Sport Sciences for Health (2019) 15:453–461
https://doi.org/10.1007/s11332-019-00553-1

ORIGINAL ARTICLE

Anaerobic training and its effects on sleep quality, state, and trait anxiety in collegiate athletes

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Received: 26 November 2018 / Accepted: 15 April 2019 / Published online: 2 May 2019

Abstract

Aim The present study was undertaken to evaluate the effects of different anaerobic training regimes on state anxiety, trait anxiety, and sleep quality among collegiate athletes.

Methods Thirty-six collegiate soccer players fulfilling the eligibility criteria were randomly divided into three groups: complex training ($n=12$; BMI 22.95 ± 1.76 kg/m^2), contrast training ($n=12$; BMI 22.05 ± 2.03 kg/m^2), and control ($n=12$; BMI 22.27 ± 1.44 kg/m^2). Athletes from the complex or contrast group were trained for 6 weeks (3 days/week). The complex group performed four different exercises, each comprised of strength [80% of one repetition maximum (1RM)] and power components alternately. The contrast group performed the same strengthening exercises alternately at two different intensities (40% and 80% of 1RM). No supervised training was given to control group. All athletes were tested for their state anxiety, trait anxiety, and sleep quality before and after 6 weeks of training.

Results 3×2 mixed ANOVA revealed significant difference in time effect ($p \leq 0.001$) and time × group interaction ($p \leq 0.001$) for state anxiety while non-significant difference was found in the group effect. There was no significant difference found between the groups for sleep quality and trait anxiety.

Conclusions The results of the present study demonstrated that anaerobic exercises have a positive impact to reduce state anxiety. Therefore, coaches should utilize these findings and implement anaerobic exercises to the training regime for reduction of anxiety among athletes.

Keywords Plyometrics · Post-activation potentiation · Complex training · Contrast training agility · Vertical jump

Abbreviations

SQ	Sleep quality
SWS	Slow-wave sleep
REM	Rapid eye movement
TST	Total sleep time
1RM	One repetition maximum
PSQI	Pittsburgh Sleep Quality Index
STAI	State-Trait Anxiety Inventory
HPA AXIS	Hypothalamic–pituitary–adrenal axis

Introduction

Exercise influences multiple physiological and psychological functions in human body, and among them sleep and anxiety are the most important. Sleep and anxiety are bi-directional, with studies reporting that long-term sleep deprivation leads to pathological anxiety [1, 2], while, people suffering from anxiety disorders are also sleep deprived [3].

Sleep is an important modality for athletes to restore central nervous system with full body recovery [4]. In athletic population, sleep disturbances are commonly seen after training or competition, which may adversely affect the recovery process and cognitive functions [4]. Athletes who compete internationally are 84% more likely to have poor sleep quality than college athletes who compete at the regional level [5]. Further, while international athletes with good sleep quality have shown greater vigor, national athletes with poor sleep quality show more confusion, depression, and fatigue [5]. Elite athletes in particular, have been identified as having poorer quality and quantity of sleep, in

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comparison to the general population, with a prevalence of sleep disturbance ranging from 13 to 70% [6]. Juliff et al. [7] found that within a sample of 283 elite Australian athletes, 64% reported poor sleep prior to an important competition. The primary reasons for such poor sleep patterns could be nervousness, deteriorations in mood and/or confidence and elevations in physical and mental stress [8].

Sleep disturbance is known to hamper cognitive performance of an individual as well as increase the risk of exercise-induced injuries and reduction in coordination during individual or team sports. Learning and motor memory are correlated with slow-wave sleep (SWS), rapid eye movement (REM) sleep [9] and coupling of sleep spindles with hippocampal ripples [10] during the night. The outcome is overnight systems-level plastic reorganization within the brain, involving enhanced activation in the primary motor cortex [11]. Nishida and Walker [10] stated that motor skill improvements are mainly related to stage-2 non-REM sleep ($r = 0.55$, $p = 0.04$), in addition to the density ($r = 0.65$, $p = 0.01$) and power ($r = 0.57$, $p = 0.04$) of S2 waves.

