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Effects of inspiratory muscle training combined with circadian rhythm-based running programs in male children football players

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Abstract

Background Inspiratory muscle training (IMT) has been suggested to improve respiratory function and physical performance; however, evidence in preadolescent athletes remains limited. This study examined whether adding IMT to circadian rhythm-based running programs would enhance functional and pulmonary outcomes in preadolescent male football players with at least two years of regular football training experience.

Methods Seventy-five boys aged 10–12 years were categorized into morning-, intermediate-, or evening-type groups based on Morningness–Eveningness Scale for Children (MESC) scores, and each chronotype group was assigned to its corresponding running protocol with or without IMT. Over six weeks, participants completed circadian rhythm-based running sessions, and IMT groups performed graded-load IMT. Pre- and post-intervention assessments included agility, six-minute walk test (6MWT), pulmonary function (FVC, FEV₁, FEV₁/FVC), and respiratory muscle strength (MIP, MEP).

Results Agility and 6MWT performance improved most in the morning running + IMT group compared with evening running + IMT and running-only groups ($p < 0.001$). FVC and FEV₁ increased in both IMT groups, though gains were modest and smaller in the running-only group. Improvements in MIP and MEP did not differ significantly between groups.

Conclusion Combining IMT with morning running produced the greatest improvements in agility and functional performance, while pulmonary gains were moderate and respiratory muscle strength changes were comparable across groups. IMT may provide additional benefit when integrated into chronotype-aligned morning training in preadolescent football players.

Trial registration Effects of Inspiratory Muscle Training Combined with Circadian Rhythm-based Running Programs in Children Football Players, *ClinicalTrials.gov*: NCT06817486, Date: 27-01-2025.

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Keywords Inspiratory muscle training, Chronotype, Endurance running, Pulmonary function, Agility, Pediatric football players

Introduction

The term biological rhythms refers to the periodicity of physiological and behavioural processes within an organism. Among these, the circadian rhythm, which operates on an approximately 24-hour cycle, is fundamental to human health and performance [1, 2]. Numerous physiological variables, including sleep–wake cycles, body temperature, blood pressure, and hormone secretion, fluctuate in accordance with this rhythm. Proper synchronization of these biological processes with environmental cues maintains homeostasis, whereas disturbances in circadian alignment may lead to metabolic, cardiovascular, and neuroendocrine dysfunctions [3, 4].

The influence of circadian rhythm on exercise performance has been well documented in adults, showing variations in physical capacity across the day depending on environmental and physiological factors such as temperature, metabolic status, and hormonal activity [5–7]. Typically, athletes tend to exhibit superior performance during the late afternoon or evening, when body temperature and neuromuscular activation are at their peak [8, 9]. However, research focusing on children and adolescents is limited. Given that growth, maturation, and hormonal regulation differ substantially from those of adults, circadian rhythm may affect performance and recovery in children in unique ways that warrant further investigation [10, 11].

Children also exhibit distinct respiratory characteristics compared with adults, including smaller lung volumes, narrower airways, and relatively weaker respiratory muscles, which together increase ventilatory demand during exercise [12, 13]. As a result, young athletes are more prone to inspiratory muscle fatigue, particularly during high-intensity or endurance-based activities [14]. These developmental differences underscore the importance of designing age-specific interventions to improve respiratory muscle efficiency and reduce fatigue.

Inspiratory muscle training (IMT) has emerged as an effective and non-invasive strategy to strengthen the diaphragm and accessory respiratory muscles, resulting in improved ventilatory efficiency and exercise tolerance [15–17]. IMT enhances respiratory muscle perfusion and delays fatigue by attenuating the respiratory metaboreflex, thereby allowing greater oxygen delivery to the working muscles [18]. Respiratory metaboreflex refers to a physiological mechanism in which fatigue of the inspiratory muscles triggers sympathetic vasoconstriction, reducing blood flow to limb muscles and accelerating peripheral fatigue. When incorporated into sport-specific training, IMT may also improve trunk stability and

postural control, thus enhancing motor performance such as agility and balance [19, 20].

Although both circadian rhythm–based training and IMT have been studied individually, the integration of these two approaches in pediatric populations remains largely unexplored. The combination may be particularly valuable for children who experience marked diurnal variation in physiological performance and higher respiratory workloads compared with adults. Synchronizing IMT and running exercises with each athlete's chronotype could therefore maximize adaptation efficiency, reduce fatigue, and promote optimal physical development [21–23].

Given this rationale, the present study aimed to investigate the effects of inspiratory muscle training combined with circadian rhythm–based running on lower-limb strength, agility, and respiratory parameters in preadolescent male football players. It was hypothesized that supplementing running exercises with IMT would lead to greater improvements in functional and respiratory outcomes compared with running alone.

Materials and methods

Study design

This study employed a parallel three-group, pretest–posttest non-randomized (quasi-experimental) design. Participants were allocated to chronotype-specific running protocols according to their Morningness–Eveningness Scale for Children (MESC) scores (morning, intermediate, evening) [14]. All procedures were conducted following the Declaration of Helsinki, and ethical approval was obtained from the Gümüşhane University Scientific Research Ethics Committee (Date: 20.09.2024; Decision No: E-95674917-108.99-282503) [15]. Written informed consent was obtained from all participants and their parents prior to the commencement of data collection. A certified athletics coach assisted in planning and supervising the running interventions.

