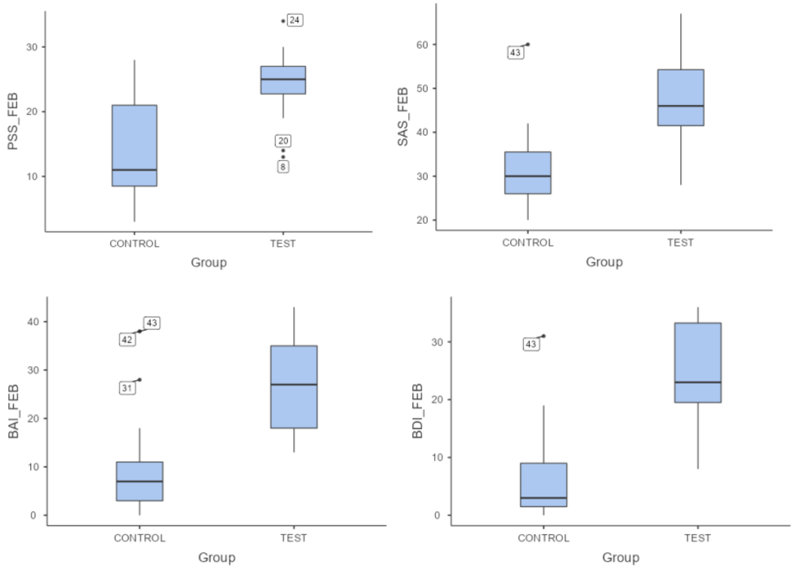

Figure 7. *Boxplot presentation of the distribution of baseline scores on the PSS, SAS, BAI, and BDI psychometric scales in February in the control and training groups, showing the median, interquartile range, overall range, and outliers*

Three months later, in May, the psychometric profiles of the two groups showed a substantial change (Fig. 8). The differences observed at the beginning of the study decreased markedly and, in most cases, lost statistical significance. On the Perceived Stress Scale (PSS), the two groups no longer differed substantially ($t(45) = -0.192$, $p=0.848$), and the effect was negligible (Cohen's $d = -0.056$). A similar trend was observed for the remaining scales: SAS ($p=0.496$), BAI ($p=0.414$), and BDI ($p=0.166$), all of which were characterized by low effect sizes. Although a tendency toward higher

BDI scores persisted in the training group, the difference did not reach statistical significance.

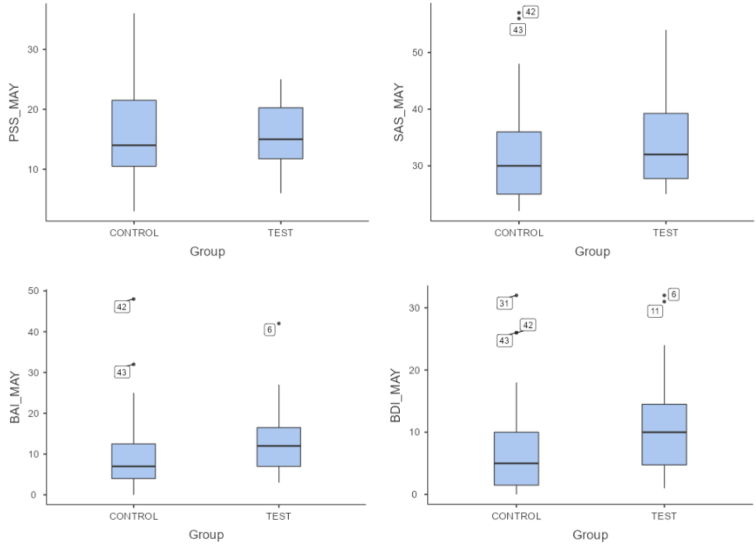

Figure 8. *Boxplot presentation of the distribution of scores on the PSS, SAS, BAI, and BDI psychometric scales in May in the control and training groups, showing the median, interquartile range, overall range, and outliers*

The observed dynamics in the results support the effectiveness of the program applied in the training group. The pronounced psychological distress characteristic of participants before the start of the program was reduced to levels comparable with those of the control group. This normalization of psychometric parameters may be interpreted as indirect evidence of the positive effect of BF training on the subjective perception of stress, anxiety, and depression. The program appears to contribute to stabilization of the emotional state and improvement of mental well-being in the population studied, supporting findings from other studies (Ratanasiripong et al., 2012; Goessl et al., 2017; Pizzoli et al., 2021).

3.1.2. Change Over Time (February → May)

3.1.2.1. PSS

To assess the effect of the BF program on perceived stress among participants in the training group, a comparative analysis of PSS results was performed (Table 13). The mean PSS score in February was 24.4, indicating a moderate-to-high level of perceived stress at the beginning of the study. After completion of the program in May, the mean score decreased significantly to 15.8, indicating substantially lower levels of perceived stress.

The paired-samples t-test showed a statistically significant difference between the two measurements ($t(23) = 6.62$, $p<0.001$). A Cohen's d value of 1.35 indicated a very large effect, meaning that the program had a strong and practically significant effect on reducing subjective stress among participants in the training group.

3.1.2.2. SAS

Anxiety levels among participants in the training group were assessed using the SAS (Table 13). In February, the mean SAS score was 47.8, suggesting a moderate-to-high level of anxiety at the initial stage of the study. In May, the mean score decreased significantly to 34.3, demonstrating a substantial reduction in anxiety after the program.

Statistical analysis using the paired-samples t-test revealed a highly significant difference between the two measurements ($t(23) = 9.13$, $p<0.001$), and the results were also confirmed by the Wilcoxon test ($W = 299$, $p<0.001$), which was appropriate for the non-normal distribution observed in the May data (Shapiro-Wilk $p=0.009$). The results statistically demonstrate that BF training was effective in reducing anxiety in the training group.

3.1.2.3. BAI

To track changes in experienced anxiety before and after the program, participants in the training group also completed the BAI questionnaire (Table 13). The mean BAI score in February was 27.2, corresponding to moderate-to-high anxiety. In May, a significant decrease to 13.4 was observed, indicating a transition to mild or absent anxiety in most participants. The substantial difference in mean scores was confirmed by the paired-samples t-test ($t(23) = 8.07$, $p<.001$) and the Wilcoxon test ($W = 300$, $p<.001$).

The substantial decrease in anxiety among participants in the training group after the program demonstrates the high effectiveness of the applied BF protocol in addressing anxiety symptoms.

3.1.2.4. BDI

To assess the effect of the program on depressive symptoms, participants in the training group completed the BDI (Table 13). The mean BDI score in February was 23.8, indicative of moderate depression. In May, it decreased to 11.8, corresponding to mild or borderline depression on the scale, with some participants showing an almost complete absence of symptoms (minimum score = 1). Statistical analysis confirmed a significant difference in both the paired-samples t-test ($t(23) = 6.28$, $p<.001$) and the nonparametric Wilcoxon test ($W=297$, $p<.001$).

The results clearly demonstrate that the BF program was highly effective in reducing depressive experiences among participants in the training group, with changes that were not only statistically significant but also clinically significant according to the magnitude of the effect.

Parameter	Measurement time	Mean	SD	t (df)	p	Wilcoxon W	Cohen’s d	Rank bis. corr.
PSS	February	24.4	4.62	6.62 (23)	< 0.001	275	1.35	0.989
	May	15.8	5.33					
SAS	February	47.8	9.81	9.13 (23)	< 0.001	299	1.86	0.990
	May	34.3	8.62					
BAI	February	27.2	9.65	8.07 (23)	< 0.001	300	1.65	1.000
	May	13.4	9.31					
BDI	February	23.8	8.92	6.28 (23)	< 0.001	297	1.28	0.980
	May	11.8	8.67					

Table 13. *Dynamics of PSS, SAS, BAI, and BDI in the training group*

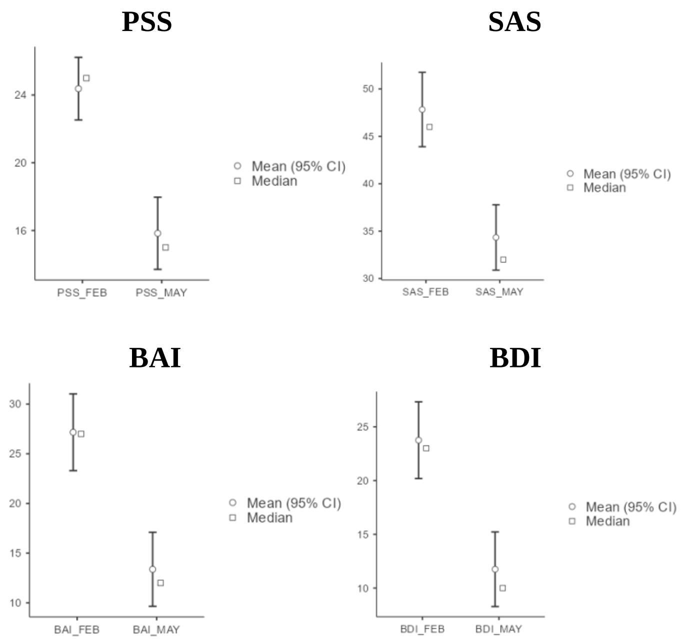

Figure 9. *Changes in the means and medians of psychometric parameters in the training (BF) group between February and May*

The results of the comparative analyses over time (February–May) showed a clearly expressed and statistically significant improvement in all psychometric parameters studied among participants in the training group (Fig. 9). The reduction in perceived stress, anxiety, and depression was not only statistically significant ($p<.001$ for all parameters) but also accompanied by exceptionally large effect sizes (Cohen's d ranging from 1.28 to 1.86), demonstrating very high practical significance of the observed differences. The results fully support previously published data from independent studies concerning the effect of BF training on the mental state of students (Ratanasiripong et al., 2012; van der Zwan et al., 2015; Goessl et al., 2017).

The analysis showed that, within the training program, participants achieved significant psycho-emotional improvement, expressed as a reduction in perceived stress, anxiety, and depressive symptoms—an effect also observed in other countries following similar studies (Ratanasiripong et al., 2012; van der Zwan et al., 2015; Munafò et al., 2016; Ravada et al., 2023; Saito et al., 2024).

In the context of the study, the data convincingly support the effectiveness of the BF program as a tool for addressing chronic stress and its psychopathological manifestations in high-stress populations such as international medical students. The results not only confirm the hypothesis regarding the beneficial effect of BF training but also emphasize its potential as a sustainable and clinically meaningful program applicable in educational and healthcare settings (Goessl et al., 2017; Castro Ribeiro et al., 2023).

3.1.3. Time-by-Group Interaction

To assess whether the program had an effect on the parameters studied in the training group compared with the control group, the interaction between time and group was analyzed using repeated-measures ANOVA with two factors: time (February → May) and group (control versus training).

3.1.3.1. PSS

Two-way ANOVA identified a statistically significant interaction between the factors ‘time’ and ‘group’ with respect to perceived stress ($F(1,45)=23.8$, $p<.001$, $\eta^2=0.136$), indicating that the change over time differed between the training and control groups and supporting the effectiveness of the applied program. Effects were also found for the factors ‘time’ ($F(1,45)=10.4$, $p=.002$, $\eta^2=0.064$) and ‘group’ ($F(1,45)=11.7$, $p=.001$, $\eta^2=0.154$), demonstrating both an overall change in stress levels between February and May and an overall difference between the two groups. Post hoc analysis showed that, at the beginning of the study, the training group had significantly higher levels of perceived stress than the control group ($p<.001$), whereas at the end of the intervention period this difference was no longer present ($p=0.997$). In addition, a significant reduction in stress levels was observed only in the training group ($p<.001$), while no substantial change was found in the control group ($p=0.657$). Despite the significant Levene’s test at the first measurement ($p=.002$), the results should be considered sufficiently robust given the similar numbers of participants in the two groups.

This pattern of stress reduction is consistent with published data indicating that BF and HRV-BF training programs lead to significant reductions in stress and anxiety, including in student populations and controlled educational settings (Ratanasiripong et al., 2012; Goessl et al., 2017; Ravada et al., 2023).

3.1.3.2. SAS

The ANOVA identified a statistically significant time-by-group interaction for anxiety levels (SAS), $F(1,45)=58.5$, $p<.001$,

η^2=0.105, indicating that the change between February and May differed between the training and control groups and supporting the effectiveness of the applied program. A significant main effect of time was also found, $F(1,45)=43.5$, $p<.001$, $\eta^2=0.078$, as well as a significant main effect of group, $F(1,45)=13.0$, $p<.001$, $\eta^2=0.165$, demonstrating both an overall change in anxiety over time and an overall difference between the two groups. Assumption testing found no violations of homogeneity of variance at either the first (Levene's $p=.283$) or the second measurement (Levene's $p=.788$), supporting the reliability of the analysis. Post hoc comparisons showed that, at the beginning of the study, the training group had significantly higher anxiety levels than the control group ($p<.001$), whereas at the end of the intervention period this difference was no longer present ($p=.902$). In addition, a significant reduction in anxiety was observed only in the training group ($p<.001$), while no statistically significant change was found in the control group ($p=.881$).

These results support previously published data showing that HRV-BF training programs can reduce anxiety and related psycho-emotional stress, including among adolescents and students (Gholami Tahsini et al., 2017; Lehrer et al., 2020; Wang et al., 2024).

3.1.3.3. BAI

Consistent with the data published in the systematic review by Alneyadi et al. on BF-based programs for anxiety, the meta-analytic findings of Lehrer et al. regarding the beneficial effect of HRV-BF on mental health, and randomized studies in students reporting reduced anxiety, the present study also found a convincing effect on anxiety symptoms reported using the BAI (Gholami Tahsini et al., 2017; Lehrer et al., 2020; Alneyadi et al., 2021; Wang et al., 2024). The ANOVA showed a statistically significant time-by-group interaction, $F(1,45)=49.6$, $p<.001$, $\eta^2=0.091$, demonstrating that the change between February and May followed a different course in the training and control groups. In addition, an effect of time, $F(1,45)=38.1$, $p<.001$, and an effect of group, $F(1,45)=12.3$, $p=.001$, were found, indicating that anxiety levels changed significantly over time and also differed overall between the two groups. Levene's test found no violations of homogeneity of variance at either measurement ($p>.60$), supporting the reliability of the analysis. Post hoc comparisons showed that, at the beginning of the study, the training group had significantly higher anxiety levels than the control group ($p<.001$), whereas at the end of the program this difference was no longer present ($p=.842$). At the same time, a significant decrease over time was observed only in the training group ($p<.001$), while no statistically significant change was recorded in the control group ($p=.928$).

The results show that the applied BF program led to a substantial reduction in anxiety symptoms over the three-month period, consistent with published data from student populations (Goessl et al., 2017).