Exercise is an emerging non-pharmacological intervention for poor sleepers with many studies supporting its promise to improve sleep quality [12]. In a meta-analysis by Kubitz et al. [13], acute effects of exercise (aerobic and anaerobic) on sleep were discussed, showing increase in stage 3 and stage 4 sleep, improvement in slow-wave sleep, increase in total sleep time and REM sleep latency, and decreases in sleep onset latency and REM sleep. In the same study, chronic effects of exercise (aerobic and anaerobic) were also listed demonstrating increases in slow-wave sleep and total sleep time, as well as decreases in sleep onset latency, REM sleep and awake time [13]. The chronic effects were larger than acute effects, suggesting that long-term involvement maximizes the beneficial effects of exercise. This has also been shown to be true for the effects of exercise on anxiety and depression [14].

Anxiety is the result of an individual's negative viewpoint, when under stress. Physical activity may stimulate complex body systems and trigger a cascade of events resulting in greater resilience to anxiety disorder [15]. A study conducted in 2010 by Conn [16] on healthy individuals, used different forms of physical activities to reduce their anxiety levels. Literature supports the notion that a single bout of aerobic exercise that lasts for around 15–20 min has anxiolytic effects on state anxiety, and multiple bouts of 50–60 min aerobic exercise produce anxiolytic effect on trait anxiety [17]. Studies showed that aerobic exercise resulted in notable decrease in self-report anxiety sensitivity [18, 19]. It has also been reported that significant reduction in state anxiety and systolic blood pressure occurred 60 min following a bicycle ergometery session [20]. Although limited findings indicate the anxiolytic effects of resistance exercising, Brand et al. [21] reported that chronic vigorous resistance exercise is positively related to adolescents' sleep and psychological functioning.

At present, exercise therapy is surpassing medication and psychotherapy in reducing anxiety and sleep deprivation. People (especially athletes) use different types of exercise as a tool to reduce anxiety and enhance sleep quality (SQ), because regular physical workout is known to promote healthy sleep cycles and is believed to have antidepressant and anxiolytic effects, while being acknowledged to protect against the detriments of stress [21, 22].

In light of what has been reported in the existing literature, it is apparent that aerobic exercise promotes better sleep, improves sleep quality and reduces anxiety levels in various populations. However, the efficacy of resistance exercises on SQ and anxiety among collegiate athletes remains unclear, thereby creating a need to explore the effects of various anaerobic exercises on sleep and anxiety. For this reason, we hypothesized that different anaerobic training affects sleep quality and anxiety differently. The purpose of the present study, therefore, was to evaluate the effects of different anaerobic exercises on sleep quality and state- and trait-anxiety among collegiate athletes.

Materials and methods

Subjects

After approval from the Institutional Ethics Committee of Jamia Millia Islamia and trial registry with clinical trials registry of India (Indian Council of Medical Research; registration number-CTRI/2017/09/009631), 36 male players from a college soccer group were selected for the investigation. Eligibility criteria were: players must have an experience of at least 4–5 years of playing at inter-university level, be able to squat 1.5 times their body weight (lower limb strength) and do five repetitions of the back squat with 60% of body weight in 5 s or less (lower limb speed) as per the guidelines of National Strength and Conditioning Association [23]. After informing athletes about the aim, risks, and the benefits of the research, written informed consent was obtained before commencement of the study procedures. Participants were enrolled during off-season to eliminate the effect of any other sport-specific drills and competitive play. All athletes, being habitual of evening training, were considered as evening types (E-types). They were randomly allocated to a complex group ($n = 12$; BMI 22.95 ± 1.76 kg/m^2), a contrast group ($n = 12$; BMI 22.05 ± 2.03 kg/m^2) or a control group ($n = 12$; BMI 22.27 ± 1.44 kg/m^2) through block randomization with a variable block size and the allocation ratio of 1:1:1 using a list of random numbers generated by Ralloc software before familiarization. Pre (or baseline) measures were taken after familiarization period

and post-readings were taken after 6 weeks of respective training (Fig. 1).

Experimental procedure

Both complex and contrast group members underwent familiarization for 2 weeks incorporating six sessions, each session including three sets of 12 repetitions. The intensity during this period was maintained at 40–60% of one repetition maximum (1RM). It was necessary to adapt the athletes to proper technique, to reduce the risk of possible injuries during the execution of the main training protocol. All participants were given 72-h rest (after familiarization) and then a baseline assessment for sleep quality and state- and trait-anxiety was carried out via questionnaires.

