ORIGINAL ARTICLE

HIGH-INTENSITY INTERVAL TRAINING VS. MODERATE-INTENSITY INTERVAL TRAINING AND EFFECTS ON STRESS MARKERS

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ABSTRACT

Objective: To find out the acute impact of High-Intensity Interval Training (HIIT) versus Moderate-Intensity Interval Training (MIIT) on salivary cortisol, heart rate variability (HRV), and state anxiety in young, healthy adults.

Study Design: Single-visit, open-label, randomized controlled trial.

Place and Duration of study: Khyber Medical University, Peshawar, Pakistan, 06 months (July to December 2025).

Methodology: Fifty physically active participants (18–30 years of age) were randomly distributed into two groups of 25 participants each, HIIT and MIIT group. Pre- and post-exercise assessments compreised salivary cortisol, heart rate variability(HRV) parameters including Root Mean Square of Successive Differences (RMSSD), Standard Deviation of NN Intervals (SDNN) & low-frequency to high-frequency (LF/HF) ratio, as well as the State-Trait Anxiety Inventory (STAI)-State Anxiety scale. HIIT comprised 10 sets of 1-minute cycling at 85–95% with active recovery. MIIT comprised 10 sets of 1-minute cycling at 55–65% with passive recovery.

Results: HIIT significantly increased salivary cortisol from 0.27± 0.07 to 0.36± 0.08 µg/dL (+32.8%, $p < 0.001$), whereas MIIT induced a decrease from 0.26 ± 0.08 to 0.24 ±0.07µg/dL (-9.3%, $p = 0.041$). The group-by-time interaction for cortisol was significant ($p < 0.001$, $\eta 2p=0.38$, large effect). HIIT decreased RMSSD and SDNN while increasing the LF/HF ratio ($p < 0.001$), indicating sympathetic dominance. Conversely, MIIT increased RMSSD (+18.2%, $p = 0.004$), indicating parasympathetic activation. Both modalities reduced state anxiety, but MIIT demonstrated a significantly greater reduction than HIIT (–20.9% vs. –12.2%, $p = 0.012$).

Conclusion: HIIT elicits a strong acute physiological stress reaction marked by HPA-axis and sympathetic dominance, whereas MIIT promotes parasympathetic reactivation and superior short-term anxiolytic effects. Exercise prescriptions should align with individual stress profiles.

Keywords: *Anxiety; Autonomic nervous system; Exercise therapy; Heart rate variability; High-Intensity interval training; Hydrocortisone; Stress, Physiological*

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INTRODUCTION

Stress is recognized as a major factor in adverse health outcomes, and it is more commonly associated with young adults and university students, who are exposed to academic, social, and environmental stressors. Chronic stress has been shown to be linked to alterations in autonomic nervous system function, dysregulation of hypothalamic-pituitary-adrenal (HPA) axis, and a higher risk of anxiety & mood disorders [1]. It is important to establish lifestyle interventions that can modulate stress biomarkers. Structured interval training, a form of physical activity, has been recognized as an effective strategy in modulating physiological and psychological markers of stress [2].

High-Intensity Interval Training (HIIT) and Moderate-Intensity Interval Training (MIIT) are two popular modalities of exercise. These modalities differ in training intensity and production of physiological stress. HIIT activates the sympathetic nervous system (SNS), which leads to the release of cortisol via the HPA axis, by inducing a rapid increase in catecholamines, heart rate, and metabolic rate [3,4]. Although the acute stress response is beneficial for improving resilience and metabolic fitness, it also

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temporarily increases physiological stress, as indicated by the increase in salivary cortisol[5]. Conversely, MIIT induces a mild autonomic response, resulting in increased parasympathetic activity and psychological relaxation, thereby reducing the release of cortisol and the feeling of stress [6, 7].

Salivary cortisol is a commonly used non-invasive marker for HPA axis activity, particularly for acute responses to exercise-induced stress [8]. An increase in the level of exercise intensity leads to increased cortisol levels, whereas moderate exercise can cause a drop or maintain the level of cortisol, depending on the duration of training and fitness level of the participants[9]. Heart rate variability (HRV) is another potent marker for the autonomic nervous system (ANS) balance. HIIT has been reported to cause a drop in HRV due to increased sympathetic activity, whereas MIIT leads to increased HRV due to increased parasympathetic activity [10, 11].