3.1.3.4. BDI

In support of meta-analytic evidence regarding the beneficial effect of HRV-BF on depressive symptoms, as well as findings from published studies in students and adults, the present study identified a marked effect of the BF training program. The ANOVA showed a statistically significant time-by-group interaction, $F(1,45)=37.4$, $p<.001$, $\eta^2=0.106$, emphasizing that the change in BDI scores between February and May followed a different course in the training and control groups and supporting the effectiveness of the intervention. A significant effect of time was also found, $F(1,45)=18.8$, $p<.001$, $\eta^2=0.053$, demonstrating an overall change in depression levels between the two measurements, along with a significant effect of group, $F(1,45)=23.4$, $p<.001$, $\eta^2=0.244$, indicating overall differences between the two groups irrespective of time. Assumption testing found no violations of homogeneity of variance ($p>0.05$), supporting the reliability of the analysis. Post hoc comparisons showed that, at the beginning of the program, the training group had significantly higher BDI scores than the control group (difference = −17.71, $p<.001$), whereas at the end of the training period this difference was no longer statistically significant ($p=0.501$). At the same time, a significant decrease over time was observed only in the training group (difference=12.00, $p<.001$), while no statistically significant change was found in the control group ($p=0.602$).

The data show that the BF program led to a substantial reduction in depressive symptoms over the three-month period. The ANOVA analyses for each psychometric instrument (PSS, SAS, BAI, and BDI) revealed a consistent pattern of significant interactions between time (before and after the program) and group (training versus control). A statistically significant 'time × group' interaction was found for all scales, demonstrating that the program's effect manifested differently in the two groups. Specifically, the training group showed a significant decrease in perceived stress, anxiety, and depressive symptoms between February and May, whereas no substantial changes were recorded in the control group.

In addition, effects of time and group were observed in all cases, strengthening the conclusions regarding the effectiveness of the applied program on the mental well-being of participants in the training group. The effect size (η^2) in all cases ranged from small to medium, which is typical of behavioral programs of a similar nature (Goyal et al., 2014; Heber et al., 2017; Dawson et al., 2020; Amanvermez et al., 2022).

3.2. Laboratory Parameters

3.2.1. Cortisol

An ANOVA was conducted to analyze changes in salivary cortisol levels between two time points (February and May) among participants in the training and control groups.

No statistically significant effect of time was found, indicating no significant change in cortisol levels among all participants over the February–May period (p=.662). The effect of the 'group' factor was also not significant, suggesting no substantial overall difference between the training and control groups (p=.550).

No statistically significant time-by-group interaction was found either (p=0.656), indicating that the change in the 'time' factor did not differ between the two groups and, accordingly, that no conclusion could be drawn that the program affected cortisol levels. Levene's test results demonstrated no significant differences in variability between the groups in February (p=0.192) or May (p=0.696), confirming homogeneity of variance and thus supporting the reliability of the analysis.

Post hoc analysis found no significant differences between the groups at either time point or in the dynamics of change over time within the groups. All p-values were above 0.80, further supporting the conclusion that there were no statistically significant effects (Fig. 10).

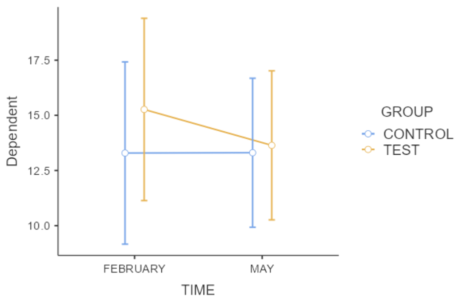

Figure 10. *Time-by-group interaction for cortisol*

The data obtained support the conclusion that, although the program was effective with respect to the psychometric parameters, it did not lead to a statistically significant change in salivary cortisol levels by the end of the study period. The results are consistent with evidence that BF and related programs improve subjective stress and mental well-being without producing clear between-group differences in cortisol (Hunter et al., 2019; Brinkmann et al., 2020; Pratap et al., 2020).

At the same time, other studies have reported a significant reduction in salivary cortisol after BF or other relaxation programs, indicating that the effect on the HPA axis is not uniform (MacLaughlin et al., 2011; Kotozaki et al., 2014; Makaracı et al., 2023; Špiljak et al., 2024). The differences reported across studies are likely attributable to the duration and type of training program, population characteristics, the stress context, and the method of cortisol measurement. In this context, the present study's data should be interpreted cautiously, as they indicate that the study design did not reveal a statistically reliable change in the cortisol response rather than demonstrating that the intervention lacked a psychophysiological effect.

3.2.2. IgA

An ANOVA was also conducted to analyze changes in salivary IgA levels between two time points (February and May) among participants in the training and control groups.

A significant effect of the 'time' factor was observed ($p=.041$), indicating a statistically significant change in IgA levels over time among all participants. This suggests an overall trend of change in IgA levels between February and May, which has also been found in other student populations (Otsuki et al., 2004; Lester et al., 2010).

The absence of a statistically significant time-by-group interaction (p=.702) means that the two groups did not show different dynamics over time. The between-group comparison likewise showed no significant differences in IgA levels, suggesting no overall systematic difference between the two groups regardless of measurement time (Fig. 11).

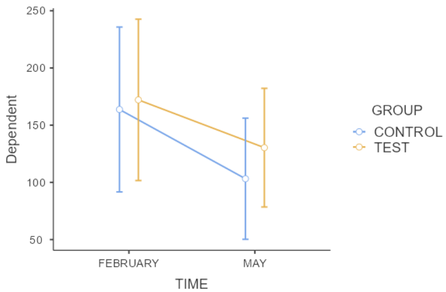

Figure 11. *Time-by-group interaction for IgA*

Analysis of the comparative tests also supports this conclusion. None of the multiple comparisons between different time points and groups reached statistical significance (all p-values>0.3), confirming the absence of a time-by-group interaction and the absence of a program-specific effect on IgA levels.

Although a change in IgA levels over time was identified, the absence of a significant time-by-group interaction does not allow this dynamic to be interpreted as a specific effect of the BF program. A similar pattern has been described in other intervention studies in which changes in psychological parameters or general temporal dynamics were observed without a specific between-group effect on mucosal IgA (Berger & O’Brien, 1998; Díaz-Silveira et al., 2020; MacLaughlin et al., 2011). This suggests that the recorded IgA changes more likely reflect the influence of nonspecific factors related to the academic context, measurement timing, and the natural variability of the marker than a direct effect of the BF training program (Jemmott et al., 1983; Seizer et al., 2024).

4. Longitudinal Analyses

Longitudinal analyses are an approach to the statistical evaluation of data collected from the same participants at two or more consecutive time points. Their principal advantage is that they make it possible to track the dynamics of the parameters studied over time and to determine whether significant changes occur within the study groups (Caruana et al., 2015).

The use of longitudinal analyses makes it possible to assess not only the presence of changes in psychometric, physiological, and laboratory parameters, but also whether these changes are associated with the effect of the applied training program, the influence of time, or the interaction between the two factors. This enables a more in-depth and methodologically grounded clarification of the effects of the BF program on the parameters studied.

4.1. Linear Trend in HRV over Time

Heart rate variability (HRV) is a sensitive marker of the ANS that reflects the balance between sympathetic and parasympathetic activity. Parameters such as SDNN, RMSSD, mean heart rate (MHR), mean heart rate range (MHRR), normalized coherence, and mean inter-beat interval (IBI) provide valuable information about the physiological response to stress and the effectiveness of interventions such as HRV-BF (Lehrer and Gevirtz, 2014).

The analysis aimed to track changes in these parameters over time and determine whether the number of completed sessions had a statistically significant effect on them.

A linear mixed model (LMM) was used in Jamovi (GAMLj module) to investigate trends over time. This approach allows modeling of the dependence between measurements from the same participant and controls for the influence of individual differences (Table 14).

Parameter	Slope (b)	SE	95% CI	p	R^2 Marginal	R^2 Conditional	ICC
Normalized Coherence	0.0095	0.075	[-0.137; 0.156]	0.900	0.000019	0.359	0.350
MHRR	0.213	0.171	[-0.121; 0.546]	0.225	0.00188	0.450	0.441
Mean Heart Rate	0.157	0.147	[-0.131; 0.445]	0.287	0.0009	0.189	0.182
SDNN	0.0095	0.075	[-0.137; 0.156]	0.900	0.000019	0.359	0.350
RMSSD	0.666	0.564	[-0.451; 1.780]	0.255	0.00271	0.34958	0.326
Mean IBI	0.666	0.564	[-0.451; 1.780]	0.255	0.00271	0.34958	0.326

Table 14. *Results of the linear mixed models for the dynamics of HRV parameters over time*

4.1.1. Normalized Coherence

In the context of HRV, coherence refers to the degree of consistency, synchronization, and rhythmic organization of fluctuations in heart rhythm, with higher coherence reflecting a more stable and regular heart-rhythm pattern (McCraty & Shaffer, 2015).

Normalized coherence is a frequency-domain parameter that reflects the relative proportion of power around the most prominent peak within the coherence range in relation to total spectral power. Analysis of normalized coherence did not identify a statistically significant effect of the ‘session’ factor ($F(1,21.3)=0.0161$, $p=0.900$), meaning that, at the specified level of statistical significance and with the available data, it cannot be concluded with high confidence that the observed change differed from random variation (Table 14 & Fig. 12). Similar findings have been reported in other studies conducted over the past five years (Timofejeva et al., 2021; Savulescu-Fiedler et al., 2025; Olarza et al., 2026).

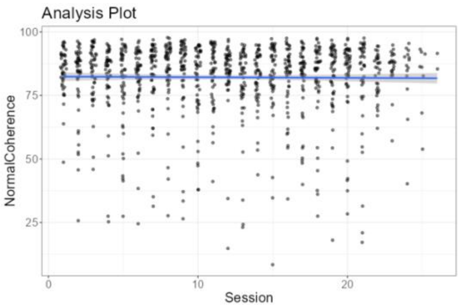

Figure 12. *Dynamics of normalized coherence across sessions with a regression trend line*

Nevertheless, graphical examination of the data revealed a clear positive trend in the mean normalized coherence values over time (Fig. 13). As shown in the figure, the slope of the trend line is positive (0.011), and the points representing the mean values for each session outline a smooth, nearly linear increase from approximately 0.50 at the beginning of the period to approximately 0.76 at the end. This indicates a gradual improvement in normalized coherence among the participants, although it did not reach statistical significance.

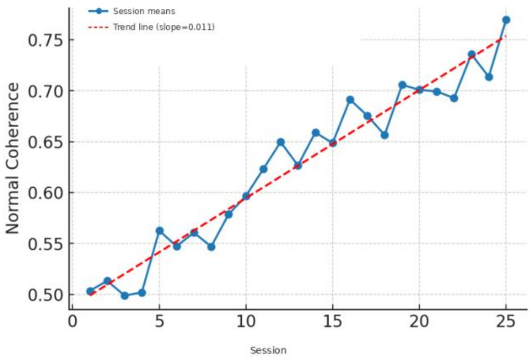

Figure 13. *Trend in mean normalized coherence values over the course of consecutive sessions*

The discrepancy between the visual trend and the statistical-test results may be explained by several factors: (1) a relatively small number of participants and sessions in relation to the variability in the data; (2) individual differences in response to the program, which increase variance; and (3) possible nonlinear effects not captured by the linear model.

However, from a practical perspective the visual trend is meaningful because it shows a sustained pattern of improvement that may become statistically significant in a larger sample or over a longer follow-up period. The combined use of statistical tests and graphical analysis is particularly important when interpreting data in psychophysiological research, where some observed changes may be practically or clinically meaningful without necessarily reaching statistical significance.

4.1.2. Mean Heart Rate Range (MHRR)

Mean heart rate range (MHRR) is a time-domain heart rate variability parameter expressed in beats per minute and calculated as the mean difference between the maximum and minimum heart rate within each respiratory cycle. In other words, it reflects the amplitude of acceleration and deceleration of heart rhythm during breathing (Shaffer & Ginsberg, 2017). Analysis of MHRR using a linear mixed model showed that the effect of the ‘session’ factor was not statistically significant ($F(1, 20.3)=1.56$, $p=0.225$) (Table 14 & Fig. 14).

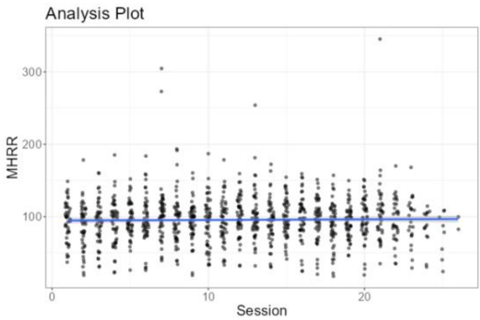

Figure 14. *Dynamics of MHRR across sessions with a regression trend line*

Nevertheless, the visual presentation of the data revealed a clearly expressed positive trend over time (Fig. 15). The slope of the regression line was positive, and the mean MHRR values gradually increased from the initial sessions toward the end of follow-up. This trajectory suggests that participants may have developed a wider heart-rate range in response to the program, consistent with the physiological hypothesis of improved variability and adaptability of the cardiovascular system during prolonged HRV biofeedback. Similar results have also been observed in other studies of BF (Dolgilevica et al., 2025; Firth et al., 2022; Six Dijkstra et al., 2023).

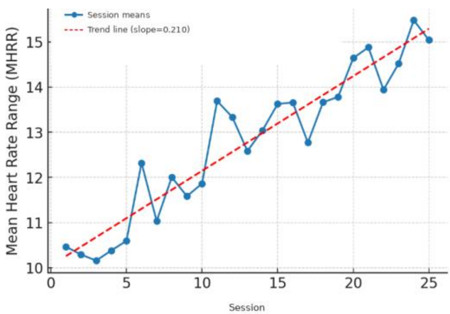

Figure 15. *Trend in mean MHRR values over the course of consecutive sessions*

The discrepancy between the visually positive trend and the absence of statistical significance may be explained by several factors: (1) the sample size, which is relatively small for this type of analysis; (2) high individual variability in MHRR, also reflected in the relatively wide confidence intervals; and (3) the possible presence of nonlinear effects that a linear model cannot capture.

The practical interpretation of the results suggests that the positive trend in MHRR toward physiological improvement may attain statistical significance in a larger sample or over a longer follow-up period. This is relevant to clinical applicability because a greater heart-rate range is an indicator of better autonomic regulation and adaptive capacity of the body.

4.1.3. Standard Deviation of NN Intervals (SDNN)

SDNN is one of the principal time-domain HRV parameters and reflects overall autonomic regulation of heart rhythm (Shaffer & Ginsberg, 2017). This parameter was analyzed using a linear mixed model to assess whether overall HRV changed among participants over the course of the sessions (Table 14). The study found no statistically significant effect of the 'session' factor on this parameter (F(1, 21.3)=0.0161, p=0.900) (Fig. 16).