Interventions

The exercise was conducted in the evening between 7 and 9 pm, thrice a week for 6 weeks (total 18 sessions). Each session comprised of 10 min of warm-up, followed by the training regime, and then 8 min of cool-down. Training regimes for the complex and contrast groups are described in Table 1. The exercises were identical with respect to their biomechanics for both groups. Proper technique of exercise was ensured by the researcher before advancing them. Progression was done by increasing weights (strength exercises) and height (plyometric exercises). The control group did not receive any specific exercise protocol; however, they were allowed to continue their soccer training routine.

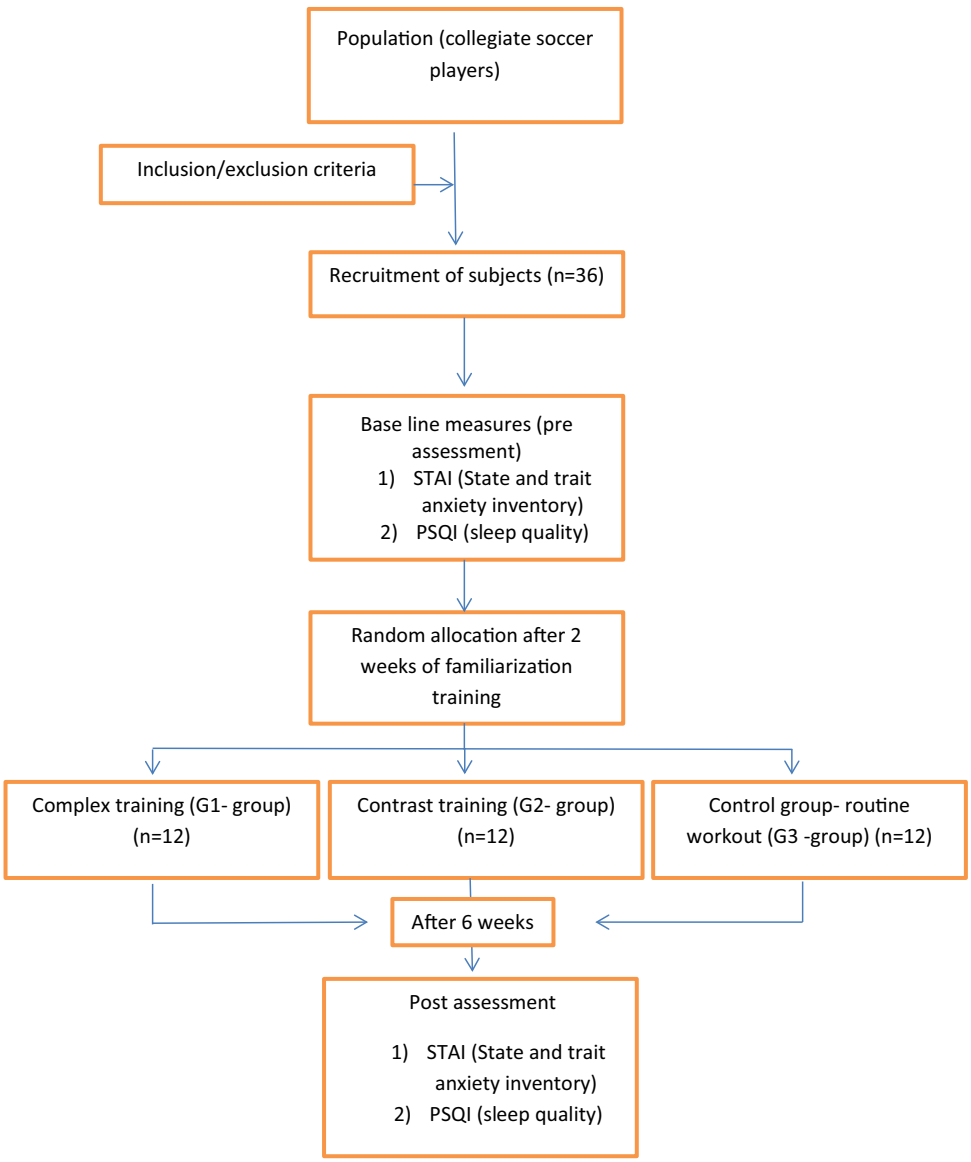

Fig. 1 Study flow diagram

Table 1 Exercise protocols

Phase 1: warm-up (10 min)		
2–3 min jogging and low-intensity strides, jumping, and bounding		
5–7 min dynamic stretching of lower limb muscles (quadriceps and hamstring calf)		
Phase 2: main phase		
Group	Exercise	Set × reps
Complex training	Squats (80% 1RM)	3 × 12
	Depth jumps	3 × 12
	Barbell lunge (80% 1RM)	3 × 12
	Split squat jumps	3 × 12
	Lateral lunge (80% 1RM)	3 × 12
	Lateral hops	3 × 12
	Calf raise (80% 1RM)	3 × 12
	Calf jumps	3 × 12
Contrast training	Squats(40% 1RM)	3 × 12
	Squats(80% 1RM)	3 × 12
	Barbell lunge (40% 1RM)	3 × 12
	Barbell lunge (80% 1RM)	3 × 12
	Lateral lunge (40% 1RM)	3 × 12
	Lateral lunge (80% 1RM)	3 × 12
	Calf raise (40% 1RM)	3 × 12
	Calf raise (80% 1RM)	3 × 12
*Recovery time between repetitions and sets were 30 s and 60 s, respectively		
Phase 3: cool-down		
5 min walk		
3 min stretching		

Testing evaluation

Participants attended three data collection sessions. On their first visit, they were evaluated for complete physical fitness including body mass index. All the athletes filled two questionnaires, Pittsburgh Sleep Quality Index (PSQI) for their sleep quality and state-trait anxiety inventory (STAI) to assess anxiety levels before and after 6 weeks of training for all groups.

STAI is more widely used in research and clinical practice. It has self-report scales for assessing state and trait anxiety. The S-anxiety scale (STAI Form Y-1) comprises of 20 statements that assess how an individual feels—right now, at this moment. The trait anxiety scale (STAI Form Y2) comprises of 20 statements that assess how individuals usually feel. The STAI-Y1 and -Y2 scales are printed on opposite sides of a one-page test sheet.

