

Comprehensive evaluation of various training protocols for youth: effects on body composition, hemodynamics, and motor performance

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Published in

Frontiers in Physiology

Frontiers in Sports and Active Living



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ISSN 1664-8714
ISBN 978-2-8325-6621-3
DOI 10.3389/978-2-8325-6621-3

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Comprehensive evaluation of various training protocols for youth: effects on body composition, hemodynamics, and motor performance

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Citation

Domaradzki, J., Alvarez, C., Danek, N., Koźlenia, D., eds. (2025). *Comprehensive evaluation of various training protocols for youth: effects on body composition, hemodynamics, and motor performance*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-6621-3

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RECEIVED 09 June 2025
ACCEPTED 23 June 2025
PUBLISHED 03 July 2025

CITATION

Alvarez C and Domaradzki J (2025) Editorial:
Comprehensive evaluation of various training
protocols for youth: effects on body
composition, hemodynamics, and motor
performance.
Front. Physiol. 16:1644031.
doi: 10.3389/fphys.2025.1644031

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Editorial: Comprehensive evaluation of various training protocols for youth: effects on body composition, hemodynamics, and motor performance

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KEYWORDS

cardiometabolic disease, high-intensity interval training (HIIT), young, adults, human performance, moderate-intensity aerobic exercise

Editorial on the Research Topic

Comprehensive evaluation of various training protocols for youth: effects on body composition, hemodynamics, and motor performance

1 Introduction

There is a worrying increase in worsening cardiometabolic health conditions, including arterial hypertension ([Organization, 2025](#)) and diabetes ([WHO, 2024](#)), in several countries; different exercise training modalities, including moderate-intensity continuous (MICT), high-intensity interval (HIIT), resistance (RT), and concurrent training (CT), are widely recommended as preventive measures and treatment tools ([Kanaley et al., 2022](#); [Bull et al., 2020](#); [Pescatello et al., 2019](#)). In both young (i.e., children and adolescents in school environments) and adult populations, there is a need to increase the amount of exercise performed for these groups, as there is strong evidence for exercise treating these abnormalities; when performance is high, other specific exercise strategies are useful to optimize performance. In the present Research Topic, we aimed to summarize current exercise strategies for youth and adults at cardiometabolic risk or for performance optimization.

2 Short school-based interventions are effective in improving physical fitness and motor performance

[Domaradzki et al.](#) have, in their PEER-HEART study, examined ($n = 307$) adolescents to test two sets of 8-weeks of traditional HIIT and a plyometric HIIT (HIPT) in physical education classes. Both groups saw significantly reduced total body fat percentage and

systolic/diastolic blood pressure (SBP/DBP) while increasing maximum oxygen uptake (VO₂max). The authors conclude that brief HIIT or HIPT sessions are feasible for improving cardiovascular health in adolescents.

Jovanović *et al.* analyzed a 12-week school-based Tabata HIIT warm-up routine (2×/week, 4-min bouts), which replaced standard physical education class warm-ups, for (n = 30) Serbian boys (16 years), with 30 controls. Shuttle-run distance and estimated VO₂max increased in both groups, but improvements were ~1.4 fold larger with HIIT. HIIT additionally boosted standing long jump and countermovement jump performance and right handgrip strength (HGS) versus control. The authors conclude that brief Tabata HIIT is an efficient way to enhance cardiorespiratory fitness and lower limb power in adolescent boys.

Sun *et al.* have examined an 8-week randomized trial dividing (n = 18) sedentary, normal weight Chinese adolescents into thrice-weekly HIIT, MICT, or control groups. Both exercise modalities similarly reduced body fat mass and visceral fat area versus baseline. Only HIIT lowered waist-to-hip ratio and elicited marked falls in SBP (−6) and DBP (−11 mmHg) as well as triglycerides (−30%). The authors conclude that short, school-friendly HIIT is a more effective and time-efficient prescription than volume-matched MICT for reducing adiposity and cardiometabolic risk in sedentary youth.