Participants

Seventy-five preadolescent male football players aged 10–12 years, each with at least two years of regular training experience, volunteered for the study. Participants were randomly assigned to one of three groups based on their scores on the Morningness–Eveningness Scale for Children (MESC): Morning Running + IMT (MRG + IMT), Evening Running + IMT (ERG + IMT), Running Only (RG). A priori power analysis was conducted using G*Power 3.1 software [16] to determine the required sample size. Based on an estimated large effect

size ($d = 0.8$; $\alpha = 0.05$; power = 0.95; $\eta^2 p = 0.8$), a minimum of 20 participants per group was required. To compensate for potential attrition, 25 players were included in each group.

The inclusion criteria were as follows: (a) Candidates must have at least two years of active football training, (b) be between the ages of 10 and 13, (c) be in good general health, (d) have no known respiratory or cardiovascular conditions, (e) provide written informed parental consent. The following criteria were used to determine exclusion from the study: The subject's medical history includes the following: (a) A history of lung disease or a current upper respiratory tract infection, (b) A history of current injury, (c) Medication use that may affect breathing or performance, (d) Individuals with $FEV1/FVC < 70$, (e) Participation in similar studies within the last six months.

Procedures

Data collection was performed at the Gümüşhane University Kelkit Aydın Doğan Vocational School Human Performance Laboratory. Each player visited the laboratory three times for measurement sessions—before, mid-way through, and after the 6-week training intervention. These visits included familiarization, pretest assessments, and posttest assessments. During the first visit, participants completed the MESC questionnaire and underwent baseline testing, including pulmonary function tests (PFT), maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), the 6-Minute Walk Test

(6MWT), and an agility test. The same measurements were repeated during the third visit, following completion of the 6-week intervention. All tests were conducted between 09:00 and 12:00 under standardized conditions, and participants were instructed to abstain from vigorous activity 24 h prior to each assessment (Fig. 1).

Body composition measurement

The height of the participants was measured using a standard height meter (Seca 769, Seca, Hamburg, Germany) with an accuracy of 0.1 centimeter while leaning against a wall without shoes. Body weight was measured using a digital scale (Beurer, model GS27) with an accuracy of 0.1 kg before and after the IMT program, which was itself incorporated into the 6-week running program. The participants' body weight was measured in kilograms (kg) without shoes and wearing shorts and a T-shirt to avoid affecting the results [17].

The Morningness-Eveningness scale for Children (MESC)

The Morningness-Eveningness Scale for Children (MESC), developed by Carskadon et al. (1993), is a tool designed to assess the daily preferences of school-age children [18]. The scale under consideration consists of 10 items with four or five response options. Scores on the scale range from 10 to 43. Carskadon et al. (1993) established 21 and 35 as the cut-off points for the scale. A positive correlation has been demonstrated between children's preference for mornings and their score on the scale. That is to say, as the score on the scale increases,