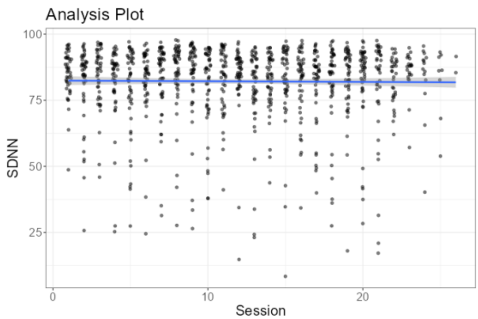

Figure 16. *Dynamics of SDNN across sessions with a regression trend line*

Nevertheless, the graphical presentation of the data revealed a weak positive trend in SDNN over time, although with a minimal slope (Fig. 17). The mean values across sessions remained relatively stable, without sharply expressed fluctuations, indicating maintenance of a similar level of overall heart rate variability during the program. A similar effect has been observed in a number of other studies (Brinkmann et al., 2020; Firth et al., 2022; Meeuwsen et al., 2021).

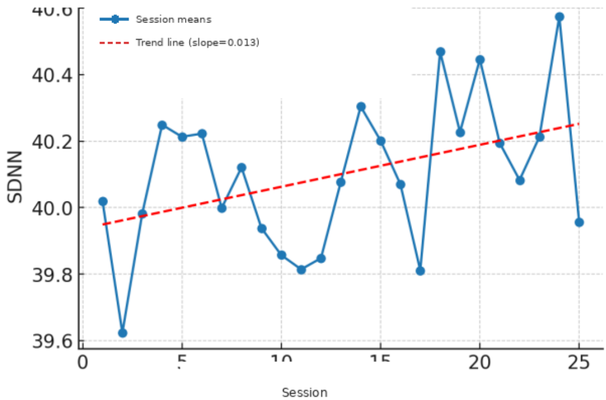

Figure 17. *Trend in mean SDNN values over the course of consecutive sessions*

SDNN is an integrative parameter reflecting both sympathetic and parasympathetic activity and is strongly influenced by recording duration and the overall state of the ANS. The absence of a significant change may be attributable to several factors: (1) the effect of the training program on this parameter may require a longer period before a change becomes observable; (2) individual differences between participants were relatively large, increasing the standard error; and (3) SDNN may be less sensitive to short-term adaptive changes than more specific HRV parameters such as RMSSD.

Despite the absence of statistical significance, the maintenance of stable SDNN values throughout the follow-up period may be interpreted positively because no reduction in variability was observed—a phenomenon often associated with chronic stress and impaired autonomic regulation. In this context, the stability of SDNN may be regarded as an indicator of preserved autonomic balance during the BF program.

4.1.4. Root Mean Square of Successive Differences (RMSSD)

RMSSD is one of the most frequently used time-domain HRV parameters and is regarded as a sensitive marker of parasympathetic activity (Shaffer & Ginsberg, 2017). Analysis of this parameter using a linear mixed model aimed to determine whether vagal regulation of heart rhythm changed among participants over the course of the sessions (Table 14). No statistically significant effect of the 'session' factor on this parameter was found (F(1, 22.6)=1.36, p=0.255) (Fig. 18), but the graphical presentation of the results showed a weak positive trend over time, with mean RMSSD values gradually increasing (Fig. 19).

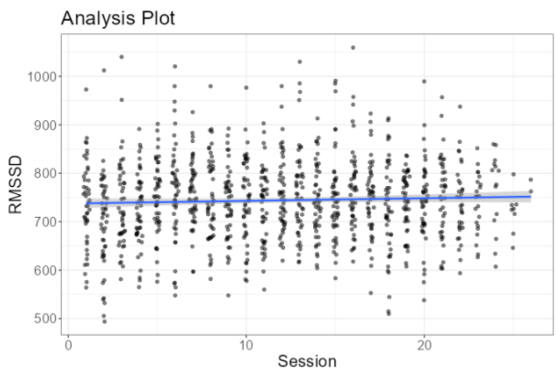

Figure 18. *Dynamics of RMSSD across sessions with a regression trend line*

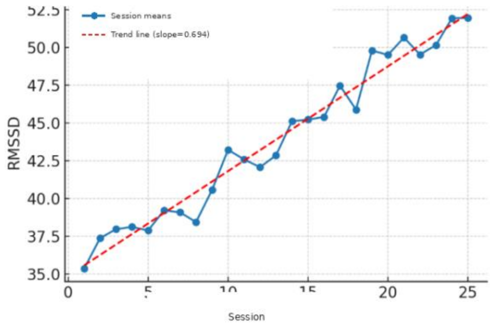

Figure 19. *Trend in mean RMSSD values over the course of consecutive sessions*

It is possible that some participants experienced improvement in parasympathetic activity and in the capacity for rapid adaptation of heart rate, although the effect was not strong enough to reach statistical significance within the sample.

RMSSD is a sensitive parameter reflecting short-term fluctuations in heart rhythm associated primarily with vagal regulation. A number of authors use it as a principal indicator of recovery processes and relaxation effects in BF training programs (DeGiorgio et al., 2010; Grässler et al., 2021; Zeng et al., 2023). In this context, the observed upward trend may be regarded as a possible positive effect of BF on the ANS that could become more evident with longer follow-up or a larger sample.

4.1.5. Mean Heart Rate (MHR)

Mean heart rate (MHR) is a basic physiological parameter reflecting the overall level of cardiac activation and may indirectly reflect changes in autonomic regulation during the training program (Shaffer & Ginsberg, 2017). A linear mixed model was applied to determine whether a systematic change in this parameter occurred over the course of consecutive sessions (Table 14). The analysis found no statistically significant effect of the 'session' factor on MHR (F(1, 21.1)=1.17, p=0.287) (Fig. 20).

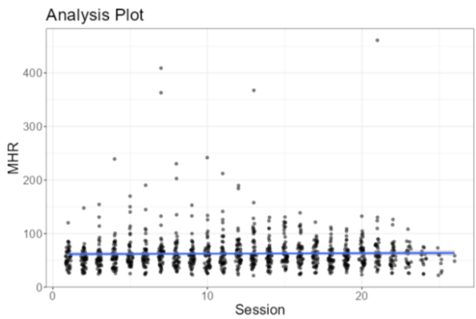

Figure 20. *Dynamics of MHR across sessions with a regression trend line*

The graphical presentation of the results showed a slight upward trend in mean MHR values across sessions, but the slope of the trend line was relatively small and fluctuations between individual sessions remained within a narrow range (Fig. 21). This indicates relative stability in mean heart rate throughout the entire BF training-program period.

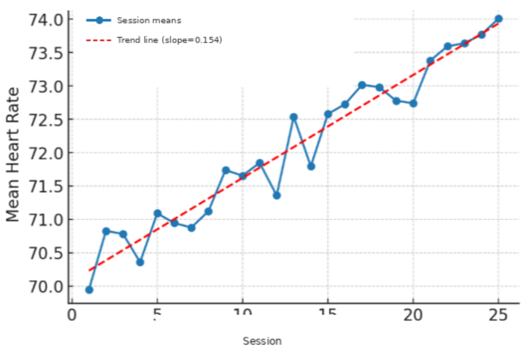

Figure 21. *Trend in mean MHR values over the course of consecutive sessions*

The stability of MHR may be interpreted as preservation of cardiovascular homeostasis, without abrupt or sustained changes in the baseline heart-rate level. The observed upward trend could be associated with gradually increasing engagement in the training, increased arousal, or adaptive responses, but these effects remained insufficiently pronounced to reach statistical significance. A similar pattern has been described in other studies of HRV-BF. For example, Nashiro and colleagues reported that, within a five-week randomized intervention, MHR during training remained similar across conditions despite clear differences in cardiac oscillations and some HRV parameters. This supports the idea that BF may influence regulatory mechanisms without producing pronounced changes in baseline heart rate (Nashiro et al., 2022). In the same direction, Lehrer noted in a systematic review and meta-analysis of HRV-BF that improvements in respiratory sinus arrhythmia and baroreflex regulation are not necessarily accompanied by a substantial change in mean heart rate, which is compatible with the interpretation of preserved cardiovascular stability (Lehrer et al., 2020).

4.1.6. Mean Inter-Beat Interval (IBI)

IBI is a time-domain parameter reflecting the mean duration between consecutive heartbeats and is inversely related to heart rate (Shaffer & Ginsberg, 2017). A linear mixed model was applied to assess possible changes in this parameter over the course of the intervention and found no statistically significant effect of the ‘session’ factor ($F(1, 22.6)=1.36$, $p=0.255$) (Table 14 and Fig. 22).

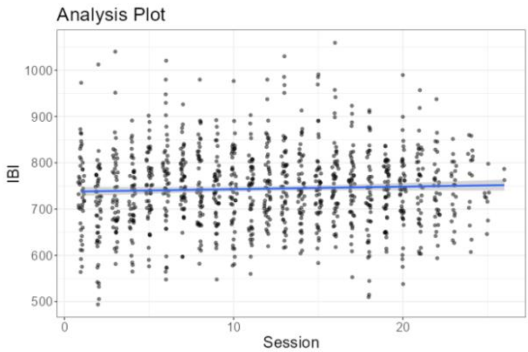

Figure 22. *Dynamics of IBI across sessions with a regression trend line*

The graphical presentation of the results showed a weak upward trend, with mean IBI values gradually increasing over the course of the sessions (Fig. 23). This pattern suggests a slight lengthening of the interval between heartbeats, which may be interpreted as a sign of increased parasympathetic tone and more efficient cardiac function in some participants.

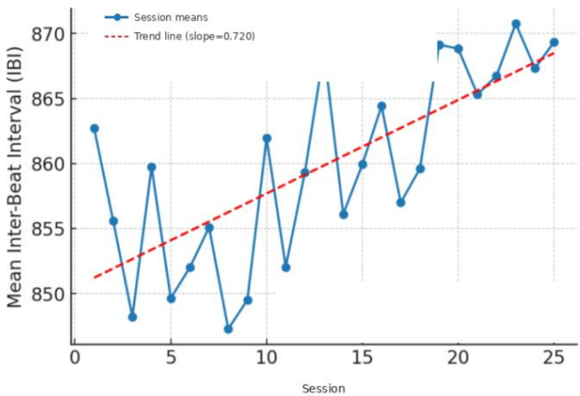

Figure 23. *Trend in mean IBI values over the course of consecutive sessions*

Higher IBI values are associated with a lower mean heart rate and are often regarded as an indicator of better autonomic regulation and cardiovascular adaptation (McCraty & Shaffer, 2015). Nevertheless, the trend identified in the study was too weak and statistically nonsignificant to permit definitive conclusions.

In the context of the scientific literature, these results are comparable with other studies in which short-term HRV-BF training programs did not produce statistically significant group-level changes in the principal HRV parameters, although trends toward stabilization or improvement were observed in participant subgroups and among individuals more engaged with the program (Firth et al., 2022; Hirten et al., 2024; Meeuwsen et al., 2021). This supports the hypothesis that more intensive or longer protocols, as well as better control of adherence to training, could reveal more clearly expressed positive effects.

From a practical perspective, the relative stability of HRV parameters in a sample at high risk of stress may be regarded as a potentially favorable outcome, insofar as chronic psychosocial stress is usually associated with reduced HRV and signs of autonomic dysregulation (Kim et al., 2018; de Vries et al., 2022; Benítez-Agudelo et al., 2025). Accordingly, preservation or a slight increase in these parameters, although statistically nonsignificant, may have practical value.

4.2. Nonlinear Trend (Quadratic Component)

To assess the effect of the 11-week BF program on the participants' physiological parameters, linear mixed models with an added quadratic component (Session and Session^2) were applied. This approach makes it possible to investigate not only the presence of a linear trend (a gradual increase or decrease in parameters over time), but also nonlinear changes associated with a potential plateau or a change in the direction of the dynamics during training.

Indicator	Linear effect (b, SE, p)	Quadratic effect (b, SE, p)	$F(df_1, df_2)$, p – linear	$F(df_1, df_2)$, p – quadratic	R^2 marginal	R^2 conditional	Summary of trend	Approximate peak/plateau*
Normalized Coherence	−0.298 (0.224), p=0.184	+0.0129 (0.0089), p=0.145	F(1,608)=1.77, p=0.184	F(1,1001)=2.12, p=0.145	0.00141	0.36059	No convincing linear/ quadratic effect	≈ session 12 (n.s.)
SDNN	−0.298 (0.224), p=0.184	+0.0129 (0.0089), p=0.145	F(1,608)=1.77, p=0.184	F(1,1001)=2.12, p=0.145	0.00141	0.36059	No significant changes	≈ session 12 (n.s.)
MHRR	+0.992 (0.473), p=0.036*	−0.0321 (0.0186), p=0.078	F(1,547)=4.40, p=0.036*	F(1,1009)=3.12, p=0.078	0.00340	0.45110	Significant linear increase, with indication of a "plateau"	peak ≈ sessions 15–16
Mean HR	+1.707 (0.572), p=0.003*	−0.0647 (0.0230), p=0.005*	F(1,1046)=8.90, p=0.003*	F(1,1032)=7.88, p=0.005*	0.00688	0.19370	Significant increase, followed by slowing/decline	peak ≈ session 13
RMSSD	+4.288 (1.374), p=0.002*	−0.153 (0.053), p=0.004*	F(1,464)=9.75, p=0.002*	F(1,1033)=8.36, p=0.004*	0.00773	0.35453	Significant increase, reaching a plateau	peak ≈ session 14
Mean IBI	+4.288 (1.374), p=0.002*	−0.153 (0.053), p=0.004*	F(1,464)=9.75, p=0.002*	F(1,1033)=8.36, p=0.004*	0.00773	0.35453	Significant lengthening, reaching a plateau	peak ≈ session 14

Table 15. Linear and quadratic time effects in the dynamics of the HRV indicators

4.2.1. Detailed Interpretation by Parameter

No clearly expressed or statistically confirmed change in normalized coherence was found over the course of the program. Visually, a tendency toward slight improvement in coherence may be observed in some participants, but this change was not sufficiently consistent at the group level. This means that the number of completed sessions alone did not lead to a sustained increase in cardiac coherence. The values of this parameter are more likely to be influenced to a greater extent by individual characteristics of autonomic regulation, baseline coherence, and differences in how participants master breathing and self-regulation techniques. Therefore, in the sample used, normalized coherence remained a relatively stable parameter, without convincing evidence of a cumulative effect of the training sessions on it.

SDNN showed an almost identical pattern: no significant linear or quadratic effects and a low marginal R^2, with a substantial ICC ≈ 0.35. As a global indicator, SDNN is often more inert and more sensitive to recording duration and conditions. In the context of the study, it may be noted that the three-month period was most likely insufficient to induce clearly expressed group-level changes in overall variability.

For **MHRR**, the model with a quadratic component identified a statistically significant positive linear effect over time and a tendency toward a negative quadratic component ($p=0.078$). This means that the heart-rate range widened as the sessions progressed, but the rate of widening gradually slowed and reached a ‘plateau’ around sessions 15–16. In practical terms, this model may be interpreted as an early increase in the parameter followed by leveling off of the change in the later sessions.