In acknowledgment of the STAI S-anxiety scale, subjects mark a number on the standard test sheet (at the right side of each statement) that more suitably explain the level of their emotions: (1) not at all, (2) somewhat, (3) moderately so, (4) very much so. In acknowledgment of the T-anxiety scale, subjects are advised to signify how they usually feel by rating the frequency of their emotional anxiety on a four-point scale: (1) almost never, (2) sometimes, (3) often, (4) almost always.

Sleep quality

Pittsburgh Sleep Quality Index (PSQI) is a valid measure of sleep quality [24]. It is a self-rated questionnaire which assesses sleep quality and patterns of sleep in adults over a period of 4 weeks. It consists of 19 individual items that are grouped into seven components—sleep quality, sleep latency, sleep duration, sleep efficiency and sleep alteration, use of sleeping pills and daytime dysfunction. It can be used as a screening tool to differentiate between poor sleepers and good sleepers [24]. We used the sleep-quality component for this research. The subjects filled the PSQI a day before the training independently. Reassessment was done 2 days after training on PSQI.

Statistical analysis

Data were analyzed using SPSS version 21. Normality of the data was ascertained using Shapiro–Wilk test. The demographic characteristics and baseline criteria measures were compared using one-way ANOVA and were found to be comparable between the groups for state and trait anxiety; however, non-parametric test Kruskal–Wallis was applied for sleep quality.

Participants in each group were tested using 3 × 2 mixed ANOVA with groups (complex, contrast, and control) and time effect (pre and post) for all the test variables. A p value of < 0.05 was considered as significant.

Results

The descriptive characteristics including age, weight, height, BMI were found to be comparable as assessed by one-way ANOVA (Table 2).

The results for STAI-Y1 showed a significant difference regarding the time effect ($p < 0.001$*). Moreover, time × group interaction analysis for STAY-Y1 also demonstrated a significant value with $p < 0.001$*. However, when group comparison analysis was done, STAI-Y1 showed no difference in either of the groups compared (Table 3).

Pre and post comparison of STAI-Y2 yielded a non-significant relationship when analyzed for either, time effect, group effect or time × group effect (Table 3).

SQ was also assessed using 3 × 2 mixed ANOVA, but non-significant values were obtained for all the effects including time, group and time × group interaction. The results implicate that there is a similar effect from all training on sleep and none in particular proved to be superior (Table 3).