3 The effects of different exercise modalities on body composition and hemodynamic parameters in youth

Min-Seong Ha studied a 16-week after-school combined exercise program (sports games plus MICT and RT), which enrolled (n = 33) Korean boys (11–12 years) with (n = 16) and without obesity (n = 17). In the obesity group, body fat percentage was reduced from 37.6% to 29.1% and muscle mass increased by ~31%. C-peptide and resistin fell markedly (−1.6 and −3.0 ng mL^{−1}), while insulin growth factor (IGF-1) and growth hormone increased by ~20%–25%. C-peptide and IGF-1 were thus considered mechanistic markers of training responsiveness in adolescent boys with obesity.

Nowak *et al.* have carried out a cross-sectional analysis of (n = 495) male academy footballers (12–16 years) producing normative percentile charts for speed, endurance, and power tests. Running times over 5 m, 10 m, and 30 m, standing long-jump distance, and maximal aerobic speed (MAS) from the 30–15 intermittent fitness test were recorded during training blocks from 2018 to 2022. The sharpest gains appeared between ages 13 and 14: sprint times improved by 0.087–0.215 s (5–10 m) and 0.438–0.719 s (30 m), long-jump length rose 31–48 cm, and MAS increased 0.3–0.6 m s^{−1}. Percentile grids (P3–P97) allow coaches to benchmark individual progress and detect outliers in development trajectories. These charts provide a practical tool for tailoring youth-soccer conditioning, optimizing load, and mitigating injury risk throughout adolescence.

Amare *et al.* conducted a randomized 8-week trial assigning (n = 24) inactive, overweight/obese Ethiopian men (~49 years) to MICT, RT, or CT. All groups saw lowered fasting blood glucose, insulin resistance, SBP/DBP, and waist-to-hip ratio compared to their baselines. RT and CT produced larger fasting glucose declines than MICT, and insulin resistance was reduced with RT compared

with MICT. SBP dropped most in CT and RT. The exercise modality explained up to 57% of the variance, summarizing that short-term RT or CT are effective exercise modalities for cardiometabolic risk reduction in overweight and obese middle-aged men.

4 The physiological mechanisms underlying exercise responses and support strategies

Feige *et al.* examined (n = 11) elite fin swimmers (children <17 years and adults 17–29 years) during dynamic apnea dives of 25–100 m. Transcutaneous pulse oximetry and heart-rate monitoring during repeated pool dives showed a stereotypical diving response-bradycardia during immersion followed by tachycardic rebound at surfacing. Longer apnea duration, rather than swim speed, produced the greatest falls in oxygen saturation, whereas higher speeds chiefly intensified cardiovascular workload. The authors conclude that real-life dynamic apnea evokes age-dependent cardiorespiratory stress and provide benchmark data to guide training and risk assessment in pediatric and adult divers.

Wu *et al.* studied (n = 90) male college athletes who completed 7 days of moderate-intensity training and were randomized to true transcranial pulse current stimulation (tPCS) or control. Daily 20-min tPCS at 1.5 mA was delivered immediately post-exercise, and fatigue was reported by subjective fatigue scale (RPE), functional near-infrared spectroscopy (fNIRS) cerebral oxygenation, and blood biomarkers. Compared with controls, the tPCS group reported lower RPE scores and showed smaller post-exercise drops in oxygenated hemoglobin concentration (Oxy Hb) and rises in deoxyhemoglobin concentration (HHb), total hemoglobin concentration (HbTot), and hemoglobin concentration difference (HbDiff). The authors conclude that brief daily tPCS is an effective, non-invasive countermeasure against the accumulation of exercise-induced fatigue by preserving central neural function.

Zhuan *et al.* applied a randomized crossover study testing whether adding three blood-flow-restriction (BFR) modes (continuous low, intermittent medium, and intermittent high to high-intensity [75% 1RM]) squat sessions boost lower limb and core muscle activation. Twelve RT college men performed three sets of eight deep squats under each BFR condition and a no-BFR control while electromyography, thigh circumference, and RPE were recorded. All BFR modes elevated vastus lateralis and vastus medialis maximum voluntary contraction during the first two sets versus the control. The authors conclude that continuous low-pressure BFR offers the most stable posterior-thigh engagement, while intermittent high-pressure BFR optimizes spinal-extensor activation and perceived exertion, making both viable add-ons to heavy squat training.