Aside from physiological biomarkers, the use of the State-Trait Anxiety Inventory (STAI) also helps to evaluate the mental state of an individual. Exercise has been proven to reduce state anxiety, regardless of the exercise intensity[12]. However, emerging data suggest moderate exercise intensity is more beneficial for anxiety relief, whereas high exercise intensity increases anxiety before exerting its benefits [13, 14].

Despite considerable scientific literature on the effects of exercise on stress physiology, direct comparisons of the effects of HIIT versus MIIT on physiological (cortisol, HRV) and psychological (anxiety) stress markers are still limited, particularly in young adults. Additionally, the majority of the literature has concentrated upon the chronic effects of exercise, whereas the effects of single exercise-session are of considerable interest for its prescriptions and stress management in daily life [15, 16].

Understanding the stress response to HIIT versus MIIT is of particular interest for the design of exercise programs for university students, who have different levels of fitness and may have different responses to exercise intensity. The effects of a single bout -exercise may give immediate insights into which type of exercise modality is the best for stress management.

This study examined the acute effects of a single bout of HIIT versus MIIT on salivary cortisol, HRV parameters, and state anxiety in healthy university students. This study offers a comprehensive assessment of exercise-induced stress responses by examining both physiological and psychological markers of stress. This will help in developing recommendations for reduction of stress by employing exercise interventions of optimal intensity.

CAPSULE SUMMARY

The acute impact of High-Intensity Interval Training (HIIT) versus Moderate-Intensity Interval Training (MIIT) on salivary cortisol, heart rate variability (HRV), and state anxiety was determined in young, healthy adults. HIIT elicited a strong acute physiological stress reaction, whereas MIIT promotes parasympathetic reactivation and superior short-term anxiolytic effects. Exercise prescriptions should be align with individual stress profiles.

METHODOLOGY

This parallel group experimental study was conducted as a single-visit open-label randomized controlled trial at the Exercise Physiology Laboratory, Department of Physiology, Khyber Medical University (KMU), Peshawar, from July to December 2025. The study had the approval from the Institutional Review Board (IRB), Ref No: KMU/IBMS/2025/205. Participation was entirely voluntary, and the confidentiality of the participants was maintained throughout. From the participants, informed consent was taken. Participants had the freedom to leave the study any time.

The acute effects of HIIT and MIIT on physiological and psychological stress markers were compared. Using computer-generated randomisation sequences, participants were assigned at random to one of the two intervention groups and were assigned to a single bout of exercise, either HIIT or MIIT. Opaque sealed envelopes were used to ensure allocation concealment. Because of the nature of the exercise interventions, it was not possible to blind participants and investigators. Pre- and post-intervention salivary cortisol, HRV, and anxiety levels were measured. This study was carried out under controlled conditions and lighting to minimize stress induced by external factors. The test was conducted between 8:00 am and 12:00 pm to account for the diurnal variation of cortisol levels and HRV.

Study participants were recruited from the constituent colleges of KMU through classroom announcements, electronic messages, and social media. The study setting was equipped with standard exercise machines, HRV recording devices, and salivary sample collection facilities. One orientation session was conducted before data collection to familiarize the study participants with the exercise machines.

Eligible participants included full-time university students, between 18 and 30 years of age, who were physically active, with at least two exercise sessions per week for a minimum of 30 minutes per session, and without any medical contraindications to high-intensity exercise, as well as without any acute illness, infection, or injury during the study period.

Exclusion criteria included individuals with chronic conditions such as cardiovascular, respiratory, and endocrine disorders, as well as those who were using medication that could affect cortisol, autonomic, and anxiety responses, smokers, and individuals who consumed caffeine within 12 hours of testing, irregular sleep patterns with less than 5 hours of sleep before testing, and those females who were menstruating at the time of cortisol sampling.