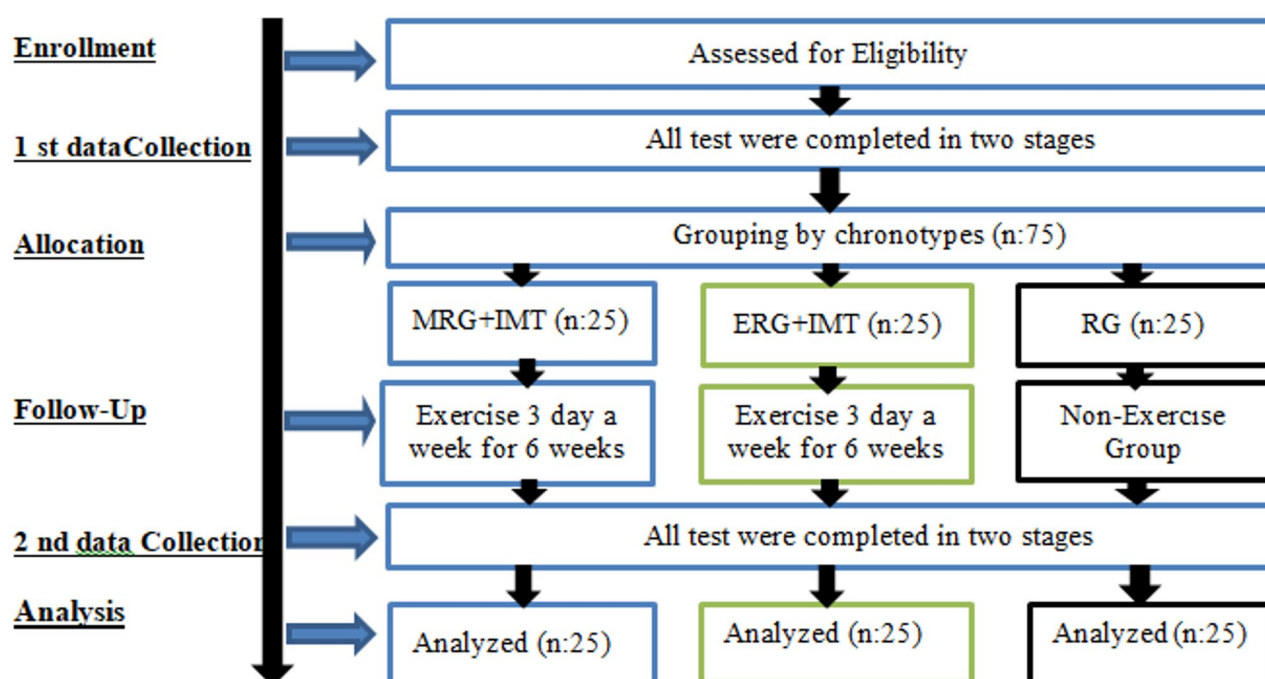


Fig. 1 Experimental design

the preference for mornings concomitantly increases. Scores ranging from 22 to 34 points are indicative of an intermediate type, while scores of 21 or below are associated with an evening type. Scores of 35 points and above are considered to indicate a morning type. The validity and reliability of the Turkish version of the scale were previously examined by Önder and Beşoluk (2013), who determined a Cronbach's alpha value of 0.72 [19]. In the context of the study, subjects who attained scores below 21 points were allocated to the evening group. Those who scored between 22 and 34 points were designated as the control group, which comprised individuals who engaged exclusively in jogging. Subjects who scored 35 points and above were assigned to the morning group.

505 agility test

This test consists of measuring the time taken to complete the last 5 m of a 15-meter track. The time within the first 10 m from the start of the test is not included in the test score. When the next 5 m distance is passed for the first time, the recording begins and stops when the same distance is covered in return [20].

Six-Minute Walk Test (6MWT) protocol

The 6MWT test is a reliable and valid functional test that can be used to assess exercise tolerance and endurance in healthy children [21]. The rationale behind our selection of this particular evaluation method stems from its status as the most pragmatic and ergonomic approach to appraising the submaximal extent of functional exercise capacity [22]. The Six Minute Walk Test (6MWT) instructions were provided to all participants by an exercise physiologist. The participants were instructed to walk as fast as possible while maintaining a comfortable pace for the duration of six minutes along a pre-measured straight path. During the test, the assessor recorded the time and provided standardized encouragement at each minute interval. The assessor also tallied the number of laps completed by each participant. At the sixth minute, the participants halted at their position on the path, and the assessor recorded the total distance traversed for the final lap. The evaluator then calculated and recorded the total distance walked over the 6 min [23].

Pulmonary function tests

FEV1, FEV1/FVC (Tiffenau index), and FVC capacity were analyzed via a CPFS/D USB spirometer from MGF Diagnostics (Saint Paul, Minnesota, USA). Measurements were taken between 15:00 and 17:00 for all participants to obtain the highest spirometric throughput [24]. Participants with FEV1/FVC < 75%, any chronic or pulmonary disease, medication that could affect lung function, or a history of upper respiratory tract infection were excluded from the study. Lung function tests were

performed with the participants in the standing position. During the tests, the participants wore a nose clip and were instructed to hold their lips tightly around the mouthpiece to prevent air from escaping [17].

Respiratory muscle strength

Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured via a hand-held portable oral pressure meter (MicroRPM, CareFusion Micro Medical, Kent, UK) according to the American Thoracic Society and European Respiratory Society guidelines [25]. With the appropriate filters and holders in place, the nasal airway was closed with a clip. The mouthpiece assembly included a 1 mm hole to prevent glottic closure and minimize the contribution of the buccinator muscles during inspiration. Inspiratory and expiratory maneuvers were performed in the standing position, with MIP and MEP measurements starting at the residual volume and total lung capacity, respectively, and continuing for at least 1 s. The measurements were repeated until there was a 5% difference between the two best results, and the results were recorded as the mean cm H₂O [26].

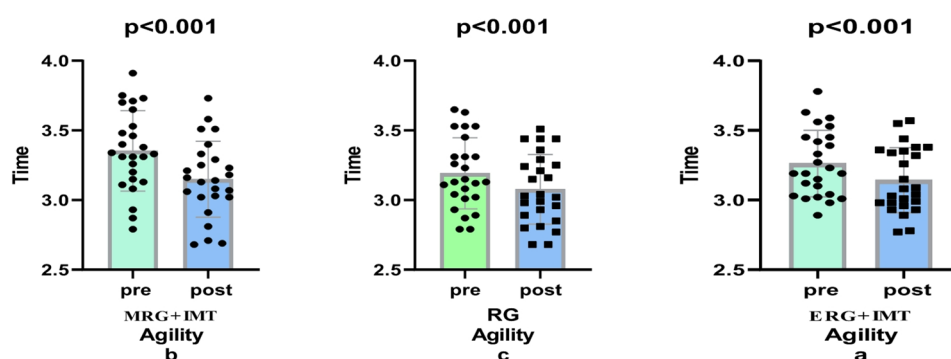
Running training

The morning running sessions were performed between 08:00 and 10:00, the evening sessions were performed between 18:00 and 20:00, and the control group, which only jogged, performed their sessions between 12:00 and 15:00 [27]. The exercise intensity for each participant in the running groups was determined as 50% of their heart rate (HR) calculated using the Karvonen formula: Target HR = [(220 - age - rest HR) × intensity] + rest HR. HR was monitored from the first week of training using a telemetric heart rate monitor (Polar M400, Finland). As environmental conditions can affect airway epithelial responses during high-intensity exercise [28], all participants performed continuous running exercises on a soccer field in Kelkit, Gümüşhane, Turkey (altitude: 1373 m). Each session lasted 50 min, including a 10-minute warm-up and cool-down each, and was performed at the set target HR three days per week over six weeks. All sessions were supervised by trained coaches. Warm-up and cool-down routines included static stretching and light exercises targeting relevant muscle groups. Coaches were responsible for monitoring athletes' running technique and speed, ensuring safety, and providing motivation. Both groups were adequately hydrated throughout the sessions to prevent dehydration [29]. The 50% HRR target was selected to provide a sustainable, submaximal training load appropriate for preadolescent athletes, prioritizing safety and minimizing excessive fatigue while still eliciting aerobic adaptations. This intensity is commonly used in pediatric exercise interventions and was monitored continuously by telemetric HR monitors.