5 Conclusion and future directions

Taken together, all these studies support the concept that different exercise training modalities such as MICT, HIIT, RT, CT or other variations of these, increase the cardiorespiratory fitness and modify positively the body composition of young populations in school environments, dynamic apnea reduces diving risk, tPCS

is effective in decreasing exercise-induced fatigue, and low-pressure BFR modes improve heavy squat training performance.

Nevertheless, several open Research Topic remain:

- There is a pressing need to explore inter-individual variability in exercise response and identify predictors of responsiveness in the context of responders and non-responders to the exercise stimuli.
- Future work should compare different durations, intensities, and modalities of training in diverse populations to identify the optimal dose for effective response in various morphological and physiological features.
- Long-term follow-ups are needed to assess whether effects observed in postintervention measurements translate into adult health benefits over long-term periods.
- Personalized and context-specific interventions (e.g., biological, social-economic, etc.) are needed to study the various conditions, diversity, and their effects.

Author contributions

CA: Writing – review and editing, Writing – original draft, Conceptualization. JD: Methodology, Writing – review and editing, Investigation, Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

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Acknowledgments

The editors would like to thank the authors who have submitted their research to this Research Topic. The Editors also acknowledge all reviewers for their contribution and valuable time spent reviewing the manuscript.

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OPEN ACCESS

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RECEIVED 22 May 2024

ACCEPTED 15 July 2024

PUBLISHED 05 August 2024

CITATION

Zhuan S, Zhu Y, Zhou J, Lei S, Wang X and Li J
(2024), Enhancing lower limb and core muscle
activation with blood flow restriction training: a
randomized crossover study on high-intensity
squat exercises.
Front. Physiol. 15:1436441.
doi: 10.3389/fphys.2024.1436441

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Enhancing lower limb and core muscle activation with blood flow restriction training: a randomized crossover study on high-intensity squat exercises

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Objective: The primary objective of this study was to assess the impact of high-intensity deep squat training integrated with various blood flow restriction (BFR) modalities on the activation of lower limb and core muscles.

Methods: A randomized, self-controlled crossover experimental design was employed with 12 participants. The exercise protocol consisted of squat training at 75% of one-repetition maximum (1RM), performed in 3 sets of 8 repetitions with a 2-min inter-set rest period. This was conducted under four distinct BFR conditions: continuous low BFR (T1), intermittent medium BFR (T2), intermittent high BFR (T3), and a non-restricted control (C). Surface electromyography (EMG) was utilized to collect EMG signals from the target muscles during the BFR and squat training sessions. The root mean square (RMS) amplitude standard values were calculated for each squat set to quantify muscle activation levels, with these values expressed as a percentage of the maximum voluntary contraction (%MVC). Rating of Perceived Exertion was evaluated after each squat set, and leg circumference measurements were taken.

Results: 1) During the first two sets of deep squats, the %MVC of the vastus lateralis and vastus medialis in all compression groups was significantly higher than that in the control group ($p < 0.05$). Furthermore, in the first set, the %MVC of the vastus lateralis in Group T3 was significantly higher than in Group T2 ($p < 0.05$). In the third set, the %MVC of the vastus medialis in Groups T1 and T3 was significantly lower than in the first two sets ($p < 0.05$). 2) Group T1 showed an increased activation of the biceps femoris and semitendinosus muscles in the second and third sets, with %MVC values significantly greater than in the first set ($p < 0.05$). Group T2 only showed an increase in biceps femoris activation in the third set ($p < 0.05$). Group T3 significantly increased the activation of the biceps femoris and semitendinosus muscles only in the first set ($p < 0.05$). 3) No significant differences were observed in the changes of rectus abdominis % MVC among the groups ($p > 0.05$). In the first set, Group T3's erector spinae % MVC was significantly higher than the control group's; in the second set, it was significantly higher than both Group T2 and the control group's ($p < 0.05$). 4) After

training, a significant increase in thigh circumference was observed in all groups compared to before training ($p < 0.05$). 5) For RPE values, Group T2's post-squat values were significantly higher than the control group's after all three sets ($p < 0.05$). Group T1's RPE values were also significantly higher than the control group's after the third set ($p < 0.05$). Groups T1, T2, and C all had significantly higher RPE values in the second and third sets compared to the first set ($p < 0.05$).