The sample size was determined using G*Power software

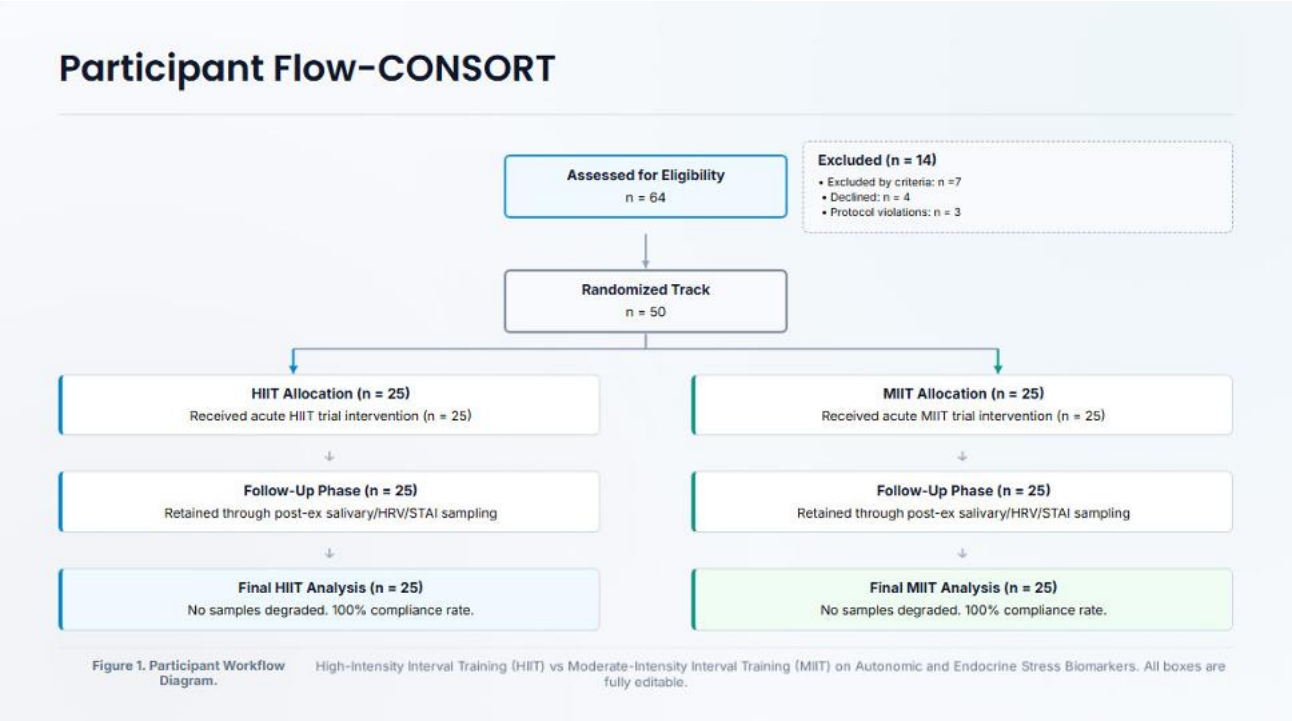

Figure 1. CONSORT Participant Flow Diagram.

(version 3.1), for a repeated measures mixed ANOVA (interaction within x between). The calculation involved two groups (HIIT and MIIT), two measurements (pre- and post-exercise), the level of significance (α) at 0.05, the statistical power (1 − β) at 0.80, correlation among repeated measures at 0.50, and the non-sphericity correction (ε) at 1.0. The effect size (Cohen's f = 0.25) was estimated assuming a medium effect size as recommended by Cohen when similar interaction effect sizes in the literature cannot be used [17]. Even though there are no studies that report the effect size of a similar interaction (effects of HIIT versus MIIT on salivary cortisol, HRV, and anxiety), exercise interventions have shown moderate to large group-by-time interaction effects in autonomic variables. For instance, Ketelhut et al found significant interaction effects on HRV parameters after HIIT interventions corresponding to effect sizes larger than the conventional medium[18]. Thus, a medium effect size (f = 0.25) was chosen a priori. With such assumptions, the required sample size comes out to be 40. Keeping in mind the possibility of attrition and sample loss, the sample size target was increased by 20%, resulting in a final target of 50 participants.