Table 1 Descriptive statistics of the participants

	Age		Height		Weight	
	Mean	S.D	Mean	S.D	Mean	S.D
MRG + IMT (n:25)	11.40	0.61	145.2	4.79	37.12	5.53
ERG + IMT (n:25)	11.28	0.74	146.56	5.53	38.72	6.33
IMT (n:25)	10.92	0.70	144.28	4.92	35.88	5.22

SD Standard deviation

**Fig. 2** Comparison of agility scores by groups**Inspiratory Muscle Training (IMT)**

Inspiratory muscle training (IMT) was performed using the POWERbreathe® device (POWER® Breathe Classic, IMT Technologies Ltd., Birmingham, UK) [9, 11]. The IMT protocol was administered twice daily, in the morning and evening, for a period of six weeks, with the intervention occurring five days per week. Each training session comprised 30 breathing cycles (totaling 60 breathing cycles per day), and participants performed these exercises separately in the morning and evening [30]. Prior to the administration of the POWERbreathe® device, the resistance setting was calibrated to 40% of the participant's maximum inspiratory pressure (MIP), as reported in the study by Çelikel et al. (2025) [17]. The initial MIP value was increased by 10% on a weekly basis [17]. The training sessions were overseen by a certified trainer, who ensured that participants adhered to proper form. The morning IMT sessions were held between 8:00 and 10:00, and the evening sessions were held between 17:00 and 20:00 [27]. All IMT sessions were supervised at least once weekly by a certified trainer; during supervised sessions, trainers directly observed technique and recorded completion. Participants maintained daily training logs, checked weekly by coaches. POWERbreathe device usage was logged by coaches during supervised sessions; additionally, heart-rate monitors and attendance records for running sessions were used to cross-verify overall session compliance. We collected logs and attendance; mean adherence was 94%.

Statistical analysis

Data were analyzed using IBM SPSS Statistics 24. Descriptive statistics are presented as mean ± SD.

The normality of data was assessed using the Kolmogorov–Smirnov test. For within-group comparisons, paired-samples t-tests were used; for between-group and interaction effects, a two-way repeated-measures ANOVA (time × group) was applied. Cohen's d effect sizes were calculated and interpreted according to Cohen's thresholds: 0.20 = small, 0.50 = medium, 0.80 = large [31]. Statistical significance was set at $p < 0.05$.

Results

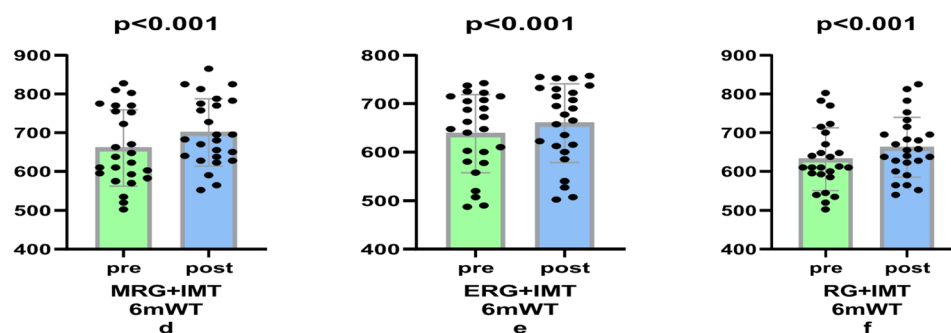
The study sample comprised 75 male football players who were assigned to one of three groups based on their scores on the Morningness-Eveningness Scale for Children (MES): morning running + IMT group (MRI + IMT), evening running + IMT group (ERG + IMT), or IMT only group. Table 1 presents a comprehensive overview of the subjects' demographic and physical characteristics.

The study revealed that the incorporation of inspiratory muscle training into a 6-week running training program led to a more significant enhancement in agility scores in preadolescent children within the MRG + IMT (e.s.: 0.714, 5.97%) group when compared to the ERG + IMT (e.s.: 0.511, 3.68%) and RG (e.s.: 0.431, 3.44%) groups ($F(2-72): 11.204, p < 0.001, np2:0.237$, Fig. 2a-b-c).

The study demonstrated that the incorporation of inspiratory muscle training into a 6-week running training program led to a more significant enhancement in 6MWT scores in preadolescent boys within the MRG + IMT (e.s.: 0.430, 5.93%) group when compared to the ERG + IMT (e.s.: 0.269, 3.41%) and RG (e.s.: 0.384, 4.79%) groups ($F(2-72): 8.971, p < 0.001, np2:0.199$, Table 2; Fig. 3d-e-f).