Table 2 Comparison of demographics and outcome measures at baseline

	Complex, mean ± SD ($n = 12$)	Contrast, mean ± SD ($n = 12$)	Control, mean ± SD ($n = 12$)	One-way ANOVA (p value)	Kruskal–Wallis (p value)
Age (years)	22.00 ± 2.41	20.83 ± 2.08	21.50 ± 1.83	0.411	–
Height (cm)	173.38 ± 5.34	171.13 ± 5.95	171.15 ± 4.75	0.507	–
Weight (kg)	69.30 ± 5.15	64.9 ± 8.47	65.04 ± 6.15	0.207	–
BMI (kg/m^2)	22.95 ± 1.76	22.05 ± 2.03	22.27 ± 1.44	0.431	–
STAI Y1 pre	35.92 ± 5.08	37.58 ± 3.84	35.08 ± 4.94	0.418	–
STAI Y2 pre	36.75 ± 7.38	41.41 ± 6.69	40.33 ± 7.74	0.274	–
SQ pre	1.33 ± 0.49	1.00 ± 0.60	1.08 ± 0.66	–	0.38

BMI body mass index, *STAI Y1* State and Trait Anxiety Inventory Y1, *STAI Y2* State and Trait Anxiety Inventory Y2, *SQ* sleep quality

Table 3 Results (mean ± SD) of intervention groups (complex and contrast) and control group before and after 6 weeks using 3×2 mixed ANOVA

Variables	Complex (CLX)	Contrast (CST)	Control (CRL)	Time effect (p value)	Group effect (p value)			T×G interaction (p value)
					CLX vs CST	CST vs CRL	CRL vs CLX	
STAI-Y1				< 0.001*	1.00	1.00	0.412	< 0.001*
Pre-test	35.92 ± 5.08	37.58 ± 3.84	35.08 ± 4.9					
Post-test	30.16 ± 4.48	31.25 ± 4.45	36.58 ± 5.6					
STAI-Y2				< 0.885	0.297	1.00	0.62	< 0.972
Pre-test	36.75 ± 7.38	41.41 ± 6.69	40.33 ± 7.7					
Post-test	36.66 ± 6.00	41.66 ± 6.97	40.41 ± 8.0					
SQ				0.109	0.867	1.00	1.00	< 0.361
Pre-test	1.33 ± 0.49	1.00 ± 0.60	1.08 ± 0.66					
Post-test	0.91 ± 0.28	0.83 ± 0.83	1.08 ± 0.51					

STAI Y1 State and Trait Anxiety Inventory Y1, *STAI Y2* State and Trait Anxiety Inventory Y2, *SQ* Sleep quality, *CLX* Complex group, *CST* Contrast group, *CRL* Control group

STAI-Y1 showed significant difference with time effect ($p < 0.001$) and group × time interaction ($p < 0.001$); however, group effect showed no significant difference after 6 weeks between all three groups in state anxiety. Mixed analysis of variance revealed that there was no significant difference between the groups for STAI-Y2 inventory for trait anxiety and PSQI measure of sleep quality (SQ) (Table 3).

Discussion

The main finding of the study was that anaerobic exercises reduce state anxiety. Both the complex and contrast groups showed reduction in the state anxiety level, although trait anxiety was unchanged. In addition, the statistical analysis of PSQI revealed no change in sleep-quality scores among all groups.

The present study showed a positive impact of exercise on state anxiety and the results were consistent with Petruzzello et al. [14], who showed that exercises (aerobic or anaerobic) have a positive effect on state anxiety but not on the measures of trait anxiety. Similarly, Wipfli et al. [25] demonstrated that exercises (effect sizes for aerobic and anaerobic were − 0.40 and − 1.15, respectively) are more effective for stress reduction than other non-pharmacological interventions; their meta-analysis demonstrated the anxiolytic effect of exercise is similar to pharmacological treatments or cognitive behavioral therapy [26]. Moreover, there are very less reported side effects of exercise as compared to pharmacological therapies [25, 27]. Therefore, exercise should be preferred over other conventional treatment for anxiety reduction.