Figure 1 shows the flow of participants from the stages of screening, recruitment, random assignment into either the HIIT group (n=25) or MIIT group (n=25), to the different follow-up periods, and finally to the analysis stage. In HIIT, subjects were subjected to 5 minutes of warm-up, with a light cycle workout at 30-40% of HRmax.

The main HIIT routine entailed 10 cycles of 1-minute cycling at 85-95% of Hrmax, followed by 1 minute of recovery at 40-50% HRmax (for a total of 20 minutes). After this, there was a 5-minute cool -down period with light cycle exercises. The total time duration for the intervention process was exactly 30 minutes.

Regarding MIIT, participants completed a 5-minute warm-up of light cycling at 30–40%HRmax. The MIIT protocol consisted of 10 intervals of 1-minute cycling at 55–65%HRmax, interspersed with 1-minute passive recovery intervals (totalling 20 minutes). The session concluded with a 5-minute cool-down phase of light cycling, resulting in an identical total intervention duration of 30 minutes. Active recovery was incorporated into the HIIT protocol to maintain the prescribed high exercise intensity across successive intervals and to align with established HIIT programming recommendations. In contrast, passive recovery was employed during MIIT because the exercise intensity remained within the moderate range, and additional activity during recovery could have increased the overall exercise workload beyond the intended moderate-intensity stimulus. The recovery modalities were therefore selected to preserve the distinct physiological characteristics of each training protocol.

Saliva samples were collected using sterile saliva tubes. The samples were collected at pre-exercise (10 minutes seated rest). The second sample was collected at post-exercise (20 minutes after the end of the exercise, which is the time at which the cortisol levels peak)[19]. Before the experiment, participants were prohibited from eating, drinking, or brushing their teeth for an hour.

Salivary cortisol concentrations were determined by using a human cortisol enzyme-linked immunosorbent assay

(ELISA) kit, Calbiotech (Catalog No. CO368S; 96-test format, 12 × 8 breakable strip wells) according to the instructions of the manufacturer. The assay had a standard range of 20–400 ng/mL, an analytical sensitivity of 20 ng/mL, and required a sample volume of 25 µL per well. Cortisol concentrations were calculated from the standard curve generated for each assay and expressed as µg/dL. The ELISA kits were stored at 2–8°C until use.

Continuous electrocardiographic R-R intervals were recorded using a validated chest strap heart rate monitor, linked to the COSMED system. Short-term 5-minute stationary recordings were captured at pre-exercise (following a 10-minute seated stabilization period) and post-exercise (at a designated 5-minute quiet resting window). Time-domain metrics extracted included the Root Mean Square of Successive Differences (RMSSD) and the Standard Deviation of NN Intervals (SDNN), while frequency-domain analysis calculated the low-frequency to high-frequency (LF/HF) ratio.

Acute psychological state anxiety was evaluated using the State subscale of STAI, developed by Spielberger[20]. A validated regional translation of the 20-item scale was utilized, where elevated values correlate directly with higher perceived state anxiety. In this study population, the instrument demonstrated high internal consistency and reliability, achieving a baseline Cronbach's alpha (α) of 0.94.

The participants reported to the lab following a 12-hour abstinence from caffeine, stress, and strenuous exercise. After a 10-minute rest, baseline saliva cortisol, HRV, and STAI-state measures were recorded. The participants then performed the exercise task for which they were randomly assigned. The saliva samples and HRV recordings were collected, following the standardized time intervals post-exercise. The procedures were performed by trained lab staff who were unaware of the hypothesis.

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots. Independent-samples t-test for continuous variables and Chi-square tests for categorical variables were utilised to compare the baseline physiological and demographic characteristics between groups.