Table 2 RMA analyses of the parameters

Group →		MRG + RMT	ETG + RMT	IMT	F	p	np2
Agility	Pre	3.35 ± 0.29	3.26 ± 0.24	3.19 ± 0.26	11.204	$p < 0.001$	0.237
	Post	3.15 ± 0.27	3.14 ± 0.23	3.08 ± 0.25			
6mwt	Pre	660.80 ± 97.98	638 ± 80.71	632.1 ± 80.55	8.971	$p < 0.001$	0.199
	Post	700.70 ± 87.19	659.80 ± 81.4	662.40 ± 77.22			
FVC	Pre	2.01 ± 0.27	1.89 ± 0.20	2.01 ± 0.23	8.039	$p < 0.001$	0.183
	Post	2.59 ± 0.35	2.41 ± 0.29	2.25 ± 0.20			
FEV ₁	Pre	1.83 ± 0.22	1.80 ± 0.16	1.84 ± 0.21	4.597	0.013	0.113
	Post	2.35 ± 0.30	2.26 ± 0.25	2.14 ± 0.19			
FEV ₁ /FVC	Pre	0.87 ± 0.03	0.93 ± 0.05	0.89 ± 0.03	7.145	0.001	0.166
	Post	0.92 ± 0.04	0.95 ± 0.03	0.91 ± 0.04			
MIP	Pre	67.72 ± 11.88	70.68 ± 14.81	71.52 ± 10.76	1.578	0.213	0.042
	Post	92.40 ± 9.12	95.08 ± 9.82	92.20 ± 10.32			
MEP	Pre	79.68 ± 12.45	82.72 ± 14.44	84.16 ± 13.66	2.515	0.088	0.065
	Post	99.80 ± 10.79	104.40 ± 11.6	100.04 ± 12.16			

**Fig. 3** Comparison of 6MWT scores by groups

In the study, although MRG + IMT (e.s.: 1.856, 28.85%, $p < 0.001$) and ERG + IMT (e.s.: 2.192, 27.51%, $p < 0.001$) groups provided similar improvement in FVC scores in preadolescent children, the RG group (e.s.: 1.114, 11.94%, $p < 0.001$) provided less improvement (F (2–72):8.039, $p < 0.001$, np2:0.183, Table 2; Fig. 4g–k–n). FEV₁ scores improved more in the MRG + IMT (e.s.: 1.977, 28.41%, $p < 0.001$) group than in the ERG + IMT (e.s.: 2.192, 25.55%, $p < 0.001$) and RG (e.s.: 1.498, 16.30%, $p < 0.001$) groups (F (2–72):4.597, $p = 0.013$, np2:0.113, Table 2; Fig. 4h–l–o). FEV₁/FVC scores improved more in the MRG + IMT (e.s.: 1.414, 5.74%, $p < 0.001$) group than in the RG (e.s.: 0.566, 2.24%, $p < 0.001$) and ERG + IMT (e.s.: 0.485, 2.15%, $p = 0.129$) groups (F (2–72):7.145, $p = 0.001$, np2:0.166, Table 2; Fig. 4i–m–p).

MIP scores improved similarly in the MRI + IMT (e.s.: 2.330, 36.44%), ERG + IMT (e.s.: 1.942, 34.52%), and RG (e.s.: 1.961, 28.91%) groups (F(2–72):1.578, $p = 0.213$, np2:0.042, Table 2; Fig. 5q–r–s). MEP scores improved at a similar rate in the MRI + IMT (e.s.: 1.727, 25.25%) group as in the ERG + IMT (e.s.: 1.655, 26.2%) and RG (e.s.: 1.228, 18.86%) groups (F (2–72):2.515, $p = 0.088$, np2: 0.065, Table 2; Fig. 5t–u–v). Although large within-group effect sizes were observed for MIP and MEP, these reflect pre–post changes within groups. However, the group ×

time interaction ANOVA did not reach statistical significance, indicating that the magnitude of change did not differ significantly between chronotype groups. Therefore, the observed within-group improvements should be interpreted cautiously and independently from between-group statistical comparisons.

Discussion

The main aim of the present study was to examine the effects of inspiratory muscle training supplemented with six weeks of circadian rhythm-based running exercises on 6MWT, agility, and respiratory parameters in preadolescent male soccer players. To the best of our knowledge, this is the first study to examine this objective in preadolescent male soccer players. The primary findings of this study indicated that the incorporation of inspiratory muscle training into a six-week running training program resulted in a substantial enhancement in agility scores among preadolescent children. The MRG + IMT group demonstrated a more pronounced improvement in comparison to the ERG + IMT and RG groups. In the 6-minute walk test (6MWT), the MRG + IMT group demonstrated a superior performance enhancement in comparison to the ERG + IMT and RG groups. A notable enhancement in FVC and FEV₁ scores was