Conclusion: All BFR modalities significantly enhanced the activation level of the anterior thigh muscles, with the continuous low BFR mode demonstrating a more stable effect. No significant differences were found in the activation level of the rectus abdominis among the groups. However, the intermittent high BFR mode was the most effective in increasing the activation level of the erector spinae muscles. While BFR did not further augment leg circumference changes, it did elevate subjective fatigue levels. The RPE was lowest during squatting under the intermittent high BFR condition.

KEYWORDS

blood flow restriction training, squat, lower limb muscles, core muscles, degree of muscle activation, rating of perceived exertion

Introduction

Blood Flow Restriction (BFR) is a technique that involves the use of cuffs applied proximally on the limbs to restrict venous blood return during physical activity, thereby diminishing arterial blood flow to the muscles without completely preventing it. This method is designed to enhance the metabolic stress response, surpassing that of traditional training approaches (Jia et al., 2019a; Lin et al., 2023). In recent years, BFR has risen in prominence across competitive sports, physical conditioning, and medical rehabilitation settings due to its efficacy in achieving training outcomes similar to those of high-intensity exercises, even when combined with low-intensity efforts (Shen et al., 2022). BFR has been shown to significantly improve muscle fitness parameters, such as strength, quality, and functionality, when compared to non-BFR training methods (Shen et al., 2022).

The heightened effectiveness of BFR can be ascribed to several mechanisms. Notably, BFR prompts an increased reliance on anaerobic metabolism, which can rapidly fatigue type I muscle fibers, necessitating the recruitment of high-threshold type II muscle fibers (Manini et al., 2011). Additionally, the pressure applied can activate afferent nerve centers, initiating a stress response cascade that may lead to the inhibition of a motor neurons (Yasuda et al., 2006). This results in a greater recruitment of muscle fibers to maintain force production and mechanical output, enhancing muscle activation and adaptability in exercise participants.

Despite the established benefits, there are limitations in the current BFR modalities, with a dearth of research in certain areas. Prevalent research tends to focus on low-intensity (20%–30% 1RM) combined with medium to high pressure (200–300 mmHg) (Liang et al., 2020), medium-intensity (30%–50% 1RM) with low to moderate pressure (100–200 mmHg) (Suga et al., 1985), or high-intensity (75% 1RM) with low pressure (100–150 mmHg) (Yuan, 2018; Lu et al., 2020; Zheng and Zhou, 2021). There is a scarcity of research on high-pressure and high-resistance modes, possibly due to safety concerns regarding potential injuries to cardiovascular function and muscles from sustained high-pressure training (Jia

et al., 2019b). However, some studies have begun to explore the application of high BFR in moderate and high-intensity resistance training by varying the restriction mode, reporting improvements in muscle size and strength (Davids et al., 2021; Torma et al., 2021). These studies, however, have not fully elucidated the impact on neuromuscular adaptations during training, particularly concerning changes in muscle electrophysiological signals.

In light of these findings, the present study introduces an intermittent compression intervention to assess the effects of high-intensity squat training with block pressures on the activation levels of the lower limbs and core muscles. The study also incorporates the subjective fatigue index as a measure to evaluate the practicality of this combined training approach. The revised aim of this study is to quantify the effects of high-intensity squat training integrated with specific BFR modalities—continuous low-pressure BFR (T1), intermittent medium-pressure BFR (T2), and intermittent high-pressure BFR (T3)—on the following specific metrics: Muscle activation levels of targeted lower limb muscles (e.g., quadriceps, hamstrings) and core muscles (e.g., rectus abdominis, erector spinae) as measured by Surface Electromyography (EMG); Changes in muscle girth to evaluate muscle volume and potential congestion; Subjective fatigue levels assessed using the RPE scale. We propose two hypotheses regarding the integration of BFR with high-intensity squat training: Hypothesis 1: The combination of high-intensity squat training with various BFR modalities will augment the activation of the lower extremity and core muscles. Hypothesis 2: BFR training will attenuate subjective perceptions of fatigue in comparison to unrestricted training protocols.