Within-group pre-to-post changes in physiological and psychological outcomes were assessed using paired-samples t-tests. The primary study outcomes were analyzed using a 2 × 2 repeated-measures mixed-design ANOVA, with Group (HIIT vs. MIIT) as the between-subject factor and Time (Pre vs. Post) as the within-subject factor. The Group × Time interaction term was considered the primary effect of interest, indicating whether changes over time differed between the intervention groups. Effect sizes were reported as partial eta-squared (η^2p), with values of 0.01, 0.06, and 0.14 representing small, medium, and large effects, respectively. Statistical significance was set at $p < 0.05$.

Table 1. Baseline Characteristics of Participants (n=50)

Variables	HIIT (n = 25) n(%)	MIIT (n = 25) n(%)	t / χ2 value	p-value
Gender Male Female	 13(52) 12(48)	 12(48) 13(52)	0.080	0.79
Variables	HIIT (n = 25) mean±SD	MIIT (n = 25) mean±SD	t / χ2 value	p-value
Age (years)	22.8 ± 2.1	23.1 ± 2.3	-0.485	0.56
BMI (kg/m²)	23.7 ± 2.6	23.4 ± 2.9	0.385	0.68
Resting HR (bpm)	75.6 ± 6.8	76.4 ± 6.5	-0.425	0.62
Salivary Cortisol (µg/dL)	0.265 ± 0.07	0.258 ± 0.08	0.330	0.72
RMSSD (ms)	34.8 ± 10.9	35.2 ± 11.3	-0.127	0.88
SDNN (ms)	48.1 ± 12.4	49.3 ± 13.1	-0.333	0.81
LF/HF ratio	2.41 ± 0.8	2.38 ± 0.7	0.141	0.87
STAI-State	42.6 ± 7.2	43.1 ± 6.9	-0.251	0.78

BMI, Body Mass Index; HR, Heart Rate; STAI, State–Trait Anxiety Inventory; RMSSD, Root Mean Square of Successive Differences; SDNN, Standard Deviation of NN Intervals; HRV, Heart Rate Variability; ms, milliseconds.

Table 2. Pre- and Post-Exercise Salivary Cortisol Levels (n=50)

Group	Pre-exercise (µg/dL) mean±SD	Post-exercise (µg/dL) mean±SD	% Change	Within p-value	Interaction p-value (Time X Group)	Effect size (η^2_p)
HIIT	0.27 ± 0.07	0.36 ± 0.08	32.80	<0.001	<0.001	0.38 Large
MIIT	0.26 ± 0.08	0.24 ± 0.07	−9.3	0.041		

HIIT, High-Intensity Interval Training; MIIT, Moderate-Intensity Interval Training; µg/dL, micrograms per decilitre.

Table 3. Pre and Post Exercise HRV parameters (n=50)

Variable	Group	Pre-exercise mean±SD	Post-exercise mean±SD	% Change	p-value (within group)	Interaction p-value (Time X Group)	Effect size (η^2_p)
RMSSD (ms)	HIIT	34.8 ± 10.9	24.3 ± 8.7	−30.2	<0.001	<0.001	0.44 Large
	MIIT	35.2 ± 11.3	41.6 ± 12.1	18.20	0.004		
SDNN (ms)	HIIT	48.1 ± 12.4	39.7 ± 11.6	−17.5	0.01	<0.001	0.26 Large
	MIIT	49.3 ± 13.1	53.9 ± 14.0	9.40	0.03		
LF/HF ratio	HIIT	2.41 ± 0.8	3.12 ± 1.0	+29.5	<0.001	<0.001	0.32 Large
	MIIT	2.38 ± 0.7	1.92 ± 0.6	−19.3	0.002		

RMSSD, Root Mean Square of Successive Differences; SDNN, Standard Deviation of NN Intervals; LF/HF ratio, low-frequency to high-frequency HRV ratio; HIIT, High-Intensity Interval Training; MIIT, Moderate-Intensity Interval Training; ms, milliseconds.