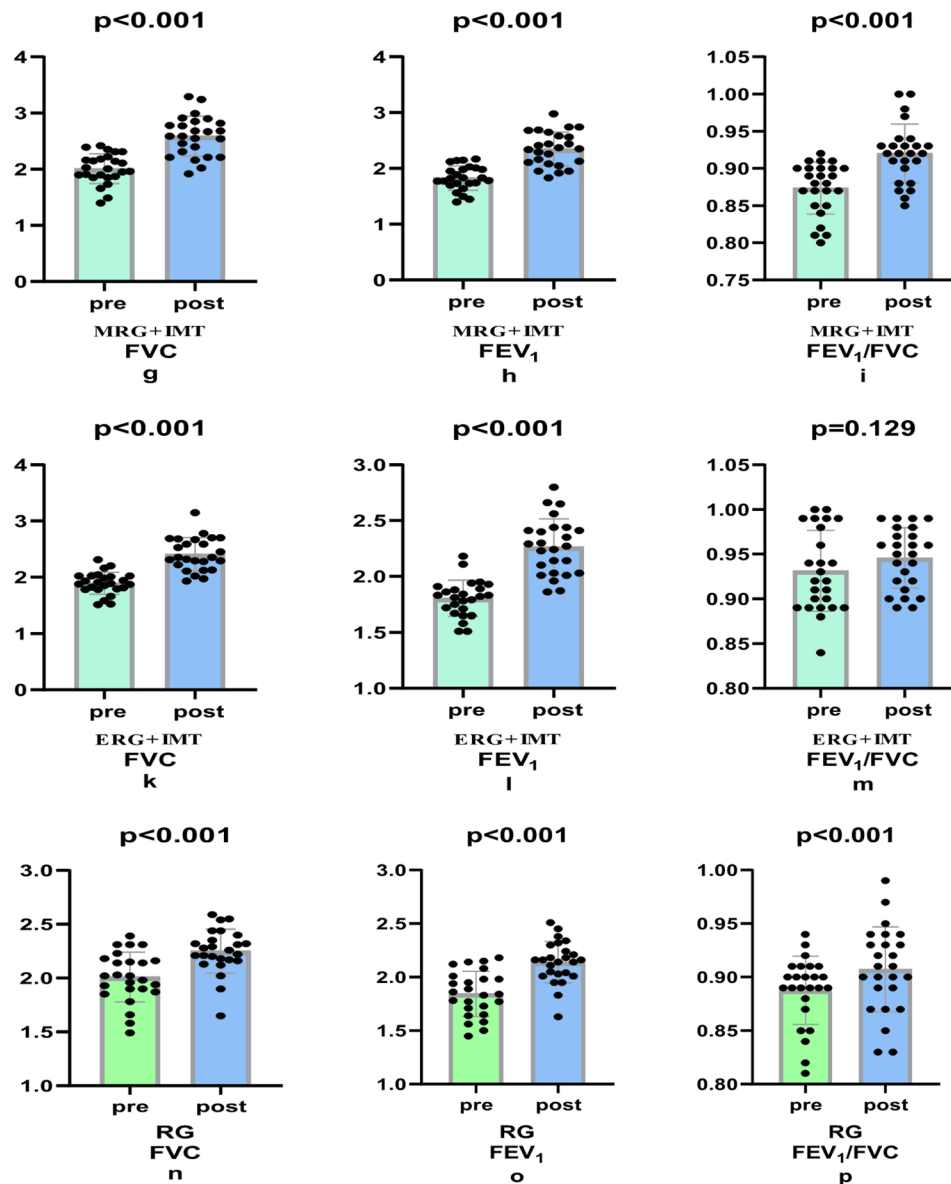


Fig. 4 Comparison of Pulmonary functions by groups

evident in the MRG+IMT and ERG+IMT cohorts. However, these enhancements were more modest in the RG group. The FEV₁/FVC ratio was significantly higher in the MRG+IMT group compared to the other groups, indicating an improvement in respiratory efficiency. The maximum inspiratory (MIP) and expiratory (MEP) pressure scores demonstrated comparable improvement across the study groups. These findings suggest that inspiratory muscle training, especially in combination with morning running training, is effective in the development of physical performance and respiratory parameters in preadolescent boys.

Previous studies using IMT protocols have found that they improve respiratory muscle strength, endurance,

and exercise performance [32–34]. This study seeks to answer the question of how the use of this effect, in addition to circadian rhythm-based running exercises, affects performance. The study findings show that inspiratory muscle training significantly increases agility and respiratory capacity, particularly when combined with morning maximum running protocols. In this study, IMT applied in addition to circadian rhythm-based running exercises significantly improved agility testing in preadolescent male soccer players. A significant increase in agility performance was observed, especially in the MRG+IMT group. Increasing inspiratory muscle strength improves lower extremity coordination by supporting trunk stability, which results in better performance in agility