Many physiological and psychological theories have been introduced to account for this mood enhancement as a result of exercise. Among those physiological theories, the effect of exercise on endorphins action within the central nervous system has gotten more attention [28]. While quite a few studies found that exercise (40–80% VO_2 max) increases plasma endorphins [29, 30], they failed to establish a correlation between endorphins concentration and mood enhancement. Still, studies did find that an endorphin

antagonist like naloxone was unable to completely reverse the mood enhancement after long-distance running exercises (mean weekly mileage = 41.5 miles) [31]. Another known fact is that glucocorticoids like cortisol are elevated due to stress [32] even low-intensity chronic exercise can affect morning cortisol level [33], and these elevations activate hypothalamic–pituitary–adrenal (HPA) axis which increases anxiety. Many studies have reported that physical activity interacts with the HPA system to reduce depressive and anxiety-related symptoms by its attenuated response to stressful stimuli. Cortisol concentration is also affected by the time of day for exercising and chronotype of the athletes; these factors also eventually affect mood and anxiety [34]. Athletes in this study were habitual evening trainers and so trained in the evening during the study, which could be a reason for athletes showing reduction in anxiety following exercise bouts.

Different types, duration, and intensities of exercise also have varying effects on anxiety, as demonstrated by various studies. Some report that 10 min brisk walking is enough to increase energy and reduce tension [35], and others indicate that more than 1 h of aerobic exercise improves mood and reduces anxiety [36]. Petruzzello and colleagues [14] conducted a meta-analyses for three measures of anxiety (state, trait, and physiological) and found that the first 20–30 min of running at 75% VO_2 max had the largest effect on physiological measures of anxiety and more than 40 min of aerobic exercise had largest effects on trait anxiety but similar to state anxiety and physiological measures. Wipfli and associates [25] reported session durations of 60–90 min to be the most effective. With regard to various intensities, Farrell and colleagues [37] stated that exercises at 60–80% of VO_{2max} reduce anxiety but found no such effect with 40% VO_{2max}. Similarly, cycling at 60–70% of HRR reduced state anxiety, while 30% HRR or rest showed no such changes [38]. Moreover, while some studies revealed that programs lasting 4–12 weeks have largest effect sizes [25, 26], contrary Kugler et al. [39] did not identify program length as a moderator for the anxiolytic effect of rehabilitation exercise programs. Regarding the frequency of exercise, consistent 3–4 sessions per week showed greatest effect in certain studies [25], when others found greatest effect with three or five sessions per week, but not four [26].

Thus arises the question as to what/which intensity of exercise gives optimal results/effects on anxiety? And the answer can be found in a study done by Zervas et al. [40], in which one group was allowed to choose their own intensity of aerobic exercises and the results were compared with low-, moderate- and high-intensity exercise groups. It was found that the self-selection group improved more than others; these results supported the flow theory, which states that "flow"—a pleasurable state of consciousness—can only be achieved when individual competencies are realistically matched against the challenges of the task at hand [41]. This means that when an individual is unable to overcome the given task or situation, the most likely results are frustration and anxiety. On the other hand, they get bored when the task is of very low intensity or not challenging enough [42]. Consequently, some individuals find discomfort with high-intensity exercises; when others, more fit and active individuals who categorize themselves as physically active, take pleasure in exercising and exhibit positive mood enhancement post exercise [43]. From this theory one might conclude that individual optimal exercise intensity induces mood enhancement. It is possible that individuals who are more accustomed to exercise sensation feel more comfortable at higher intensities. But this is not always true as most studies failed to support this rationale and were unable to find differences between more or less active people [36, 44].

Various hypotheses also exist for exercise-induced anxiolytic effects. The thermogenic hypothesis postulates that mood enhancement after exercise is subjective and happens due to rise in body temperature [45], as rise in temperature was directly related to increase in anxiety and anxiolytic effects were apparent after body temperature dropped down [45, 46]. Petruzzello et al. [46], on the other hand, mentioned that reduction in anxiety after exercise could be due to variation in brain activity rather than core body temperature. Another hypothesis depends upon distraction and proposes that reduction of anxiety is not due to some specific activity or action, rather it is due to time-out or break from daily activities or stressors, distracting the thoughts and freeing the individual from disturbing thoughts or worries [45, 47]. However, we do not support this view as our study did not measure the instant results after or during exercises, instead we made assessments pre and post exercise protocols and in resting condition. Instead of these hypotheses we can agree on the mastery hypothesis which states that the completion of an important and effortful task brings about a sense of mastery or achievement, thereby improving mood [48] this bears a striking similarity to Bandura's theory of self-efficacy, that conclude that mood enhancement after exercise was due to subjects' enthusiasm to having reached or overcome the assigned task [49]. Similarly, the confidence and self-efficacy of our athletes enhanced after training, which causes reduction in anxiety. Certainly, it would be impossible to disconnect this theory in the majority of this field of research [50].