Group	Pre-exercise mean±SD	Post-exercise mean±SD	Mean Change	% Change	Within p-value	Interaction p-value	Interaction Effect Size (η^2_p)
HIIT	42.6 ± 7.2	37.4 ± 6.8	−5.2	−12.2	<0.001	0.024	0.10 (Medium)
MIIT	43.1 ± 6.9	34.1 ± 6.5	−9.0	−20.9	<0.001		

STAI-State, State Anxiety subscale of the State–Trait Anxiety Inventory; HIIT, High-Intensity Interval Training; MIIT, Moderate-Intensity Interval Training.

Table 4. Pre and Post Exercise State Anxiety levels (n=50)

RESULTS

All 50 participants completed the study without any adverse effects. The compliance rate for the exercise protocol was 100%. The results also showed that the participants in the HIIT and MIIT study groups were similar in terms of demographic and physiological factors such as age, gender, BMI, heart rate, salivary cortisol level, RMSSD, and anxiety level. No significant difference was found in the baseline characteristics of study participants ($p > 0.05$) (Table 1).

There was a marked divergence in the endocrine stress reaction between the two groups following the intervention. The HIIT group showed a marked increase in salivary cortisol levels after the exercise, representing a 32.8% increase. Conversely, the MIIT group showed a mild decline in salivary cortisol levels after the exercise, representing a 9.3% decline. This indicates that high-intensity intervals triggered a marked endocrine stress reaction, whereas moderate-intensity intervals triggered a mild endocrine calming effect (Table 2).

Significant changes in autonomic responses were noted between the two groups. The HIIT group showed a reduction in HRV indices, where the values of RMSSD decreased by 30.2%, SDNN decreased by 17.5%, and the LF/HF ratio increased. This shows an increased level of sympathetic activity. The MIIT protocol resulted in increased parasympathetic activity, where the values of RMSSD increased by 18.2%, while the values of SDNN increased by 9.4%, along with a decreased LF/HF ratio. This shows that MIIT may help in the recovery of the ANS, leading to a relaxed state (Table 3).

Both modes of exercise led to a reduction in psychological stress as measured by the STAI-State scale; however, the degree of change was significantly different. The HIIT group had a reduced STAI-State score reflecting a 12.2% reduction in anxiety. The MIIT group had a more significant change, with the reduced STAI-State score reflecting a 20.9% reduction in state anxiety. These data suggest that, although both modes of exercise have a positive effect on the enhancement of the mood state, the effect of MIIT is more significant (Table 4).

DISCUSSION

This study investigated the acute effects of both HIIT and MIIT on physiological and psychological markers of stress in healthy young adults. The results indicated that clear distinctions exist between both modes of exercise. HIIT was associated with increased salivary cortisol and decreased HRV, whereas MIIT was associated with decreased cortisol, increased HRV, and greater anxiety-reduction responses. These results support the notion that exercise intensity plays an important role in acute stress responses.

The marked rise in cortisol seen in the HIIT group is consistent with previously established evidence showing that high-intensity exercise is a strong activator of the HPA axis, resulting in cortisol secretion in response to increased metabolic demands[21,22]. Although the rise in cortisol in high-intensity exercise is physiological and adaptive, in terms of survival responses, it also represents an increased endocrine stress response[23]. In contrast, the reduction in cortisol, seen in the MIIT group, is consistent with evidence showing that moderate-intensity exercise is associated with homeostasis, decreased endocrine stress, and improved psychological recovery.The results obtained from the HRV analysis again emphasize the different responses of the ANS to the intensity of exercise. The decrease in RMSSD and SDNN, along with an increase in LF/HF, in the HIIT group suggests a sympathetic dominance along with vagal withdrawal, which is a characteristic response of the autonomic ANS to acute physiological stress[24-26].

These changes in the ANS have been found in both trained and untrained subjects who have been subjected to high-intensity exercise[27]. The increase in RMSSD and SDNN, along with a decrease in LF/HF, in the MIIT group suggests a parasympathetic dominance, which has been found in previous studies where the parasympathetic nervous system shows an enhanced response to moderate-intensity exercise[28]. This enhanced parasympathetic response might be the reason for the enhanced anxiolytic response found in the MIIT group.

From a psychological perspective, both modes of exercise resulted in lower state anxiety levels, with MIIT showing almost double the percentage of reduction compared to HIIT.