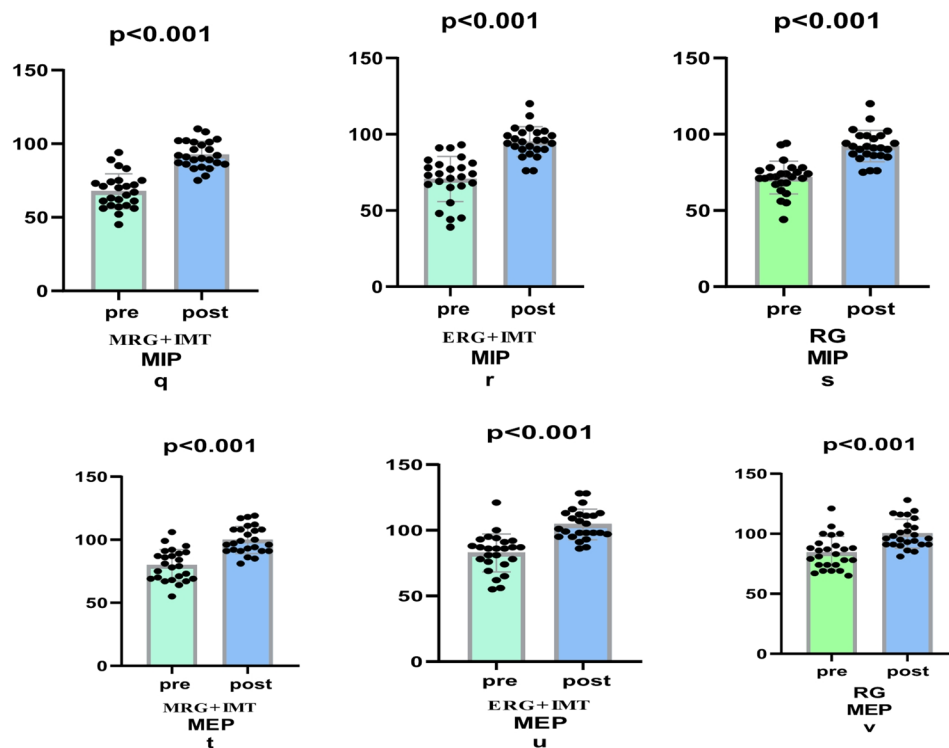


Fig. 5 Comparison of respiratory muscle strengths by groups

tests based on changing direction. Huguet et al. (1995) reported that running in the morning hours was more effective in terms of sprint performance in children aged 9–11 years, and that children's peak performance timing may differ from adults [35]. Similarly, Souissi et al. (2012) reported that morning training was more effective in terms of muscle strength and anaerobic performance gains in boys [36].

These findings are consistent with the literature supporting the positive effects of inspiratory muscle strength on dynamic balance and postural control [37]. Furthermore, it has been suggested that training in the morning hours may enhance performance by optimizing physiological responses to the circadian rhythm. The results of the study showed that the daily fluctuations in respiratory and agility parameters observed in the morning running group persisted after the training program. This suggests that parameters such as oral temperature alone are insufficient to explain time-of-day effects in anaerobic performance [38]. On the other hand, it has been reported that lower cortisol levels in the morning hours may limit athletic performance and delay peak performance [39]. It has also been emphasized in the literature that cortisol may have a supportive effect on performance under certain conditions [40]. In this context, it is stated that individual differences such as chronotype, training time, physiological status, and experience of the athlete should be taken into account when planning training programs

[41]. While adult studies often demonstrate pronounced time-of-day differences in performance and physiological responses, such findings cannot be directly extrapolated to children. Pediatric populations differ in respiratory system development, chest wall compliance, neuromuscular coordination, and hormonal maturation. Therefore, comparisons with adult literature should be interpreted cautiously, and age-related physiological distinctions must be considered. Physiological mechanisms such as circadian patterns in cortisol release, thermoregulation, or hormonal fluctuations may influence exercise performance in general, but our study did not measure hormonal variables. Therefore, any such interpretations should be considered tentative and cannot be directly supported by our findings. Further studies incorporating endocrine or thermoregulatory assessments are needed to clarify these mechanisms.

With regard to respiratory parameters, significant increases in FVC and FEV₁ scores were observed in the MRG+IMT and ERG+IMT groups. This finding suggests that inspiratory muscle training exerts a positive influence on ventilatory capacity by enhancing the strength of the diaphragm and accessory respiratory muscles [42]. The increase in the FEV₁/FVC ratio was particularly evident in the MRG+IMT group, indicating that IMT may improve respiratory efficiency with more intense exercise load. Conversely, no substantial disparities were observed between the groups in MIP and MEP

data. This finding may be explained by the comparable physical activity levels of all groups and the rapid adaptation of respiratory muscles to short-term stimuli [43].

Findings in the literature regarding the effects of circadian rhythm on pulmonary function and performance are inconsistent. Silva et al. (2006) found no significant circadian-related variation in the FEV₁ parameter in a study conducted on asthmatic children [44]. Similarly, although Boukelia et al. (2018) recommended morning hours for training in hot and humid environmental conditions, they did not observe a marked diurnal variation in FVC, PEF, FEV₁, FEF_{25–75%}, or the FEV₁/FVC ratio [45]. In parallel, Ünver and Atan (2021) reported no significant differences in respiratory muscle strength or oxygen saturation (SaO₂) levels at different time points of measurement [46]. On the other hand, some studies have demonstrated mild circadian effects. However, other studies have reported modest but measurable circadian variations in pulmonary parameters. For instance, Spengler and Shea (2000) reported small but statistically significant circadian fluctuations in FEV₁ and the FEV₁/FVC ratio in healthy adults, while FVC and PEF were unaffected [47]. In another study, Boukelia et al. (2017) found no statistically significant differences between pulmonary function tests conducted in the morning and afternoon among professional athletes; however, they noted that morning runs could increase physiological strain due to additional stressors such as fasting and cold exposure, which might in turn promote adaptive responses [27]. Additionally, Gaultier et al. (1977) identified a circadian rhythm in pulmonary resistance and dynamic lung compliance in healthy children, reporting peak values at specific times in the morning and evening (07:30, 11:30, 16:30, and 22:30) [48].

The parallel trends in the MIP and MEP data across all groups indicate the potential efficacy of shared mechanisms in the adaptation process of the inspiratory and expiratory muscle groups. As stated in the relevant literature, even brief applications of IMT have been demonstrated to elicit substantial enhancements in respiratory muscle strength [42]. However, in this study, the differences between the groups were not statistically significant, possibly because all groups were exposed to similar levels of physical activity. The training outcome corroborates the theory of training specificity, as evidenced by the morphological and functional similarity between inspiratory muscles and skeletal muscles. These muscles respond to specific physiological stimuli. The observed training effects align with the findings reported in studies on healthy adult athletes, suggesting that children of this age exhibit a comparable response to inspiratory training stimuli as adults [29].