Sleep and exercise influence each other through complex, bilateral interactions that involve multiple physiological and psychological pathways [51]. PSQI, an estimate of sleep quality, did not reveal any change in our study, thereby contradicting Brand et al. [21] and Brand et al. [52] who concluded that physical activity enhanced sleep quality as well as quantity. Brand et al. [52] reported that male soccer players who trained 12–13 h per week showed improvement

in sleep quantity as well as quality, reduction in sleep latency and sleep disturbance, and longer sleep duration when compared with controls.

Though, sleep quality is affected by many factors such as individuality, specificity, environment, competition timing (day or night game), rest-activity circadian rhythms (RARs), and exercise principles [51, 53, 54], which were not controlled in our study. RARs are involved in sleep–wake cycle control and thus compromised health status [54]. Vitale and colleagues [54] concluded that RARs is different for different sport disciplines due to their different training schedules, training loads and intensities. Sleep quality is also influenced by chronotype and can differ among individuals [55]. Chronotype is defined as circadian typology that represents the expression of an individual's circadian rhythmicity and it has categorized into three chronotypes: evening-types (E-types), morning-types (M-types), and neither-types (N-types) [56, 57]. Our study included evening-type athletes and revealed no significant difference in sleep quality. Yet, our study did not show any deterioration in sleep quality either, which can be considered a positive effect after training as previous literature suggested lower sleep quality and quantity among E-types individuals [55]. Our results are in line with the work of Vitale et al. [58] and Bonato et al. [59], who concluded that HIIT done in the evening will not hamper sleep quality if the athletes are evening-type but can affect morning-type athletes. Our training does not negatively affect sleep quality because our athletes were habitual to training in the evening and for E-types athletes those were accustomed to train late in the day, it does not hamper sleep quality [60].

It was also found that performance and status of athletes could hamper the sleep quality. Halson [61] studied junior soccer players and their sleeping quality and quantity through polysomnography and found that soccer players had greater sleep efficiency, shortened sleep onset latency, less awakenings after sleep onset, more time in stage 4 sleep, and less REM sleep. Here a question arises—does more physical activity relate to more sleep quality improvement? Unfortunately, there exists evidence for the contrary too. Schwenk et al. [62] reported that pain (48%) and sleep problem (28%) are the two most reported reasons for early retirement. Throughout their career, athletes constantly encounter many conditions that interfere with sleep and are responsible for acute and chronic sleep problems or disorders.

More careful and stern investigations are needed to find out the effects and benefits of exercise on sleep quality and quantity and on sleep patterns because the current research was not controlled for other sleep disturbing elements such as exposure to bright light, different food intake, mood, uses of various drugs such as anti-depressants and circadian rhythms. Moreover, basic metabolic rate (BMR), cardiorespiratory functions, glucose intake, and immune functions are all influenced by long-term exercise [63, 64], and it is hard to discriminate among the direct and indirect pathways involved in exercise-induced changes to sleep.

Conclusion

It can be concluded from the present study that anaerobic training positively affects psychology of athletes and assists them in reducing anxiety after training. It is also concluded that anaerobic training does not affect sleep quality negatively. Thus, it is suggested that anaerobic exercises be used to prevent anxiety disorder among athletes with maintenance of sleep hygiene.

Acknowledgements The authors acknowledge the financial support of the Centre for Physiotherapy and Rehabilitation Sciences, Jamia Millia Islamia, without which this work would not have been possible. They are grateful to the university soccer coaches for their support in recruiting players, and thankful to young soccer players who participated in the study. Sincere thanks to Irshad Ahmad (P.T.), Centre for Physiotherapy and Rehabilitation Sciences, Jamia Millia Islamia, New Delhi, for his help with statistical analysis of the data.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethics Committee of Jamia Millia Islamia and trial registry with clinical trials registry of India (Indian Council of Medical Research; registration number-CTRI/2017/09/009631) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all individual participants included in the study.

Funding None.

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