Objective and methods

The experimental study was conducted within the Physical Fitness Room at the School of Physical Education, Zhengzhou University, spanning October to November 2022. A randomized cross-control design coupled with a self-controlled methodology was implemented. Participant recruitment targeted students from the same institution, with stringent inclusion and exclusion criteria

TABLE 1 Basic information of the subjects.

Age	Height(cm)	Weight (kg)	Left leg circumference(cm)	Right leg circumference (cm)	Squat 1RM (kg)
23 ± 2	173.10 ± 5.95	79.15 ± 5.51	56.02 ± 1.42	57.24 ± 2.21	114.00 ± 21.09

applied to an initial pool of 28 candidates. This rigorous selection process culminated in the inclusion of 12 students who met the criteria, thereby comprising the final participant group for the study. [Table 1](#) provides an overview of the participants' basic information. To mitigate subjective bias inherent in the study's design, participants were not informed of the true purpose of the experiment until its conclusion. Instead, they were informed of a purported objective, which aimed to assess the impact of pressure stimulation during squat training on muscle training efficacy. The selection of male participants was intentional, considering the potential confounding effects of hormonal fluctuations and physiological differences between sexes that could influence muscle activation and training outcomes. This selection criterion was implemented to maintain consistency in the study's physiological measurements and to reduce variability that could obscure the effects of the BFR modalities on muscle activation.

Inclusion criteria encompassed: 1) a minimum of 3 years' experience in resistance training, 2) demonstrated proficiency in performing squat exercises, and 3) the capability to execute squats at a load of at least 1.2 times their body weight, as verified by one-repetition maximum (1RM) testing.

Exclusion criteria were as follows: 1) a diagnosis of lumbar disc degeneration, 2) any history of lower limb muscle or bone injuries, and 3) an inability to perform standardized squat movements or to complete the experimental procedures properly.

For Exercise Risk Assessment, a review of each participant's physical activity history was conducted. The Physical Activity Readiness Questionnaire (PAR-Q+) was administered to evaluate the individual's physical condition and to ensure the safety of the testing protocol. The exercise environment was rigorously assessed, including an inspection of the venue, equipment, and protective gear, to ensure safety standards were met.

Before engaging in the study, participants received detailed information regarding the study's objectives, methods, and potential risks, and provided their informed consent. The present study confirms that informed consent has been obtained from all participants, and the research involving human subjects is conducted in accordance with the Declaration of Helsinki; it was approved by the Ethics Committee of Zhengzhou University's School of Basic Medical Sciences, with the reference number ZZUIRB 2023-JCYXY0016.

Research methods

Experimental design and process

All subjects conducted their own controlled experiment and underwent squat training with four different BFR modes at 75% 1RM exercise intensity. The BFR modes included a continuous low BFR mode (T1), an intermittent medium BFR mode (T2), an

intermittent high BFR mode (T3), and a non-BFR mode (C). For T1, a continuous arterial occlusion pressure of 40% of the individual's arterial occlusion pressure (AOP) value was applied throughout the exercise period. For group T2, AOP was consistently applied at 50% during squat exercises, with pressure relief occurring during the inter-set rest intervals to facilitate recovery. In contrast, group T3 featured 60% AOP application during the inter-set rest intervals, while the actual squat movements were performed without pressure to allow for blood flow and reduce fatigue. (C) performed squats without any compression.

The resistance training scheme involved squatting with a load of 75% 1RM for three consecutive sets, with eight repetitions each set and a 2-min group interval. The sequence of squat tests under different compression modes was randomized and balanced, with a time interval of 72 h between each mode to prevent muscle damage caused by compression resistance training from affecting the test state of the subjects ([Sonkodi et al., 2021](#)) and reduce mutual interference effects between different modes. TheraBand BFR equipment was used for the intervention, applying a binding position at the root of the thigh near the proximal end with a binding pressure set at 30 mmHg ([Figure 2](#)). AOP values were calculated based on [Loenneke et al.'s](#) conversion standard "Reference Table of AOP Values Corresponding to Leg Circumference" ([Loenneke et al., 2015](#)), as shown in [Table 2](#). The selection of low, middle, and high occlusion pressures aligned within [Li Zhiyuan et al.'s](#) range for lower limb occlusion pressures ([Zhiyuan et al., 2021](#)). The squat 1RM test was conducted 3 days before the formal experiment. On the day of the formal test, the subjects first underwent the standard warm-up procedure with the squat 1RM test and then performed the maximum voluntary contraction test of the target muscle (MVC) and the surface myoelectric test of compressed squat. Surface EMG signals were collected during both tests, leg circumference was measured immediately after each squat, and subjective fatigue was recorded, as shown in [Figure 1](#).