MHR followed a classic nonlinear, downward-concave profile: it increased during the early sessions (significant linear effect), but the negative quadratic component indicated slowing and a subsequent slight decrease, with a calculated peak around session 13. Such a biphasic response is typical of adaptation: initial activation/toning followed by stabilization of the cardiovascular response as breathing and regulation techniques are mastered.

For **RMSSD**, a statistically significant positive linear effect and a significant negative quadratic component were observed. This outlines a pattern of an initial increase in values, followed by slowing of the change and a tendency toward stabilization around session 14. In interpretive terms, this result reflects a more pronounced early adaptation to training followed by establishment of a relatively stable level.

Mean IBI replicated the RMSSD pattern: a significant linear increase and a significant negative quadratic effect, meaning that the interval between heartbeats lengthened initially (indirectly indicating a reduction in MHR) and stabilized around session 14. This interpretation supports the proposition of increased parasympathetic tone and more optimized cardiorespiratory dynamics after mastery of the training techniques.

4.2.2. Overall Conclusions Regarding Program Dynamics

When the parameters are considered together, a coherent biphasic trajectory of adaptation emerges: during the first 14–16 sessions, there was a clear improvement in indicators related to vagal control and cardiac adaptability (RMSSD, IBI, and MHRR—positive trends; MHR—a temporary increase followed by normalization), after which the process stabilized (significant negative quadratic components for MHR, RMSSD, and IBI; a tendency for MHRR). The absence of significant changes in SDNN and normalized coherence suggests that overall variability and coherence did not show a cumulative effect within the training period. Their stability in a high-stress group sample may be interpreted positively—there was no progressive deterioration.

The variance components showed substantial interindividual differences (ICC 0.19–0.45), particularly pronounced for MHRR, RMSSD, and IBI. The results clearly indicate that participants differed both in their baseline autonomic profile and in the extent to which their cardiovascular regulation adapted over the course of the training program. Individual characteristics of autonomic regulation should therefore be taken into account when planning and interpreting the effects of the BF program.

The coherent biphasic trajectory of adaptation supports contemporary conceptual models of heart rate variability adaptation. The systematic review by Wareing et al. proposes a staged model according to which, in the early phase of a training program, breathing exercises initially enhance oscillatory coordination between physiological systems and lead to an overall increase in indices such as SDNN, whereas at a later stage the effect shifts toward more sustained regulation and stabilization of the response (Wareing et al., 2024). The pattern observed in the present study—early improvement in parameters related to vagal control and cardiac adaptability, followed by stabilization—is physiologically plausible and corresponds to the logic of staged autonomic adaptation described in the literature.

At the same time, published intervention studies most often rely on comparisons between initial and final HRV values or on a limited number of time-point measurements, making it difficult to capture the finer internal course of change. Ratajczak et al. emphasize that studies of HRV-BF are often limited by methodological difficulties, including insufficient assessment of training fidelity, and show that comparison of data only before, after 10 sessions, and after 20 sessions reveals a dose–response relationship for the training program (Ratajczak et al., 2021). The systematic analysis by Balaji and colleagues, based on approximately 1.8 million HRV-BF sessions, demonstrated that parameters such as

IBI, SDNN, and RMSSD vary systematically according to the level of coherence during training. The findings support the idea that session-by-session dynamics contain important physiological information that cannot be reduced solely to comparisons between initial and final values (Balaji et al., 2025).

Against this background, the present study examines adaptation not only as a difference between two time points, but as a process unfolding over consecutive sessions and modeled using linear mixed models for several HRV parameters. According to the available literature, this combination of multisession analysis, focus on temporal trajectory, inclusion of both classical HRV parameters (SDNN, RMSSD, IBI), heart-rate and coherence parameters, and application in a group of international medical students with high stress levels is exceptionally rare. This is precisely where the study makes its principal contribution: the effect of the training program is evaluated by tracking the manner in which autonomic adaptation develops, reaches a more pronounced physiological expression, and subsequently stabilizes over the course of consecutive BF sessions.

5. Multivariate and Factor Analyses

Multivariate and factor analyses are statistical approaches through which the relationships among multiple variables are examined simultaneously (Hazra and Gogtay, 2017). They were used to identify possible grouping of individual psychometric, physiological, and behavioral parameters into common factors and to distinguish those making the most substantial contribution to explaining the observed differences and changes.

5.1. PSS

Analysis of covariance (ANCOVA) was used to assess the effect of the training program on perceived stress (PSS). Membership in the respective group (training versus control) and sex were entered into the model as independent factors, while age and PSS values recorded in February were entered as covariates.

The overall model explained 17.7% of the variance in PSS scores in May (Table 16). A significant predictor of these final scores was the baseline PSS score recorded in February, indicating that the initial level of perceived stress had a substantial influence on the post-program results. The effect of group membership was borderline significant, with mean values showing lower scores in the training group (M = 13.26, 95% CI = 9.99–16.53) than in the control group (M = 18.12, 95% CI = 14.76–21.48). Sex and age showed no significant influence.

Effect	F(1,42)	p	partial η^2	95% CI (η^2)
Group	3.36	0.074	0.074	0.000 – 0.250
Sex	0.30	0.586	0.007	0.000 – 0.123
Age	0.17	0.683	0.004	0.000 – 0.109
PSS – February	7.61	0.009*	0.153	0.011 – 0.342
Model (overall)	F(4,42) = 2.26, p = 0.079, R^2 = 0.177			

Table 16. *ANCOVA of factors associated with perceived stress in May*

To determine whether the effect of the training program depended on baseline stress levels, the group × PSS–February interaction was added (Table 17). The new model explained 19.1% of the variance, with the February PSS score again remaining the only significant predictor.

Effect	F(1,41)	p	partial η^2	95% CI (η^2)
Group	0.05	0.823	0.001	0.000 – 0.085
PSS – February	4.97	0.031*	0.109	0.000 – 0.294
Group × PSS – February (interaction)	0.70	0.407	0.016	0.000 – 0.154
Model (overall)	F(5,41) = 1.94, p = 0.109, R^2 = 0.191			

Table 17. *ANCOVA of factors associated with perceived stress in May, including the interaction between group and baseline PSS scores*

The perceived-stress (PSS) results show that the strongest factor determining stress levels at the end of the study was the baseline level measured in February. The higher the initial scores, the higher the final scores remained. Sex and age had no substantial influence.

With respect to the effect of the BF program, the results indicate a moderate impact, with mean perceived-stress scores being lower in the training group than in the control group. Perceived stress proved relatively stable over time, and the program showed a promising but uncertain effect on its reduction. Such an effect has also been observed in other studies reporting a similar positive trend (Hernandez et al., 2019; van der Zwan et al., 2019; Lehrer et al., 2020; Benítez-Agudelo et al., 2025).

5.2. SAS

ANCOVA was performed to assess the effect of the BF program on anxiety measured using the SAS. Group membership and sex were entered into the model as independent factors, while age and February SAS scores were entered as covariates.

The overall model explained 57.7% of the variance in SAS scores in May (Table 18). The strongest predictor was the baseline anxiety level, demonstrating the high stability of the parameter over time. The effect of group membership was statistically significant and large, whereas sex and age did not demonstrate a similar effect.

The mean values show that the training group (M=28.03, 95% CI = 24.89–31.18) had significantly lower anxiety levels at the end of the program than the control group (M=39.05, 95% CI = 35.81–42.29). This provides evidence that the BF program had a substantial positive impact on reducing anxiety among participants.

Effect	F(1,42)	p	partial η^2	95% CI (η^2)
Group	18.20	<0.001*	0.303	0.089–0.483
Sex	0.09	0.764	0.002	0.000–0.095
Age	0.56	0.456	0.013	0.000–0.143
SAS – February	54.46	<0.001*	0.565	0.348–0.689
Model (overall)	$F(4,42) = 14.33$, $p < .001$*, $R^2 = 0.577$			

Table 18. *ANCOVA of factors associated with anxiety levels in May, assessed using the SAS*

The SAS data analysis showed that anxiety was relatively stable over time, with values recorded immediately before the beginning of the training program being the strongest predictor of the final results. Similar persistence of anxiety has been described in a number of longitudinal studies, including studies of medical students. The data support the understanding that baseline assessment of mental state is a key determinant of later anxiety levels (Carmel and Bernstein, 1989; Stanislawski et al., 2023).

At the same time, the BF program had a clearly expressed effect, with participants showing lower anxiety levels in May than the control group. This result confirms meta-analytic evidence demonstrating that HRV-BF leads to a significant reduction in anxiety and stress, as well as randomized studies in student populations in which BF training likewise reduced anxiety compared with control groups (Ratanasiripong et al., 2012; Lee et al., 2015; Goessl et al., 2017).

5.3. BAI

ANCOVA was performed to assess the effect of the BF program on anxiety measured using the BAI. Group membership (training versus control) and sex were entered into the model as independent factors, while age and BAI values recorded immediately before the study were entered as covariates.

The overall model explained 58.3% of the variance in BAI scores in May (Table 19). The two strongest predictors were group membership and anxiety scores immediately before the start of the program. The effect of group membership was statistically significant, demonstrating the importance of the training program in achieving the final outcomes. February BAI scores were also an exceptionally strong predictor, whereas sex and age showed no substantial influence. The mean values demonstrated a clear distinction between the two groups: the training group had significantly lower anxiety levels (M = 7.01, 95% CI = 3.64–10.38) than the control group (M = 17.56, 95% CI = 14.09–21.02).

Effect	F(1,42)	p	partial η^2	95% CI (η^2)
Group	14.98	<0.001*	0.263	0.063–0.448
Sex	0.46	0.499	0.010	0.000–0.136
Age	0.00	0.954	0.000	0.000–0.026
BAI – February	55.86	<0.001*	0.571	0.355–0.693
Model (overall)	F(4,42) = 14.69, p < .001, R^2 = 0.583			

Table 19. *ANCOVA of factors associated with anxiety levels in May, assessed using the BAI*

The BAI analysis showed that the BF program had a clearly expressed and statistically significant effect on anxiety. Participants demonstrated significantly lower anxiety levels than the control group. The effect size was large, indicating high effectiveness of BF training in reducing anxiety symptoms. The results support meta-analytic data according to which HRV-BF is associated with a significant and often clinically relevant reduction in anxiety symptoms (Goessl et al., 2017). Similar effects have also been described in other studies of student populations, where BF training led to a more pronounced reduction in anxiety than control conditions, including among nursing students, medical students, and students with high stress levels during periods of increased academic workload (Ratanasiripong et al., 2012; Gholami Tahsini et al., 2017; Ravada et al., 2023). The data support the conclusion that BF training may be an effective approach to reducing anxiety in young, high-stress academic populations.

5.4. BDI

ANCOVA was performed to assess the effect of the BF program on depressive symptoms measured using the BDI. Group membership (training or control) and sex were entered into the model as independent factors, while age and BDI scores recorded in February were entered as covariates.

The overall model explained 40.1% of the variance in BDI scores in May (Table 20). The two strongest predictors were group membership and BDI scores before the start of the training program. Here again, sex and age made no significant contribution. The mean values clearly showed that the training group had significantly lower BDI scores (M = 5.93, 95% CI = 2.05–9.81) than the control group (M = 14.16, 95% CI = 10.16–18.15).

Effect	F(1,42)	p	partial η^2	95% CI (η^2)
Group	6.34	0.016*	0.131	0.004–0.319
Sex	2.28	0.139	0.051	0.000–0.216
Age	1.02	0.318	0.023	0.000–0.167
BDI – February	21.72	<0.001*	0.341	0.119–0.515
Model (overall)	F(4,42) = 7.02, p < .001, R^2 = 0.401			

Table 20. *ANCOVA of factors associated with depression levels in May, assessed using the BDI*

The BDI results support meta-analytic evidence according to which HRV-BF is associated with a statistically significant reduction in depressive symptoms (Fernández-Alvarez et al., 2021; Pizzoli et al., 2021). The same applies to a number of studies in student populations in which BF reduced depression alongside improvements in stress and anxiety (Karavidas et al., 2007; Ratanasiripong et al., 2015; M. Li et al., 2025). In a broader context, the observed predictive role of baseline BDI scores corresponds to published data showing that the severity of baseline symptoms is a strong predictor of subsequent changes in depressive symptoms, whereas demographic factors do not always make an independent and consistent contribution (Meyers et al., 2019; Wu and Liu, 2023; M. Li et al., 2025).

5.5. Other Factors

The analysis included additional covariates reflecting health status (chronic diseases, psychiatric disorders, use of medications/supplements), social and behavioral characteristics (smoking, alcohol use, financial status, parental support), and lifestyle habits (hours of study and sleep) (Table 21).

Scale	Chronic diseases	Mental health conditions	Use of medications	Smoking	Alcohol consumption	Financial status	Parental support	Studying	Sleep
PSS	F=0.51, p=0.479	F=0.01, p=0.955	F=0.34, p=0.557	F=0.11, p=0.741	F=0.20, p=0.836	F=0.42, p=0.907	F=0.49, p=0.494	F=0.67, p=0.420	F=0.00, p=0.971
SAS	F=1.54, p=0.226	F=0.03, p=0.869	F=0.02, p=0.885	F=0.08, p=0.786	F=0.07, p=0.932	F=0.06, p=0.805	F=0.34, p=0.571	F=0.01, p=0.905	F=0.04, p=0.849
BAI	F=0.07, p=0.719	F=0.42, p=0.639	F=0.82, p=0.416	F=0.22, p=0.650	F=0.69, p=0.781	F=0.42, p=0.843	F=0.36, p=0.463	F=0.12, p=0.884	F=0.17, p=0.682
BDI	F=0.16, p=0.695	F=0.61, p=0.551	F=0.00, p=0.964	F=0.90, p=0.764	F=0.20, p=0.979	F=0.60, p=0.878	F=0.01, p=0.897	F=0.26, p=0.616	F=0.65, p=0.425

Table 21. *Influence of social, health-related, and behavioral factors on psychometric parameters*

Across the four psychometric scales, the specified factors showed no statistically significant effect. The data partially support studies in which some behavioral and social covariates lose their independent effect after multivariable adjustment. For example, Ebrahim and colleagues found no association of smoking or alcohol use with stress and anxiety among medical students (Ebrahim et al., 2024). Jeong and colleagues showed that, after multivariable regression, poor sleep quality, irregular eating, and self-rated health no longer reached statistical significance despite less favorable univariable associations (Jeong et al., 2010). In this sense, the present findings support the proposition that, when multiple related covariates are included simultaneously, some may not make an independent contribution to variation in stress, anxiety, and depression.

According to the literature, financial stress, poor sleep, pre-existing mental-health difficulties, and low social/family support are frequently associated with greater psychological distress among students, and significant effects of sex, low social support, lack of academic support, and poor sleep quality have also been described among medical students (Roy et al., 2025). Specific significant effects in the same direction have also been reported in large student and medical samples. Shao and colleagues reported that a heavy financial burden, poor sleep quality, unfavorable family functioning, and low social support were significantly associated with the development and worsening of depression and anxiety (Shao et al., 2020). Ebrahim's team found that chronic diseases, younger age, and living far from family predicted higher stress (Ebrahim et al., 2024). Fawzy and colleagues identified a significant association between female sex and higher levels of depression, anxiety, and stress (Fawzy & Hamed, 2017). Therefore, the absence of a statistically significant effect in the present analysis does not exclude the potential importance of these factors; rather, it indicates that, within the sample studied and the multivariable model used, they did not emerge as independent predictors of the final psychometric outcomes.