The Six Minute Walk Test (6MWT) results demonstrated a significant improvement in the MRG + IMT

group. This finding aligns with studies that have demonstrated the efficacy of inspiratory muscle training in enhancing functional capacity, delaying the onset of fatigue threshold, and optimizing oxygen utilization efficiency during exercise [49]. In a study conducted on a sample of primary school children, researchers observed significant differences in physical fitness tests between morning and evening sessions [50]. The findings obtained from the shuttle run and standing long jump tests indicated that fitness parameters such as strength and cardiovascular fitness exhibited diurnal variations. Conversely, Woorons et al. (2020) reported that a high-intensity training protocol involving voluntary hypoventilation at low lung volume (VHL) during cycling led to enhanced running performance in team athletes [51]. This finding suggests that respiratory adaptations may have significant effects on exercise performance.

The superior 6MWT and agility outcomes observed in the morning training group may partly be explained by hormonal regulation patterns associated with the time of day. Although cortisol levels are typically higher in the morning and have been evaluated as potential performance suppressors in some studies [39], the integration of IMT into morning running sessions in the present study may have mitigated these effects by promoting respiratory and neuromuscular adaptation [40]. Notably, age-related disparities in thermoregulatory mechanisms have been observed. As indicated by Ahmed et al. (2008), children exhibit diminished diurnal variations in body temperature, suggesting the potential for divergent physiological responses to exercise and enhanced adaptability with respect to training scheduling [52]. It has been documented that children employ divergent strategies in comparison to adults when engaging in lower extremity motor control, underscoring the necessity for age-specific considerations in training responses [53, 54].

In conclusion, the present study demonstrated that the incorporation of inspiratory muscle training into circadian rhythm-based maximal running protocols, in the morning hours, resulted in a synergistic effect on both agility and respiratory capacity in preadolescent male soccer players. Consistent with the findings of previous studies in the relevant literature, IMT was determined to be a feasible and effective method in children. Adopting individualized approaches in designing training programs, which take into account children's physiological characteristics, chronotypes, and response to exercise, will contribute to maximizing performance improvement.

The present study demonstrates that integrating inspiratory muscle training into morning running programs can yield substantial benefits, including enhanced physical performance and improved respiratory function, in preadolescent boys. These findings suggest that IMT has

the potential to promote healthy respiratory function and support basic motor and physiological development during childhood. They also suggest that IMT should be included in training programs for practitioners.

Limitations

Although this study provides novel insights into the interaction between inspiratory muscle training and circadian rhythm-based running programs in preadolescent football players, certain limitations should be acknowledged. First, the relatively small and homogeneous sample (consisting exclusively of male participants aged 10 to 12 years with similar training backgrounds) restricts the generalizability of the findings to female athletes or children with different training experiences and maturation levels. Second, while chronotype was assessed using the Morningness–Eveningness Scale for Children, more objective measures such as actigraphy or melatonin rhythm profiling could have provided a more precise evaluation of individual circadian alignment. Third, the study duration was limited to six weeks, which may not have been sufficient to observe long-term adaptations in respiratory or performance parameters. Additionally, environmental factors such as temperature, humidity, and altitude were standardized as much as possible, but their subtle influence on pulmonary and exercise performance cannot be entirely ruled out. Finally, this study did not control for nutritional intake, sleep quality, or psychosocial stress, all of which may influence circadian rhythm and exercise performance. Future research involving larger, more diverse cohorts, longer intervention durations, and multimodal circadian assessments would strengthen the external validity and mechanistic understanding of these findings. In this study, it was hypothesized that all participants would reach maximum performance in all tests. Another limitation is the absence of monitoring for training load, sleep patterns, and dietary intake, all of which may influence circadian rhythm, recovery, and performance outcomes. Future studies should incorporate objective measures of these factors to better understand their interaction with chronotype and exercise responses.

Conclusions

The primary hypothesis of this study, which posited that IMT-augmented, circadian rhythm-based, running training interventions would enhance 6MWT, agility, and respiratory parameters in fit preadolescent male soccer players, was validated. The findings suggest that circadian rhythm running training is particularly effective in motor performance tests and certain respiratory functions, with this effect becoming more pronounced when combined with the IMT protocol. The combination of morning running training and IMT was found to be particularly efficacious in enhancing performance parameters. This

finding lends support to the notion that IMT may have a role to play in the development of training programs designed for preadolescent boys, thereby underscoring the MRG model's applicability in this context.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

Conceptualization, C.Y., E.K., S.D., A.S.; methodology, C.Y., S.D., A.S., E.K.; software, C.Y., E.K., M.H., O.A.; validation, C.Y., M.H., O.A.; formal analysis, C.Y., E.K., A.S., S.B.; investigation, C.Y., S.D., E.K., A.S., M.H.; resources, C.Y.; data curation, C.Y., M.H., O.A.; writing—original draft preparation, C.Y., E.K., S.B.; writing—review and editing, C.Y., E.K., S.D., A.S., O.A.; supervision, C.Y., E.K., S.D., A.S., M.H., O.A.; project administration, C.Y. and E.K., S.B. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Gümüşhane University (2024/7; dated E-95674917-108.99-282503). All participants had given written informed consent before the data collection began. Clinical trial number: NCT06817486, Date: 27-01-2025.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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