EMG Analysis: EMG analysis software was used to rectify, filter, smooth, and standardize the original EMG values. On the original EMG, the muscle force range was selected to cut the wave according to the start and stop time of each group of squats. The absolute value of EMG was selected for full wave rectification, and an infinite impulse response filter (IIR) was applied. The low wave was filtered at 8 Hz, and the high wave was filtered at 450 Hz ([Hermens et al., 2000](#)). Then, the root mean square (RMS) was used for smoothing processing, and the average value of RMS was taken. Finally, standardized processing was performed. The RMS value obtained in the MVC test was defined as the maximum value of the muscle force, and the average value of the muscle RMS obtained in each group of squats was divided by the RMS value during the MVC test, which is called the RMS standard value (%MVC) to reflect the degree of muscle activation ([Konrad, 2005](#)). See [Formula 1](#) for the expression of EMG_{RMS} .

TABLE 2 Reference table of AOP corresponding to leg circumference.

Leg circumference (cm)	60% AOP (mmHg)	50% AOP (mmHg)	40% AOP (mmHg)
<45–50.9	120	100	80
51–55.9	150	130	100
56–59.9	180	150	120
≥60	210	180	140

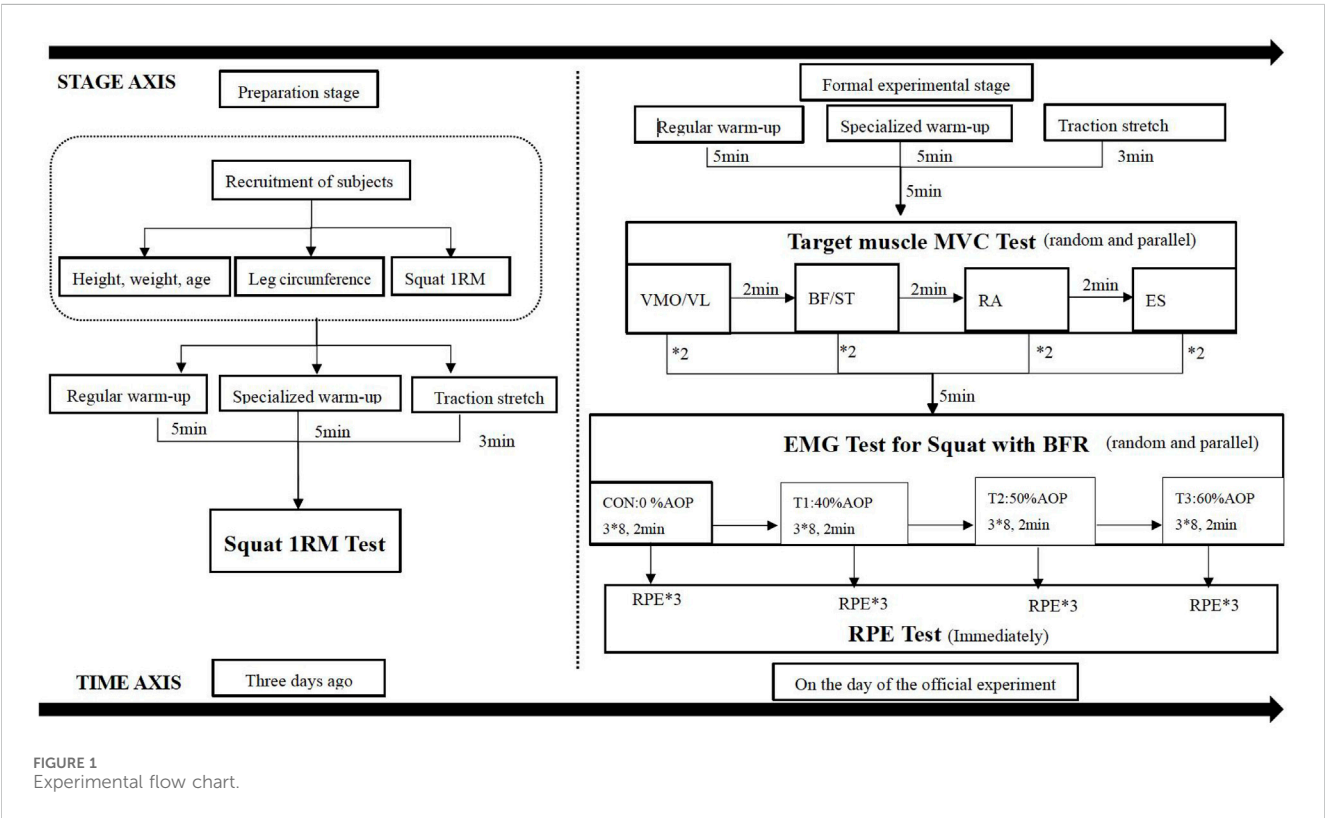


FIGURE 1 Experimental flow chart.

(1)
$$EMG_{RMS} = \sqrt{\frac{\sum_{i=0}^N Data[i]^2}{N}}$$

Main test and observation indicators

1) Squat 1RM test

The one-repetition maximum (1RM) squat test was administered following a standardized protocol 3 days prior to the main experiment. The standard squat technique was delineated as follows (Figure 3): participants were instructed to stand with their eyes facing forward, feet comfortably positioned outward, and hands gripping the barbell around the neck. They were then directed to perform squats in a controlled rhythm, lowering to the deepest point—defined by the hips dropping below the knees—before pausing momentarily at the base of the squat. Subsequently, they were to exert force in reverse and ascend back to the starting position with knees fully extended. In preparation for the 1RM test, subjects commenced with a standardized warm-up, which included a 5-min session at a resistance of 100 W and a step