It is important to emphasize that adding these factors did not change the main conclusions—the program retained a significant and stable effect on anxiety (SAS and BAI), whereas perceived stress and depression were influenced primarily by baseline values before the start of the training program. This supports the conclusion that the effect of the BF program on anxiety symptoms persists independently of the participants' health-related, social, and behavioral characteristics.

5.6. Principal Component Analysis of One-Minute HRV Parameters

To summarize the information from the HRV parameters and determine whether they formed common groups of interrelated parameters, principal component analysis (PCA) with Varimax rotation was performed on six HRV parameters: MHRR, MHR, SDNN, RMSSD, normalized coherence, and mean inter-beat interval.

Before performing PCA, the suitability of the data for this type of statistical analysis was assessed. Bartlett's test was statistically significant, $\chi^2(15)=8432$, $p<.001$, indicating sufficient relationships among the HRV parameters studied. The Kaiser-Meyer-Olkin (KMO) measure was 0.575, providing evidence of acceptable, although moderate, suitability of the data for PCA. The lowest value was recorded for normalized coherence (MSA = 0.433), while values for the remaining parameters ranged from 0.468 to 0.625.

Three principal components were extracted, explaining the major proportion of variance in the HRV parameters (Table 22). The first component grouped MHRR, mean IBI, and MHR in the opposite direction and may therefore be interpreted as a component reflecting overall cardiac dynamics and chronotropic regulation. Higher scores were associated with longer intervals between heartbeats, a greater heart-rate range, and a relatively lower MHR. The second component included primarily SDNN and RMSSD and reflected short-term heart-rhythm variability, associated predominantly with parasympathetic modulation. The third component was dominated by normalized coherence, indicating that coherence emerged as an independent psychophysiological dimension distinct from the classical time-domain HRV parameters.

Parameter	Component 1	Component 2	Component 3	Uniqueness
MHRR	0.894	–	–	0.047
Mean Inter-Beat Interval	0.977	–	–	0.012
MHR	–0.981	–	–	0.017
SDNN	0.453	0.877	–	0.020
RMSSD	0.349	0.836	–0.328	0.071
Normalized Coherence	–	–	0.970	0.028

Note: Component loadings below |0.30| are not presented in order to provide a clearer visualization of the principal relationships among the parameters.

Table 22. *Results of the principal component analysis of short-term HRV parameters*

The PCA results showed a clear differentiation of HRV parameters into three principal dimensions. The first component, associated with MHRR and mean IBI, reflected the temporal aspects of HRV. This is consistent with previous studies according to which HRV is based on variations in RR intervals, and the time-domain parameters derived from them reflect the overall dynamics and regulation of heart rhythm (de Almeida et al., 2022; Kleiger et al., 2005; Maki et al., 2024; Shaffer & Ginsberg, 2017; Shaffer & Meehan, 2020).

The second component, dominated by SDNN and RMSSD and negatively associated with MHR, may be interpreted as a vagally related or parasympathetically dominated dimension of HRV. This interpretation supports previously published evidence according to which RMSSD is a principal time-domain parameter of short-term vagally mediated variability, whereas SDNN reflects overall HRV and, in short recordings, also captures a substantial component of autonomic, including parasympathetic, regulation (Besson et al., 2025; Laborde et al., 2017; Shaffer & Ginsberg, 2017). The negative association with MHR further supports this interpretation because higher HRV, particularly at rest, is generally associated with a lower heart rate and more pronounced vagal control (Shaffer & Ginsberg, 2017; Shaffer & Meehan, 2020).

The most interesting result was observed for the third component, which reflected normalized coherence. The isolation of coherence as an independent latent factor suggests that this parameter captures an aspect of autonomic regulation that does not completely overlap with classical time-domain or vagally related HRV indices. This corresponds to the concept of 'physiological coherence,' which is often regarded in the literature as a relatively independent pattern of increased synchronization among heart rhythm, breathing, and other physiological rhythms. These effects are associated with a more optimal HRV profile, better self-regulation, and more stable emotional states (McCraty and Zayas, 2014; Pham et al., 2021). Additional support for this interpretation is provided by large-scale data from HRV-BF sessions, according to which the state of coherence is associated with a more organized heart-rhythm pattern, better spectral organization of HRV, and differences in participants' emotional state. This supports the understanding that coherence is an independent psychophysiological dimension reflecting the degree of synchronization among cardiac activity, breathing, and autonomic regulation (Balaji et al., 2025).

The isolation of coherence as a separate factor has important implications for interpreting the effects of BF interventions. It suggests that training influences traditional HRV parameters while also creating a specific state of coherence that may be considered an independent indicator of program effectiveness. In this sense, the results support the inclusion of coherence as a separate variable when analyzing the psychophysiological effects of BF programs.

5.7. Principal Component Analysis of Extended HRV Parameters

To summarize information from a larger number of extended HRV parameters and determine whether they formed common groups of interrelated variables, PCA with Varimax rotation was performed on the following parameters: VLF, LF, HF, total power, normalized coherence, intervals, duration, MHRR, MHR, SDNN, and RMSSD. Preliminary assessment showed that the data were suitable for this type of analysis. Bartlett's test was statistically significant, $\chi^2(55)=26{,}196$, $p<.001$, indicating sufficient relationships among the parameters studied.

Three principal components were extracted, explaining 86.3% of the total variance in the data. The first component, associated with 33.9% of the variance, included high factor loadings for VLF, LF, HF, RMSSD, recording duration, and MHRR. This factor may be interpreted as reflecting spectral and temporal variability of heart rhythm because it combines frequency-domain and classical time-domain parameters.

The second component explained 30.7% of the variance and was characterized by high loadings for SDNN, normalized coherence, and total power. It may be interpreted as an indicator of parasympathetic modulation and autonomic balance because it simultaneously includes time-domain (SDNN), integrative (total power), and coherence indices.

The third component explained 21.8% of the variance and was dominated by intervals and MHR, with SDNN also making a negative contribution. This factor may be regarded as an indicator of cardiovascular dynamics, reflecting baseline heart rate and interval fluctuations.

Factor analysis of the extended HRV parameters showed a clear differentiation of three principal dimensions: spectral and temporal variability, parasympathetic modulation and autonomic balance, and cardiovascular dynamics (Table 23). Cumulatively, they explained more than 86% of the variance, which is an exceptionally high value for psychophysiological data and demonstrates a stable and interpretable latent structure (Shaffer & Ginsberg, 2017; Shaffer & Meehan, 2020).

Parameter	Component 1	Component 2	Component 3	Uniqueness
VLF	0.847	–	–	0.264
LF	0.845	–	–	0.116
HF	0.863	–	–	0.223
Total Power	0.638	0.681	–	0.053
Normalized Coherence	–0.393	0.773	–	0.214
Intervals	–	–	0.974	0.007
Duration	0.794	–	–	0.365
MHRR	0.922	–	–	0.074
MHR	–	–	0.985	0.006
SDNN	0.493	0.750	–0.396	0.037
RMSSD	0.815	0.356	–	0.133

Note: Component loadings below |0.30| are not presented in order to provide a clearer visualization of the principal relationships among the parameters.

Table 23. *Results of the principal component analysis of extended HRV parameters*

The resulting three-factor structure supports published evidence according to which HRV parameters cluster into several separate but complementary autonomic dimensions rather than forming a single general and homogeneous pattern. Similar to the present results, Lucini and colleagues described several latent autonomic domains with a very high proportion of explained variance, supporting the idea that this type of data reduction is physiologically meaningful (Lucini et al., 2018). The absence of correlations among the three components in the present analysis indicates that each reflects a different aspect of autonomic regulation. For this reason, factor scores may be used as summary parameters in subsequent statistical models, similarly to the integrative autonomic indices proposed in other studies, including the ANSI developed by Sala and colleagues (Sala et al., 2017).

5.8. Comparison between Short-Term and Extended PCA

The results of the two PCA procedures—on short-term and extended HRV parameters—showed a high degree of consistency in the structure of the data. In both cases, three components were extracted that explained a substantial proportion of variance and could be interpreted as separate, physiologically meaningful patterns.

The short-term analysis identified three factors reflecting temporal variability, parasympathetic modulation, and coherence. The extended analysis supplemented this structure by including the frequency domains and total power, again extracting three factors: spectral and temporal variability, parasympathetic modulation and autonomic balance, and cardiovascular dynamics.

The similarity between the two factor structures confirms that HRV parameters consistently cluster into several principal latent dimensions regardless of whether short-term or extended recordings are analyzed. This increases the reliability of the extracted indices and supports their use in subsequent statistical models (Shaffer and Meehan, 2020).

At the same time, differences between the two analyses—such as the isolation of coherence as an independent factor in short-term recordings and its integration into the parasympathetic factor in extended recordings—emphasize the specific sensitivity of different HRV metrics to recording duration and measurement context (Table 24).

Analysis	Factor 1	Factor 2	Factor 3
Short-term HRV	Time variability (MHR, mean IBI)	Parasympathetic modulation (*SDNN, RMSSD, MHR*)	Coherence (normalized coherence)
Extended HRV	Spectral and temporal variability (VLF, LF, HF, RMSSD, duration, MHRR)	Parasympathetic modulation and autonomic balance (SDNN, normalized coherence, total power)	Cardiovascular dynamics (*Intervals, MHR*)

Table 24. *Comparative interpretation of the factor structure in short-term and extended HRV analysis*

The resulting three-factor structure supports other similar HRV models showing that time-domain, spectral, and integrative autonomic parameters cluster into relatively independent domains rather than a single common factor (Tarvainen et al., 2014; Lucini et al., 2018; Lawrence et al., 2023; Solaro et al., 2023). To date, however, no study has simultaneously derived and compared such a structure for short-term and extended HRV parameters within a multisession BF program in a high-stress group.

5.9. Results of the Linear Mixed Model for the Individual Factors

To investigate the effect of the program on the first factor (time variability), a linear mixed model was performed with fixed effects of time (beginning–end of the program), session, and their interaction (time × session), together with a random effect for participant.

The analysis showed a statistically significant effect of time ($F(1,988)=35.19$, $p<.001$), demonstrating that the values of the first factor changed substantially over the course of the program. A significant effect of session was also found ($F(25,989)=2.41$, $p<.001$), indicating that the factor's dynamics differed between individual training sessions. However, the time×session interaction was not statistically significant ($F=0.36$, $p=.999$). This means that the change from the beginning to the end of the training program was similar within each session, without specific differences among sessions.

Analysis of the variance components showed that approximately 32.5% of the variance was attributable to individual differences between participants (ICC=0.325), while the remainder was explained by within-individual fluctuations. This confirms the need to use a mixed model because a substantial proportion of the variation was associated with subject-specific differences.

The results are close to published evidence according to which HRV-BF may beneficially influence time-domain HRV parameters, although the effect does not always manifest as a clearly expressed change between the beginning and end of each individual session. In this direction are the findings of Chaitanya and colleagues, who reported improved HRV after a several-week controlled breathing program (Chaitanya et al., 2023). Similar observations were made by Ratajczak, according to whom the effect of HRV-BF accumulates gradually over the course of training

(Ratajczak et al., 2021). Against this background, the present study occupies an intermediate position: a stable effect was found on the factor summarizing time HRV, as well as an influence of the sessions themselves, but without evidence of a specific effect in each individual training session. This suggests that adaptation in our sample proceeded more as a gradual accumulation and stabilization of autonomic regulation than as a clearly expressed session-specific response.

For the second factor (parasympathetic modulation), a linear mixed model was performed with fixed effects of time (beginning–end of the program), session, and their interaction (time × session), together with a random effect for participant.

The analysis revealed a statistically significant main effect of time ($F(1,988)=10.94$, $p<.001$), indicating that parasympathetic modulation changed substantially after completion of the program. Unlike the first factor, the effect of session was not significant here ($F=0.96$, $p=.517$), suggesting that individual training sessions did not themselves explain substantial variation in factor scores. The time×session interaction likewise did not reach statistical significance ($F=0.66$, $p=.895$), meaning that the change from the beginning to the end of the training program was observed as a stable effect independent of the specific session.

The model indicated that approximately 44.5% of the variance in the second factor was attributable to individual differences between participants (ICC=0.445), a higher proportion than for the first factor. This indicates that parasympathetic modulation was more strongly influenced by subject-specific characteristics and individual reactivity of the ANS.

The results support several studies in which HRV-BF was associated with improvement in parameters reflecting vagal control and autonomic balance, without this improvement manifesting as a clearly distinguishable effect of each individual training session

(Deschodt-Arsac et al., 2018; Lin et al., 2019). A similar direction has also been described in other studies reporting favorable changes in autonomic markers and parasympathetic regulation after HRV-BF, including in groups of young people (Lehrer and Gevirtz, 2014; Simon et al., 2025). In this sense, the data support the understanding that BF may facilitate vagal control in a more sustained and cumulative manner rather than necessarily through a distinct, specific change occurring immediately after each individual session.

For the third factor (coherence/cardiovascular dynamics), a linear mixed model was also performed with fixed effects of time (beginning–end of the program), session, and their interaction (time×session), together with a random effect for participant.

The analysis found no statistically significant effect of time ($F(1,988)=1.80$, $p=0.180$), meaning that changes between the beginning and end of the program were not sufficiently pronounced. The effect of the individual session was also not significant ($F=0.90$, $p=0.602$). The time×session interaction likewise did not reach significance ($F=0.67$, $p=0.887$), indicating that the dynamics of change from the beginning to the end of the training program did not vary across individual sessions either.

Component analysis of variance showed that approximately 33.7% of the total variance was attributable to individual differences between participants (ICC=0.337), while the remainder resulted from within-individual variation. This indicates that the factor was moderately influenced by subject-specific characteristics, but the absence of significant effects of time and session suggests that these variations were relatively stable over time.

The results support studies in which coherence parameters did not demonstrate a clear short-term change despite the presence of positive effects in other autonomic or psychological dimensions (Nashiro et al., 2023; Wareing et al., 2024). At the same time, they differ from other studies in which the use of specifically targeted cardiac-coherence protocols led to improved cardiorespiratory coordination (Climov et al., 2014). In this sense, the data support the understanding that the component associated with coherence and cardiovascular dynamics probably reflects a more stable aspect of psychophysiological regulation that is less sensitive to short-term changes and may require a more targeted or longer intervention.

Analysis of the three PCA factors showed different dynamics of effects (Table 25). For the first factor (time variability), significant effects of time and session were found, confirming the program's positive impact on this aspect of HRV. The second factor (parasympathetic modulation) also showed a statistically significant change over time but without an influence of individual sessions, suggesting a more sustained effect. For the third factor (coherence/cardiovascular dynamics), no significant changes were found, suggesting that it reflects more stable physiological mechanisms.