Existing literature indicates that moderate-intensity exercises elicit optimal levels of arousal and improve affective responses without any discomfort, dyspnea, or fatigue, as observed with high-intensity exercises[29,30]. Although HIIT has been linked to long-term psychological benefits, existing literature indicates that the post-exercise period may lead to increased levels of distress or cognitive load, especially in individuals who are new to HIIT[31].

While these acute findings offer pivotal baseline evidence on single-bout responses, the long-term biological consequences remain a vital avenue for research. Future longitudinal interventional trials spanning 8 to 12 weeks are strongly recommended to evaluate whether chronic adaptations to HIIT modulate or attenuate this initial acute hyper-cortisolemic response through systemic habituation, or if sustained sympathetic stress creates an allostatic load risk in vulnerable student dynamics.

Additionally, it is also necessary to consider these biomarker changes in the regional context. The students studying in developing South Asian countries, particularly in public medical universities of Pakistan, will be facing their own set of baseline stress levels due to high competition and limited resources in the institutions. The regional environment of such stress management studies highlights that any lifestyle change must match the baseline stress of the individual to not exacerbate the already affected neuroendocrine system[31,32].

Overall, it can be concluded that there lies a conceptual

distinction between HIIT as a physiological stressor and MIIT as a modulatory stimulus. There are practical implications that result from the study. People who want to feel a strong stimulus to improve their metabolism or performance may find HIIT satisfactory. However, for people who want to alleviate stress, regulate emotions, or relieve anxiety, MIIT can be a better choice.

This study has several strengths. The randomization of the study design minimizes allocation bias. The measurement of salivary cortisol levels and HRV is a reliable method of assessing physiological responses. The measurement of the STAI-State scale allowed for a psychological measurement to be taken in addition to the biological measures.

Limitations: The study only examined the acute effects of a single session of exercise, and the chronic effects could differ significantly. Secondly, certain factors, such as the lack of blinding, level of fitness, and sleeping status, could have an impact on the levels of stress biomarkers, despite the standardized conditions. Thirdly, the study only examined young healthy adults, and the results cannot be generalized to the elderly and those with certain pathological conditions.

A potential limitation of the study is the use of different recovery modalities between the HIIT and MIIT protocols. Both active recovery for HIIT and passive recovery for MIIT may independently affect the studied parameters. While both recovery types were selected according to common exercise programs and in accordance with the planned exercise intensity range, their impacts may be difficult to separate from those of exercise intensity itself.

No clinical trial registration number is available for the current study. This may limit the transparency and reproducibility of the study.

CONCLUSION

This research highlights the significance of exercise intensity in the development of physiological and psychological stress responses in healthy adults. HIIT increases salivary cortisol levels and reduces HRV, suggesting higher levels of sympathetic and HPA activity. On the other hand, MIIT leads to lower cortisol levels, higher parasympathetic activity, and more change in state anxiety. These results indicate the importance of HIIT as a physiological stress reaction, while MIIT seems to have a more calming effect on physiological stress reactions.

Practical implications of the study show that MIIT is more appropriate for stress reduction or mood stabilization, while HIIT is more appropriate for those who want to improve metabolic responses despite a transient increase in physiological stress reactions.

ETHICAL APPROVAL: KMU/IBMS/2025/205.
CONSENT FOR PUBLICATION: *Written, informed consent was obtained from the study participants.*
AVAILABILITY OF DATA: *Data is available from the corresponding author on a justified request.*
FINANCIAL DISCLOSURE/ FUNDING: *None*
ARTIFICIAL INTELLIGENCE TOOLS DISCLOSURE: *None*
CONFLICT OF INTEREST: *None*
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AUTHORS' CONTRIBUTION

- ***Saman Tauqir:*** *Conception and design, Analysis and interpretation of data.*
- ***Shazia Shakoor:*** *Analysis and interpretation of data.*
- ***Fazeelat Hajra Kareem:*** *Acquisition of data.*
- ***Hira Faisal:*** *Drafting the article.*
- ***Miraj Ahmad Khan:*** *Critical revision.*

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