frequency of 70–80 rpm. This was followed by a 5-min period of specific warm-up exercises, involving 50% and 75% of the anticipated 1RM load. Concluding the warm-up phase was a 3-min session dedicated to muscle stretching. The 1RM test itself commenced post-warm-up, with subjects initiating the squats with a load verbally estimated at 80% of their 1RM. The load was progressively increased in increments of 4–9 kg after each set of three to five repetitions. Following a 2-min rest period, the load was incremented, and subjects performed two to three additional repetitions. This pattern continued with further 2-min rests and load increases of 4–9 kg, with an additional 2–4 kg added until the point of squat failure. The 1RM values for all subjects were ascertained across five trials, with encouragement provided to support maximal effort (Kubo et al., 2019).

2) Maximum voluntary contraction (MVC) test

The Maximum Voluntary Contraction (MVC) test was initiated 5 min post-warm-up on the day of the formal experiment. Surface electromyography (EMG) signals from the target muscles were captured using a 6-channel Delsys Trigno wireless EMG

acquisition system. In accordance with the biomechanics of squat movements, the following muscles were selected for analysis: for the thigh group, vastus lateralis (VL) and vastus medialis obliquus (VMO); and for the posterior thigh group, biceps femoris (BF) and semitendinosus (ST); for the core muscle, rectus abdominis (RA) and erector spinae (ES); totaling six muscle groups. Electrodes were strategically placed on the muscle bellies based on their anatomical landmarks.

Prior to electrode placement, the skin over the muscle bellies was prepared by shaving any hair and cleaning with alcohol to eliminate any dust or sweat, thereby reducing skin impedance and ensuring optimal sensor contact. MVC test data were collected for each muscle group following Konrad's protocol (Konrad, 2005), which involved two MVC tests per muscle. The test procedures were as follows:

1. RA: Subjects were positioned supine with the knees flexed at 30°, holding their ankles and performing a maximal voluntary contraction of the upper body while the tester applied downward resistance on the chest for 3–5 s (left panel).
2. ES: Subjects lay prone with the lower limbs secured, and maximal force was applied to the scapula while the tester provided resistance for 3–5 s (right panel).
3. VMO and VL: With the subject seated and the torso and thigh at 90-degree angles, the knee was fully extended. The tester applied downward resistance at the ankle's upper end for 3–5 s (left panel).
4. BF and ST: Subjects were positioned prone with the right knee joint bent to approximately 20° in a "C" shape. Downward resistance was applied to the upper ankle, maintained for 3–5 s, and the EMG data were collected (right panel).