Factor	Time	Session	Time × Session	ICC	Interpretation
Factor 1 Time variability	F(1,988) = 35.19, *p* < .001*	F(25,989) = 2.41, *p* < .001*	F(25,988) = 0.36, *p* = .999	0.325	Significant increase, stable across all sessions
Factor 2 Parasympathetic modulation	F(1,988) = 10.94, *p* < .001*	F(25,988) = 0.96, *p* = .517	F(25,988) = 0.66, *p* = .895	0.445	Significant increase in parasympathetic activity
Factor 3 Coherence/ dynamics	F(1,988) = 1.80, *p* = .180	F(25,989) = 0.90, *p* = .602	F(25,988) = 0.67, *p* = .887	0.337	No statistically significant changes

Table 25. *Linear mixed model for the dynamics of the extracted HRV components over the course of the training program*

6. Correlation Analysis

Correlation analysis is a statistical method for assessing the direction and strength of the relationship between two or more variables (Mukaka, 2012). It was applied to determine whether psychometric, physiological, and laboratory parameters changed in a coordinated manner and whether significant interrelationships associated with the effect of the BF program existed among them.

6.1. Psychometrics

The relationships among changes in the four psychometric scales—ΔPSS, ΔSAS, ΔBAI, and ΔBDI—were examined among participants in the training group. Table 26 presents descriptive statistics for the changes (Δ), calculated as the difference between scores at the end and at the beginning of the program. The number of valid observations (N), mean, median, standard deviation (SD), and observed range of changes, represented by the minimum and maximum values, are shown. Owing to the method used to calculate the changes, negative values indicate a decrease on the corresponding scale (i.e., improvement), whereas positive values indicate an increase (i.e., deterioration).

	ΔPSS	ΔSAS	ΔBAI	ΔBDI
N	24	24	24	24
Mean	-8.54	-13.5	-13.8	-12.0
Median	-7.50	-13.5	-12.0	-9.50
SD	6.32	7.25	8.38	9.36
Minimum	-19	-28	-30	-31
Maximum	2	1	-1	3

Table 26. *Dynamics of psychometric parameters between the beginning and end of the program*

As shown by the results, the mean Δ values for all scales were negative, indicating a reduction in perceived stress, anxiety, and depression in the training group. The medians were close to the means, suggesting no substantial asymmetry. The standard deviations (≈6–9 points) reflected moderate interindividual variability in the magnitude of change, while the recorded minimum and maximum values showed that individual participants experienced both very large improvements (e.g., up to −31/−33 points) and slight deterioration (up to +1/+3 points).

Figure 24 presents the relationships among changes in the four psychometric scales—perceived stress (ΔPSS), anxiety according to SAS (ΔSAS), anxiety according to BAI (ΔBAI), and depression (ΔBDI)—among participants in the training group. Blue indicates a positive relationship, while greater color saturation and larger marker size reflect a stronger correlation. The numbers in the cells show the values of the correlation coefficient r. The color map shows that all changes occurred in the same direction: when a participant exhibited a more pronounced decrease or increase on one scale, a similar change was generally recorded on the others. The strongest relationships were between changes in perceived stress and anxiety and between anxiety and depression, indicating close interconnection among the different dimensions of the participants' mental state. The correlation values were positive and moderate to strong ($r \approx 0.50$–0.71), supporting the understanding that improvement or deterioration in one psychometric domain is usually accompanied by a corresponding change in the others.

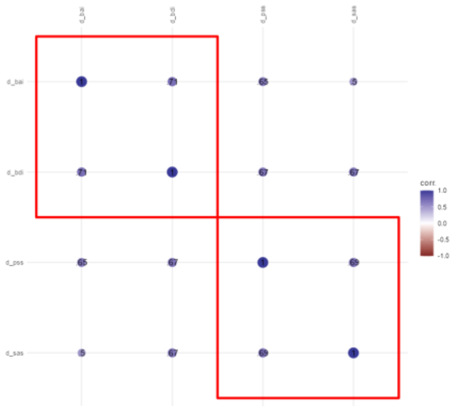

Figure 24. *Correlations among changes in perceived stress, anxiety, and depression levels (ΔPSS, ΔSAS, ΔBAI, and ΔBDI)*

The closest relationships were between changes in stress and the remaining parameters, as well as between anxiety and depressive symptoms (Table 27). This suggests that the program did not affect separate, isolated symptoms, but rather a common underlying core.

Pair of parameters	r	p
ΔPSS – ΔSAS	0.688	<0.001**
ΔPSS – ΔBAI	0.652	<0.001**
ΔPSS – ΔBDI	0.670	<0.001**
ΔSAS – ΔBAI	0.505	0.012*
ΔSAS – ΔBDI	0.666	<0.001**
ΔBAI – ΔBDI	0.706	<0.001**

Table 27. *Correlations among changes in psychometric parameters*

The pattern supports published evidence from student and medical populations. Review studies show that stress, anxiety, and depression among students generally do not develop independently but are intertwined within a common internalizing symptom configuration (Agyapong-Opoku et al., 2023). Among medical students, this overlap is particularly pronounced because the three conditions share similar academic, social, and personality-related risk factors (Mofatteh, 2020). More broadly, contemporary psychopathology literature also considers anxiety and depression to be closely related manifestations of a common internalizing domain, to which perceived stress is often added as a maintaining or amplifying mechanism (Weisz et al., 2023).

Against this background, our results support previously published evidence while also adding an important contribution to the understanding of BF. Many available studies in medical students focus mainly on the prevalence of anxiety, depression, and stress or on their risk factors at individual points in medical training. It is less common to analyze whether changes across several different psychometric instruments occur synchronously within a specific training program.

The study clearly demonstrates that the reduction in stress, anxiety, and depression was not a fragmented, partial process but a coordinated change with a visible and demonstrated effect. The results lead to the conclusion that, among international medical students, PSS, SAS, BAI, and BDI captured the same direction of change over the course of the BF program. This provides grounds for interpreting the psychometric results not merely as a collection of separate scales but as a relatively unified picture of change and improvement in the participants' condition.

6.2. Cortisol and Anxiety/Stress

The relationships between cortisol dynamics and subjective changes in anxiety and stress showed a consistent and physiologically explicable pattern. A greater increase in cortisol after the MAST (measured at 10 and 30 minutes) was associated with a smaller reduction—or a greater increase—in anxiety assessed using the BAI. The change in cortisol levels measured 10 minutes after the stress test showed a moderate positive correlation with the change in BAI scores. A similar effect was observed for the change in cortisol levels measured at 30 minutes (Fig. 25).

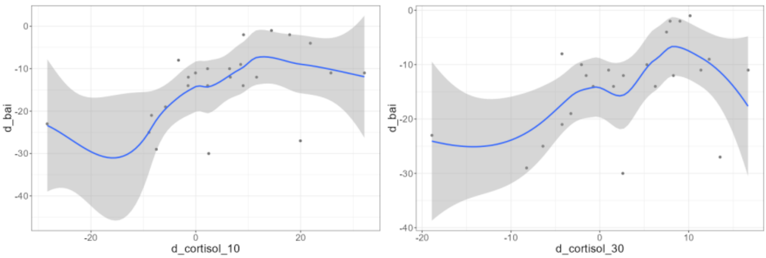

Figure 25. *Relationship between changes in cortisol levels measured at 10 and 30 minutes and changes in BAI scores*

The results show that approximately 22–26% of the variance in the change in anxiety was shared with the variance in cortisol secretion, representing a medium-sized effect.

A similar relationship was observed for PSS, although with more moderate strength. A greater increase in cortisol 10 minutes after the MAST was associated with a smaller reduction in perceived stress. This means that participants with more pronounced cortisol reactivity immediately after the stressor generally also demonstrated a less favorable change in perceived stress. For cortisol measured 30 minutes after the test, the relationship with changes in PSS was in the same direction, despite the absence of statistical significance ($p=.090$). This suggests that, if a relationship exists between cortisol reactivity and changes in perceived stress, it is more clearly expressed in the earlier post-stress window.

Overall, the relationship between cortisol reactivity and perceived stress was weaker than that with anxiety but followed the same physiologically explicable direction: a higher acute hormonal response was associated with a more limited reduction in subjective stress. In the context of multiple comparisons, this effect remained borderline, requiring cautious interpretation and supporting the conclusion that cortisol dynamics are more closely related to anxiety than to perceived stress.

The pattern supports published evidence concerning acute psychosocial stress. The MAST and related laboratory stress tests reliably increase both salivary cortisol and subjective measures of anxiety and stress, with the cortisol peak usually developing in the early post-stress window (Shilton et al., 2017). In a study using the MAST in healthy young individuals, Qi and colleagues likewise found a relationship between cortisol levels and subjective anxiety only during the early stress phase, rather than an equally strong association across all periods after the stress test (Qi et al., 2016). This is quite close to the analyzed data, in which the relationship was clearest for anxiety, particularly for cortisol 10 minutes after the stress test, whereas the effect for PSS was weaker and less consistent.

A difference of this kind between anxiety and perceived stress is physiologically justified. Anxiety, particularly at an acutely occurring moment, is more closely related to immediate HPA reactivity, whereas PSS reflects a broader cognitive appraisal of burden and is often less closely linked to short-term cortisol dynamics (Bozovic et al., 2017). Data from student populations also show that cortisol may correlate with SAS and PSS, but this relationship is not entirely uniform across different contexts and time windows (AlSarhan et al., 2024; Glenk et al., 2019).

The results obtained may be positioned as intermediate but highly compatible with the published evidence: cortisol reactivity showed a clearer relationship with anxiety than with perceived stress, and the early post-stress window proved most informative. This supports the understanding that, in training and laboratory models of stress, the dynamic cortisol response is more sensitive to acute emotional changes, particularly anxiety, than to broader and more persistent cognitive appraisals of stress.

6.3. IgA and Cortisol

To determine whether changes in mucosal immunity accompanied the stress response, the relationship between changes in salivary IgA and cortisol reactivity after the MAST was examined. The results showed a moderate negative relationship between the change in IgA and the early cortisol response measured at 10 minutes ($r = -0.436$, $p=.033$). In other words, the greater the increase in cortisol after the stress challenge, the smaller the increase in IgA or the more pronounced its decrease. For cortisol at 30 minutes, the relationship remained in the same direction but weakened and did not reach statistical significance ($r = -0.386$, $p=.062$).

This pattern corresponds to the concept of an association between the HPA axis and the mucosal immune response (Nunez et al., 2025). The closest parallel is provided by the study of Fan and colleagues, in which salivary cortisol and sIgA initially increased after acute stress, but the subsequent decrease in sIgA over the following 20 minutes was significantly associated with the increase in cortisol ($r = 0.569$, $p<0.001$) (Fan et al., 2009). More broadly, the meta-analysis by Seizer and colleagues shows that, under acute stress, sIgA generally peaks around minute 10 and returns to baseline by approximately minute 30 (Seizer et al., 2024). This provides a good explanation for why the relationship in the present study was clearer in the early post-stress window rather than 30 minutes later.

Comparison with student populations also shows that the relationship between cortisol and IgA is not unidirectional but depends strongly on context and measurement timing. Among female nursing students, Takatsuji and colleagues found an acute increase in IgA immediately after an examination without a significant change in cortisol (Takatsuji et al., 2008). Among students, Murphy and colleagues found higher cortisol levels during an examination period but a statistically nonsignificant increase in sIgA (Murphy et al., 2010). Conversely, Afrisham and colleagues reported that sIgA decreased under examination stress (Afrisham et al., 2016). Against this background, the present findings do not show a universal increase or decrease in IgA but rather demonstrate that stronger early cortisol reactivity was associated with a less favorable change in the mucosal immune response.

The data obtained support the understanding that the dynamic early cortisol response is more informative for the relationship between stress and mucosal immunity than the later window or a single value in isolation. This places the results close to studies that regard IgA as a sensitive but context-dependent marker of stress—particularly in situations where the timing of sample collection determines whether short-term activation, subsequent recovery, or relative suppression of mucosal immunity is captured (Fan et al., 2009; Murphy et al., 2010; Seizer et al., 2024).

6.4. Relationships among HRV, Biochemical, and Psychometric Parameters

Analysis of the relationships among changes in HRV, cortisol, IgA, and psychometric parameters outlined two principal groups of findings. On the one hand, physiologically expected relationships were observed: an increase in HRV, particularly in parameters associated with vagal regulation and overall autonomic adaptability, was accompanied by a more favorable cortisol profile. On the other hand, two associations also emerged whose directions did not fully correspond to the relationships most commonly described in the literature and therefore require more cautious interpretation (Kim et al., 2018; Gullett et al., 2023).

Among the expected relationships, the clearest were the negative associations between ΔRMSSD, ΔSDNN, and ΔLF, on the one hand, and ΔCortisol_before the stress test, on the other. This means that participants whose HRV improved generally also showed more favorable cortisol dynamics. Such a pattern is physiologically logical because higher HRV is regarded as a marker of better autonomic adaptability, whereas lower HRV is typical of chronic or accumulating stress (McCraty & Shaffer, 2015; Shaffer & Ginsberg, 2017). Contemporary reviews and meta-analyses consistently associate psychological stress with reduced HRV, particularly vagally mediated parameters (Kim et al., 2018). Longitudinal studies in academic settings show that, as stress increases, HRV decreases while anxiety and depressive-symptom scores rise (Benítez-Agudelo et al., 2025). Among medical students, a combination of stress, changes in HRV, and cortisol over the course of the academic year has also been described (Upadhayay et al., 2014).

This part of the results supports the understanding that more favorable autonomic regulation is associated with lower or more stable hormonal stress activation (Nunez et al., 2025). LF is the most convincing in this regard, but the similar direction observed for RMSSD and SDNN suggests that the pattern is not incidental but reflects a general physiological tendency (McCabe et al., 1985; Pagani et al., 1986).

A different picture emerges for ΔHF–ΔSAS and ΔCoherence–ΔIgA 10 minutes after the stress test. The positive association between HF and anxiety according to SAS does not correspond to the most frequently reported findings, because the literature generally associates anxiety and negative affect with reduced, rather than increased, vagally mediated HRV. This has been demonstrated both in psychophysiological reviews and in more recent studies of anxiety disorders (Gullett et al., 2023; Laborde et al., 2017; Tomasi et al., 2024). Therefore, this finding should be interpreted cautiously, as it probably reflects characteristics of the specific sample or the influence of individual observations.

The negative association between ΔCoherence and ΔIgA 10 minutes after the stress test should be interpreted in a similar manner. From a theoretical perspective, coherence is more often associated with more effective autonomic regulation and a more favorable psychophysiological state, whereas sIgA is a sensitive marker of the mucosal immune response that may increase during positive emotional states and following certain interventions (McCraty et al., 1998; Pham et al., 2021). Therefore, the negative association observed here does not follow the most intuitive pattern and instead indicates that the relationship between coherence and mucosal immunity is more complex and strongly dependent on the timing of measurement. Contemporary reviews of sIgA emphasize precisely this dependence: in acute states, sIgA is often mobilized transiently, whereas in chronic states it may be suppressed (Castro-Quintas et al., 2023).

The results are consistent with literature data concerning the relationship between HRV and cortisol regulation, according to which a more favorable autonomic profile is associated with a more stable hormonal response to stress. However, the relationships between HRV, anxiety, and IgA are more ambiguous and should be interpreted cautiously, because they are probably influenced by individual reactivity, the timing of measurement, the characteristics of the stressor, and the features of the sample.

The contribution of the present study lies in the integrated examination of autonomic, hormonal, immunological, and psychometric indicators in a high-stress population of international medical students. Some of the identified relationships follow a physiologically expected pattern, whereas others outline more complex and context-dependent interactions that may provide a basis for future research on psychophysiological adaptation to stress.

6.5. Conclusions from the Correlation Analysis

The correlation analysis outlines a consistent and physiologically meaningful pattern of relationships among the psychometric, biochemical, and HRV indicators. The correlation maps presented below show the same overall structure, but from slightly different perspectives. The first map reflects how closely the indicators change in absolute terms (Fig. 26), whereas the second examines whether the same direction of association is retained when the analysis is more robust to individual extreme values and deviations from normal distribution (Fig. 27). The similarity between the two maps indicates that the principal observed relationships are stable and are not attributable to random fluctuations in the data.

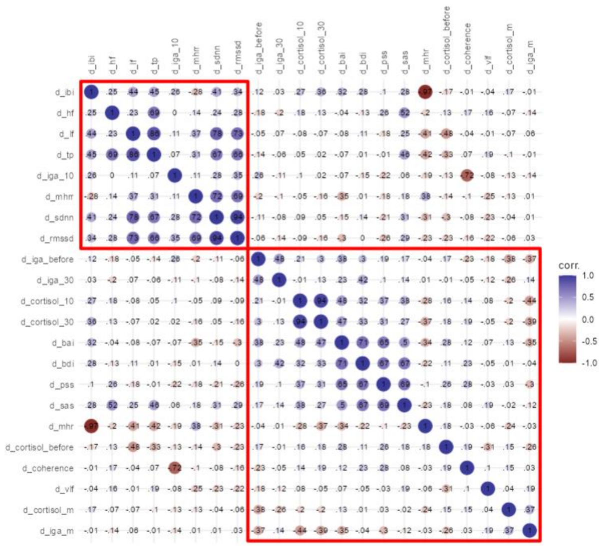

Figure 26. *Summary Pearson correlation map of changes (Δ) in psychometric, biochemical, and HRV indicators*

In the figure, larger and more intensely blue markers indicate stronger positive associations, whereas red markers indicate negative associations. Thus, groups of indicators that change in a similar direction, as well as pairs of indicators between which an inverse relationship exists, are visually distinguished.

The psychometric indicators are the most clearly expressed, with changes in perceived stress, anxiety, and depression being strongly and consistently interrelated. This means that when improvement is observed in one of the scales for a given participant, a similar positive change is usually observed in the others. Thus, the four psychometric instruments outline a common direction of reduction in perceived stress.

The second important line of results concerns the cortisol response. A greater increase in cortisol after the stress provocation is associated with a smaller reduction in anxiety and, to a more moderate extent, in perceived stress. In addition, the two cortisol indices—at 10 and 30 minutes—are very closely related to each other, indicating that they reflect the same principal reactive component of the HPA axis. The results are consistent with the concept that the dynamic hormonal response to stress, rather than merely the baseline cortisol level, is informative regarding subjective changes in anxiety and stress.

A third group of findings concerns the relationship between cortisol and mucosal immunity. The change in IgA is inversely associated with the early cortisol response, meaning that a more pronounced increase in cortisol is accompanied by a smaller increase or a more pronounced decrease in IgA. This may be interpreted as supporting the physiologically expected tension between the stress axis and the mucosal immune response.

With regard to the HRV indicators, the overall direction of the associations is also predominantly physiologically expected. Higher HRV, particularly for indicators related to parasympathetic regulation and overall autonomic adaptability, is associated with a more favorable cortisol profile. This suggests that better autonomic regulation occurs in synchrony with more favorable hormonal reactivity. At the same time, some of the associations—particularly those between HF and anxiety, as well as between coherence and IgA—do not fully follow the most commonly expected physiological direction and should therefore be interpreted more cautiously.

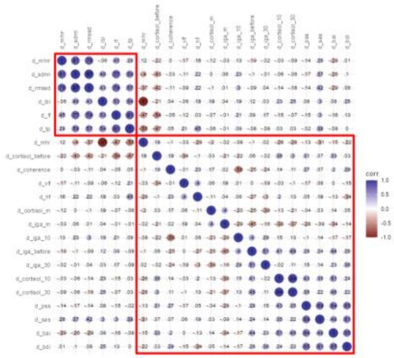

Figure 27. *Summary Spearman correlation map of changes (Δ) in psychometric, biochemical, and HRV indicators*

The Spearman map demonstrates the principal relationships observed in the Pearson analysis (Fig. 27). The clearly delineated associations among the psychometric indicators, the strong association between the cortisol measurements at 10 and 30 minutes, and the relationship between a more pronounced cortisol response to stress and a less favorable change in anxiety are retained. The negative associations between some HRV indicators and cortisol are also retained, suggesting a relationship between better autonomic regulation and more favorable hormonal stress dynamics. The similarity between the two correlation approaches indicates that the principal relationships are not determined solely by individual extreme values or deviations from normal distribution, but reflect a relatively stable pattern in the studied sample.

The correlation analysis shows that the reduction in subjective psychological stress is accompanied by more favorable biological changes, primarily along the HPA axis–anxiety/stress and cortisol–IgA pathways. The HRV indicators largely support this picture, although not all associations are equally strong or unambiguous. Thus, the results indicate that the effect of the program is not limited to subjective improvement but is embedded within a broader psychophysiological model of adaptation.

7. Regression Analysis

Regression analysis was used to assess the predictive value of one or more factors with respect to a specified outcome (Zapf et al., 2024). In the study, it was applied to determine whether baseline physiological indicators—cortisol, secretory IgA, and HRV parameters—could predict the degree of subsequent improvement on the scales for stress, anxiety, and depressive symptoms (Table 28).

Psychometric indicator	Biomarker model	R^2	Adjusted R^2	Main findings
PSS	Cortisol	0.126	-0.058	No significant predictors were identified
	sIgA	0.071	-0.124	No significant predictors were identified
	HRV	0.618	0.325	**MHR (p = 0.003) and IBI (p = 0.005) are significant predictors; SDNN shows a trend toward significance (p = 0.057)**
SAS	Cortisol	0.118	-0.068	No significant predictors were identified
	sIgA	0.085	-0.108	No significant predictors were identified
	HRV	0.496	0.108	**MHR is a significant predictor (p = 0.040); IBI (p = 0.063), SDNN (p = 0.099), and Normalized Coherence (p = 0.093) show trends toward significance**
BAI	Cortisol	0.104	-0.085	No significant predictors were identified
	sIgA	0.122	-0.063	No significant predictors were identified
	HRV	0.499	0.114	**MHR is a significant predictor (p = 0.044); IBI is at the threshold of significance (p = 0.051)**
BDI	Cortisol	0.072	-0.124	No significant predictors were identified
	sIgA	0.034	-0.169	No significant predictors were identified
	HRV	0.366	-0.121	No significant predictors were identified; the HRV model shows higher but insufficient predictive value

Table 28. *Summary of the regression models for predicting improvement in PSS, SAS, BAI, and BDI*

7.1. PSS

The HRV model demonstrates the best predictive value for improvement in PSS, in which baseline MHR and IBI are significant predictors, while SDNN shows a trend toward significance. In contrast, the cortisol and sIgA models have low explanatory power and are not statistically significant. This indicates that HRV is more informative than the laboratory indicators with respect to perceived stress.

This result supports literature data according to which HRV and HRV BF are particularly relevant to subjectively experienced stress. The meta-analysis by Goessl et al. demonstrates a significant effect of HRV BF on stress and anxiety, while the review by Kim et al. supports HRV as an objective psychophysiological indicator of stress burden (Goessl et al., 2017; Kim et al., 2018). In student populations, Tripska et al. also identified relationships between perceived stress and physiological indicators of stress and general health, which is quite close to the present results and indicates that it is the HRV profile, rather than isolated humoral markers, that carries the greatest predictive information (Tripska et al., 2022).

With regard to cortisol, the results tend to confirm studies emphasizing the heterogeneity of the relationship between perceived stress and salivary cortisol. It has been reported that individuals with higher subjective stress levels may demonstrate higher cortisol values in certain contexts, such as occupational or academic stress, and that morning cortisol in students may sometimes show a degree of predictive value for greater academic burden (van Eck et al., 1996; Murphy et al., 2010; Nijakowski et al., 2022; Sanogo et al., 2024; Špiljak et al., 2024). At the same time, systematic reviews show that the relationships between PSS scores and cortisol indices remain inconsistent, particularly when different cortisol parameters such as morning values, diurnal changes, or total daily cortisol are analyzed (Chida and Steptoe, 2009; Adam et al., 2017; Stalder et al., 2017).

This makes the absence of a stable cortisol predictor in the study entirely explicable.

With regard to sIgA, our data also support studies in which this marker is promising but strongly dependent on a number of conditions. Studies of nurses and other individuals working under high-stress conditions show that greater subjective burden may be associated with lower levels or lower secretion of sIgA (Yang et al., 2002; Mocci and Bullitta, 2006). Other data indicate associations between higher perceived stress, depressive symptoms, and changes in IgA (Castro-Quintas et al., 2023; Engeland et al., 2016). In addition, sIgA is strongly influenced by circadian rhythm, age, type of stressor, and whether the stress is acute or chronic (Chojnowska et al., 2021). Therefore, the lack of predictive value of baseline sIgA in the present models does not contradict the available evidence, but supports the understanding of a degree of biological variability.

7.2. SAS

The same tendency is observed for SAS: cortisol and sIgA do not reliably predict improvement, whereas HRV has better explanatory power ($R^2 = 0.496$; adjusted $R^2 = 0.108$), with MHR emerging as a significant predictor and IBI and SDNN showing trends toward significance. This supports the idea that, in anxiety, autonomic regulation is more closely associated with the individual response than baseline cortisol and sIgA levels.

Previous studies largely support this interpretation. Zafeiri et al. reported a significant reduction in SAS after BF therapy (Zafeiri et al., 2022). Li et al. found parallel decreases in SAS and improvements in HRV parameters such as SDNN among female students with anxiety (Li et al., 2022). The teams of Ding and Yu likewise demonstrated reductions in SAS following BF-based programs (Ding et al., 2012; Yu et al., 2017). Although the individual protocols differ, the overall concept is similar—anxiety symptoms measured by SAS are sensitive to interventions that influence autonomic regulation.

A broader scientific overview of anxiety provides even stronger support for the present HRV results. Meta-analyses by the teams of Cheng and Chalmers show that patients with anxiety disorders have lower HRV, which is consistent with the concept of impaired cardiovagal control in anxiety (Chalmers et al., 2014; Y.C. Cheng et al., 2022). It is therefore unsurprising that, in the models examined, HRV has greater predictive value for SAS than cortisol and sIgA.

Cortisol data in anxiety, however, are considerably more heterogeneous. Some studies of generalized anxiety demonstrate higher basal cortisol, whereas others, particularly in more prolonged or chronic anxiety disorders, report lower morning levels (Hek et al., 2013; Keefe et al., 2018; Mantella et al., 2008; Vreeburg et al., 2010). This means that the cortisol axis in anxiety may be either hyperactivated or partially "readapted," depending on the duration and clinical context of anxiety. In this sense, the absence of a stable cortisol predictor for SAS in the study is fully compatible with the contradictory data already published.

The relationship between anxiety and sIgA also remains inconsistent. Classical studies have found that more frequent episodes of anxiety may be accompanied by lower sIgA secretion, whereas other observations have not identified a clear correlation between anxiety and salivary IgA after relaxation procedures (Graham et al., 1988; Primo and Amorim, 2008). Therefore, the observations described here are expected rather than surprising.

7.3. BAI

The BAI analysis is analogous: cortisol and sIgA do not show reliable predictive value, whereas HRV is the most informative (R^2 = 0.499; adjusted R^2 = 0.114), with baseline MHR being a significant predictor and IBI remaining at the threshold of significance. This is particularly logical for BAI, because this scale includes pronounced somatic and autonomic manifestations of anxiety such as tachycardia, tension, sweating, and a sensation of “internal activation.”

Previous observations using BAI or closely related anxiety constructs support this interpretation. Park et al. showed that individuals with more severe anxiety, assessed by BAI, had higher cortisol concentration and secretion, whereas sIgA did not differ significantly between groups (Park et al., 2020). Liao and his team found that BAI was associated with a less favorable HRV profile during follow-up (Liao et al., 2016). Wu et al. described an association between sympathetic activation and simultaneously higher levels of anxiety and depression (Wu et al., 2022).

The meta-analyses by Cheng and Chalmers consistently demonstrate lower baseline HRV in anxiety disorders, while the meta-analysis by Goessl supports the conclusion that HRV BF improves stress and anxiety symptoms (Chalmers et al., 2014; Goessl et al., 2017; Y.C. Cheng et al., 2022). This means that the present BAI results support a number of studies according to which anxiety is closely related to autonomic dysregulation and, therefore, HRV has greater potential than cortisol and sIgA as a predictive indicator.

With regard to cortisol and sIgA, negative findings have also been observed in other studies. In individuals assessed with BAI, cortisol sometimes shows positive associations with anxiety severity, but these relationships are not sufficiently stable to serve as a universal predictor of subsequent improvement (Keefe et al., 2018; S.-H. Park et al., 2020). The evidence for sIgA is even more equivocal: some studies report lower secretion with higher anxiety, whereas others identify no substantial relationships (Graham et al., 1988; Primo and Amorim, 2008). Therefore, the result obtained—according to which HRV is a much more important predictive indicator than cortisol and sIgA for BAI—is well substantiated and supported by other research teams.

7.4. BDI

For BDI, the results differ from those observed for PSS, SAS, and BAI—neither cortisol, nor sIgA, nor HRV demonstrates stable predictive value for improvement in depressive symptoms. Although HRV has relatively greater explanatory power than the laboratory and psychometric indicators, none of them individually reaches statistical significance. This suggests that, for depressive symptoms, the baseline physiological profile is a weaker and more ambiguous predictor of the short-term response.

Meta-analyses by the teams of Pizzoli, Donnelly, and Fernández-Alvarez show that HRV BF and related programs may lead to a statistically significant reduction in depressive symptoms (Donnelly et al., 2023; Fernández-Alvarez et al., 2023; Pizzoli et al., 2021). Observations by Karavidas and his team also demonstrate the potential benefit of HRV BF in depression (Karavidas et al., 2007).

With regard to HRV as a predictor, some studies suggest the presence of such a relationship, but it is context-dependent. Huang et al. reported long-term associations between HRV and BDI (Huang et al., 2018). Carney's team found that higher nocturnal heart rate and lower HRV predicted a poorer therapeutic response in depressed patients with cardiac disease, while Kircanski showed that the predictive value of HRV may differ depending on whether depression is accompanied by a high anxiety component (Carney et al., 2016; Kircanski et al., 2019). In the present sample and design, however, HRV is not a sufficiently stable independent prognostic marker for BDI.

Cortisol data in depression likewise do not provide an unambiguous predictive picture. On the one hand, the meta-analysis by Sahu et al. shows that depression is often associated with higher cortisol levels (Sahu et al., 2022). On the other hand, results concerning prediction of therapeutic response are mixed: the teams of Fischer and Holland associate higher cortisol with a less favorable outcome or a weaker response to psychotherapy, whereas Schmalbach et al. report that more pronounced cortisol reactivity in a particular context may predict greater improvement in depressive symptoms (Holland et al., 2013; Fischer et al., 2017; Schmalbach et al., 2024). This heterogeneity partly supports the absence of a stable cortisol predictor in the study.

For sIgA, the evidence is the most limited and the most difficult to interpret. Engeland observed associations between depressive symptoms and changes in IgA, but these associations are not sufficiently consistent for sIgA to be regarded as a stable prognostic marker of improvement in depressive symptoms (Engeland et al., 2016). Thus, the negative result obtained for sIgA in relation to BDI is expected.

7.5. Conclusions from the Regression Analysis

The regression analyses performed for the four psychometric scales—PSS, SAS, BAI, and BDI—demonstrate differences in the predictive value of the physiological and biochemical indicators examined. Cortisol and secretory IgA do not demonstrate reliable predictive value with respect to reductions in perceived stress, anxiety, and depressive symptoms after the program. This result indicates that, within the sample used, the two biochemical markers do not emerge as independent predictors of psychometric improvement. In contrast, the HRV data demonstrate higher explanatory value for PSS, SAS, and BAI, with the indicators related to MHR and the IBI proving most informative. Therefore, the baseline autonomic profile may be of greater importance for the individual response to the BF program, particularly with respect to stress and anxiety.

Although the single-predictor models do not reach significance, the multivariable analyses clearly emphasize the importance of the HRV profile as a complex indicator. This corresponds to the growing understanding in the literature that autonomic regulation of cardiac activity is a central mechanism through which BF and relaxation techniques influence mental health (P. M. Lehrer & Gevirtz, 2014; McCraty & Shaffer, 2015; McCraty & Zayas, 2014).

For BDI, the results are different—all three indicators examined (cortisol, IgA, HRV) fail to demonstrate stable predictive value. This observation may be related to the different time dynamics and more complex determinants of depressive symptoms compared with stress and anxiety. The data obtained suggest that physiological markers, and HRV in particular, may be more informative for short-term psycho-emotional changes than for depressive states.

Figure 28 shows that HRV values have substantially greater explanatory power (R^2 ~0.50–0.62) compared with cortisol and IgA levels ($R^2 < 0.15$). HRV demonstrates the greatest predictive value for PSS, followed by SAS and BAI, whereas the results for BDI are weaker.

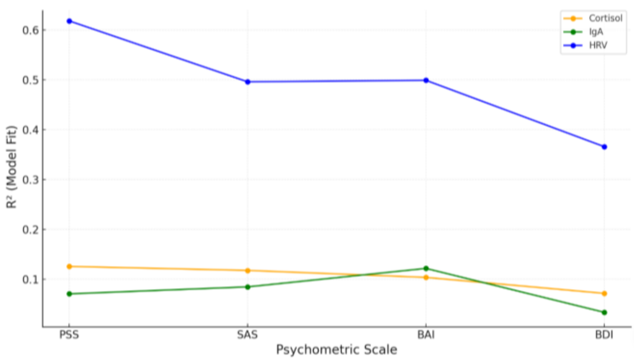

Figure 28. *Comparative predictive value of cortisol, IgA, and HRV (R^2) for the four psychometric scales (PSS, SAS, BAI, and BDI)*

V. CONCLUSION

The present study contributes to clarifying the potential of HRV-based biofeedback training as a non-pharmacological method for reducing stress and improving mental health among international medical students. The comprehensive approach applied, combining psychometric assessment, multisession monitoring of HRV, and measurement of salivary cortisol and secretory IgA, made it possible to examine the effect of the training program at psychological, autonomic, and neuroendocrine levels.

The BF program had its most pronounced effect on the participants' psycho-emotional state. After the 11-week training period, we identified a significant reduction in perceived stress, anxiety, and depressive symptoms.

The demonstrated individual variations in HRV indicators point to the need for students with more pronounced psychological distress to be included in longer or individually adapted support programs. The data show that adaptation to BF training proceeds as a complex and nonlinear process of autonomic reorganization and stabilization that cannot be assessed through a single HRV indicator. A comprehensive analysis of the HRV indicators is more informative, as these demonstrate higher predictive value for improvement in overall mental state compared with the biochemical markers examined.

In contrast to the dynamics of the HRV indicators, which were probably influenced by the baseline psycho-emotional state, capacity for self-regulation, and the presence of additional stressors, salivary cortisol and IgA showed minimal changes over the course of the training program. Nevertheless, the absence of group-level dynamics does not exclude their involvement in the stress response, insofar as relationships were identified between the early post-stress cortisol response and anxiety, as well as between changes in cortisol and secretory IgA.

On the basis of the results obtained, we confirmed the first working hypothesis, according to which BF training reduces the levels of stress, anxiety, and depressive symptoms. The second and third working hypotheses received partial confirmation, because significant but ambiguous relationships—dependent on the specific indicator and timing of measurement—were identified among the psychometric indicators, HRV, cortisol, and secretory IgA.

The data from our study indicate that HRV-based BF could become part of systematic strategies for preventing stress and mental exhaustion among medical students once university health and psychological centers integrate BF sessions as a form of psychophysiological prevention.

The method is non-invasive, requires active participation by the trainees, and enables the acquired self-regulation skills to be applied outside laboratory conditions. This is particularly important for international medical students, for whom academic requirements are combined with cultural adaptation, language difficulties, and distance from their usual social support.

Future studies with larger samples, randomized allocation, extended follow-up, and standardized protocols are needed to assess the durability of the effect and determine which participants would benefit most from such a program. The present attempt to integrate the psychological, autonomic, hormonal, and immunological aspects of the stress response represents a step forward toward the creation of individualized and scientifically grounded strategies for psychophysiological self-regulation and mental health support in the academic environment.

VI. SUMMARY OF FINDINGS

1. Analysis of the psychometric self-assessment results (PSS, SAS, BAI, BDI) demonstrates a statistically significant reduction over time in the indicators corresponding to perceived stress, anxiety, and depression in the training group. The psychological distress characteristic of the participants before the beginning of the program was reduced to levels comparable to those of the control group.

2. Participants in the biofeedback program with higher initial levels of perceived stress, anxiety, and depressive symptoms retained relatively higher values at the end of follow-up, with this relationship being most pronounced for the state of anxiety.

3. The laboratory indicators examined (salivary cortisol and IgA) did not demonstrate statistically significant changes in the training group.

   - A tendency toward a decrease in levels over time was observed for secretory IgA.
   - No such tendency was identified for cortisol.

4. The heart rate variability (HRV) indicators did not demonstrate a uniform and sustained change across all parameters during the course of biofeedback training.

   - The individual dynamics of the HRV indicators were heterogeneous, suggesting differences in sensitivity to biofeedback training and in the rate of psychophysiological adaptation.
   - No statistically significant change in coherence, MHR, SDNN, or Mean IBI was identified over the course of the individual sessions.

- MHRR and RMSSD demonstrated a significant increase over the course of the biofeedback sessions, followed by relative stabilization in the later stages of the training program.
- Assessment of the autonomic response requires a combined interpretation of heart rate, Mean IBI, time-domain variability, and coherence indicators, rather than selection of a single physiological parameter.

5. Some HRV indicators demonstrated nonlinear time dynamics during biofeedback training. The changes were more pronounced during the first 13–16 training sessions, after which no statistically significant change was observed.

   - MHR reached a maximum around session 13.
   - RMSSD and Mean IBI increased to a plateau phase around session 14.
   - MHRR demonstrated a linear increase with an indication of stabilization around sessions 15–16.

6. Statistically significant relationships were identified among HRV indicators with respect to several factors:

   - A factor combining indicators of time-domain heart rhythm variability (MHR, Mean IBI) and spectral components (VLF, LF, HF, RMSSD, Duration, MHRR);
   - A factor combining indicators reflecting parasympathetic modulation and autonomic balance (SDNN, Normalized Coherence, Total Power);
   - A factor combining coherence and cardiovascular dynamics in the long-term HRV analysis (MHR).

7. Interrelated changes in psycho-emotional indicators, cortisol reactivity, and the mucosal immune response were identified during the biofeedback program.

- Changes in perceived stress, anxiety, and depressive symptoms were interrelated, reflecting their parallel changes during follow-up.
- The early post-stress cortisol response, measured at 10 minutes, demonstrated a statistically significant relationship with anxiety, whereas the relationship with perceived stress was weaker and of borderline statistical significance.
- An inverse relationship was present in the changes in the stress markers measured at 10 minutes: the greater the increase in cortisol, the smaller the change in secretory IgA.

8. The physiological indicators demonstrated different predictive values for assessing post-training changes in perceived stress, anxiety, and depressive symptoms.

    - MHR and Mean IBI were the most significant HRV predictors of changes in perceived stress and anxiety during biofeedback training.
    - Cortisol and secretory IgA did not demonstrate reliable predictive value for the degree of reduction in perceived stress, anxiety, and depressive symptoms.
    - No stable physiological predictors were identified for depressive symptoms.

9. The heterogeneity of the relationships among the psychometric results, HRV indicators, and laboratory markers (salivary cortisol and IgA) shows that no single physiological indicator can comprehensively describe the psychophysiological response to stress and the biofeedback training program.

VII. CONTRIBUTIONS

Original Contributions:

1. For the first time, a controlled longitudinal study of the effect of HRV-based biofeedback training in international medical students was conducted, combining psychological, autonomic, and biochemical indicators of the stress response.
2. A model for psychophysiological monitoring of the individual response to biofeedback training was developed, in which data from psychometric measurements are analyzed jointly with heart rate variability indicators and salivary cortisol and IgA.
3. A structured HRV-based biofeedback training protocol was developed (an 11-week program conducted in real time, including controlled breathing, attentional focusing, and visual feedback).
4. Comparative modeling of the predictive value of HRV, salivary cortisol, and secretory IgA with respect to changes in psychophysiological state was performed, revealing the greater informativeness of baseline HRV profile indicators regarding improvement in perceived stress and anxiety.
5. A multisession approach was applied to assess autonomic regulation during the biofeedback program, enabling HRV to be monitored as individual dynamics across successive training sessions.
6. A methodological foundation was established for future multidisciplinary studies of stress and self-regulation, integrating psychometric assessment, HRV analysis, and measurement of hormonal and immunological indicators.
7. A basis was established for developing digital formats for remote monitoring and support of self-regulation through HRV-based biofeedback technologies aimed at monitoring and preventing academic stress.

Confirmatory Contributions:

1. The results support the evidence for a favorable effect of an HRV biofeedback training program on perceived stress, anxiety, and depressive symptoms in young people and student populations.
2. The understanding of the informativeness of different physiological indicators in monitoring the effect of BF training was expanded. The more pronounced dynamics of the psychometric indicators are consistent with evidence that psycho-emotional improvement after biofeedback occurs more rapidly and significantly than changes in autonomic and biochemical markers.
3. The comprehensive approach applied emphasizes the importance of combined assessment using psychometric instruments, HRV indicators, and biochemical markers, because each reflects a different aspect of the psychophysiological response to stress and intervention.
4. The absence of a specific effect of the biofeedback program on salivary cortisol and IgA is consistent with evidence of the high individual and contextual variability of these markers, which limits their use as independent indicators of short-term psycho-emotional improvement.

VIII. PRACTICAL RECOMMENDATIONS

1. Introduce screening for stress and psycho-emotional state

Periodic assessment of psychophysiological burden among students using standardized questionnaires and HRV measurements is recommended for the early identification of at-risk groups and referral to appropriate interventions.

2. Train academic staff and university psychologists in the application of biofeedback

Specialized courses and seminars for academic and counseling staff are recommended to build competencies in working with biofeedback systems and interpreting HRV results. This will ensure the sustainability and high-quality application of the method over the long term.

3. Use biofeedback to prevent burnout among students and medical personnel

Given its demonstrated effects on autonomic regulation and emotional resilience, biofeedback training may also be applied as a means of preventing occupational burnout among healthcare professionals and university faculty.

IX. PUBLICATIONS AND SCIENTIFIC COMMUNICATIONS RELATED TO THE THESIS

Publications in peer-reviewed scientific journals:

- **Panayotova G**, Pavlova S, Ivanova M, Doncheva D, Velikova M. Evaluation of academic stress among international students. J of IMAB. 2021;27(Suppl 1):94-96.
- **Panayotova G**, Nikolova S, Ivanova M, Doncheva D, Velikova M. Factors associated with increased risk of depression in international medical students. J of IMAB. 2022;28(Suppl 1):135-138.
- **Panayotova G**, Hachmeriyan A, Brüggemann K, Scholz S. Current aspects of the effect of biofeedback training on food intake and development of diabetes mellitus: a mini review. Scripta Scientifica Medica. 2023;55(4):30-34. doi:10.14748/ssm.v55i2.9845.

Scientific communications:

- **Panayotova G.**, Velikova M, Stoyanov Z. Assessing the effectiveness of long-term biofeedback training in a patient with severe depression and moderate persistent bronchial asthma: a case report. Acta Physiologica. 2024;240(Suppl 731):83. Presented at: XXIII Meeting of the Federation of European Physiological Societies and XLI Meeting of the Spanish Society for Physiological Sciences; 2024 Sep 4-6; Granada, Spain.
- **Panayotova G**, Stoyanov Z, Velikova M. Secretory IgA response to acute stress before and after 12-week biofeedback training in highly stressed individuals. Acta Physiologica. 2025;241(Suppl 735):C17-10. doi:10.1111/apha.70104.
- **Panayotova G**, Stoyanov Z, Velikova M. Effectiveness of biofeedback training on anxiety in international medical students. International Journal of Psychophysiology. 2025;213(SupplS):112688. doi:10.1016/j.ijpsycho.2025.112688.
- **Panayotova G**, Stoyanov Z, Velikova M. Effects of heart rate variability biofeedback training on stress biomarkers and allergy symptoms: a case report. International Journal of Psychophysiology. 2025;213(Suppl S):112689. doi:10.1016/j.ijpsycho.2025.112689.