3) Surface EMG test of pressurized squat

After completing the MVC test, the subject rested for 5 min, and then the tester prepared the subsequent pressurized squat surface EMG test by binding the subject. The subject followed the standard squat movements for three squat exercises. Before each squat, the camera and EMG acquisition system were turned on in preparation. Once the subject began the squat, the acquisition system began recording the EMG signal data. Based on the experimental synchronous video recording, all EMGs from the first to the third squat of each pressurized squat session were selected for subsequent data analysis.

4) Thigh circumference test

The thigh circumference test was conducted in the preparation stage 3 days before the experiment and immediately after each group of pressure squat training in the formal experiment. Subjects stood with their legs shoulder-width apart, and a tape measure was placed horizontally on the transverse line below the rear hip to measure the thigh circumference. Each leg's circumference was measured three times and averaged (Zhiyuan et al., 2021).

5) RPE test

Prior to the experimental procedures, participants were thoroughly briefed on the rating scale to be utilized for assessing perceived exertion. Following each session of squat training across varying BFR modalities, subjects were required to self-rate their perceived exertion, which was subsequently documented. The RPE scale, as adapted by Zourdos et al. (2021), was employed in this

study. This scale, tailored for evaluating the subjective fatigue levels during resistance training, ranges from 1 to 10, with the initial four levels being based on the subjective assessment of effort. Specifically, levels 1–2 denote no effort, while levels 3–4 indicate a minimal degree of exertion. The subsequent levels, 5–10, are determined by the number of repetitions in reserve (RIR): levels 5–6 suggest the capacity to perform an additional 4–6 squats post-training; level 7 corresponds to three more repetitions; level 8 to two more repetitions; level 9 to one additional repetition; and level 10 signifies maximum effort.

Statistical analysis

Statistical analysis of the electromyography (EMG) data was conducted using Excel 2010 and SPSS 17.0 software following the aforementioned procedures. Data were presented as the mean \pm standard deviation ($M \pm SD$). Two-factor repeated measures analysis of variance (ANOVA) (BFR mode \times exercise sets) was utilized to analyze the RMS standard values (%MVC) of the target muscles of the lower limbs and core muscles in different subjects under various compression modes of high-intensity squat training. Before conducting the ANOVA, Mauchly's sphericity test was performed to assess the sphericity assumption. If the test result was $p > 0.05$, aligning with the Huynh-Feldt conditions, we accepted the results of the spherical hypothesis test and proceeded with one-way ANOVA; if the test result was $p < 0.05$, indicating a violation of the sphericity assumption, we applied the Greenhouse-Geisser correction to adjust the degrees of freedom. Upon completion of repetitive measure ANOVA, multiple comparisons to adjust may cause risk of the first type of mistake, we have adopted Tukey HSD (Honestly Significant Difference) test. This method was adapted to our study design and allowed for simultaneous adjustment for group comparisons. Specifically, where ANOVA subject or interaction effects were found to be significant, Tukey HSD tests were used to determine which group differences were statistically significant. We followed standard statistical procedures and adjusted the P-value for each pair of comparisons, ensuring statistical correction and reliability of the results.

Results

A subject's raw EMG of various muscle groups during squat with BFR training (Figure 2).

Results of two-factor analysis of variance

Two-factor repeated-measures ANOVA revealed that BFR mode had a significant effect on the %MVC values of VL ($F = 20.175$, $p = 0.000$), VMO ($F = 9.267$, $p = 0.000$), BF ($F = 4.021$, $p = 0.041$), ST ($F = 8.437$, $p = 0.000$), and ES ($F = 5.593$, $p = 0.002$) ($p < 0.05$); the number of exercise sets significantly influenced the %MVC values of VL ($F = 4.956$, $p = 0.018$) and VMO ($F = 6.430$, $p = 0.006$) ($p < 0.05$); the interaction between different BFR modes and the number of exercise sets was not statistically significant for the % MVC values of all muscles ($p > 0.05$). It can be seen that different pressurization mode interventions are the main factors affecting the

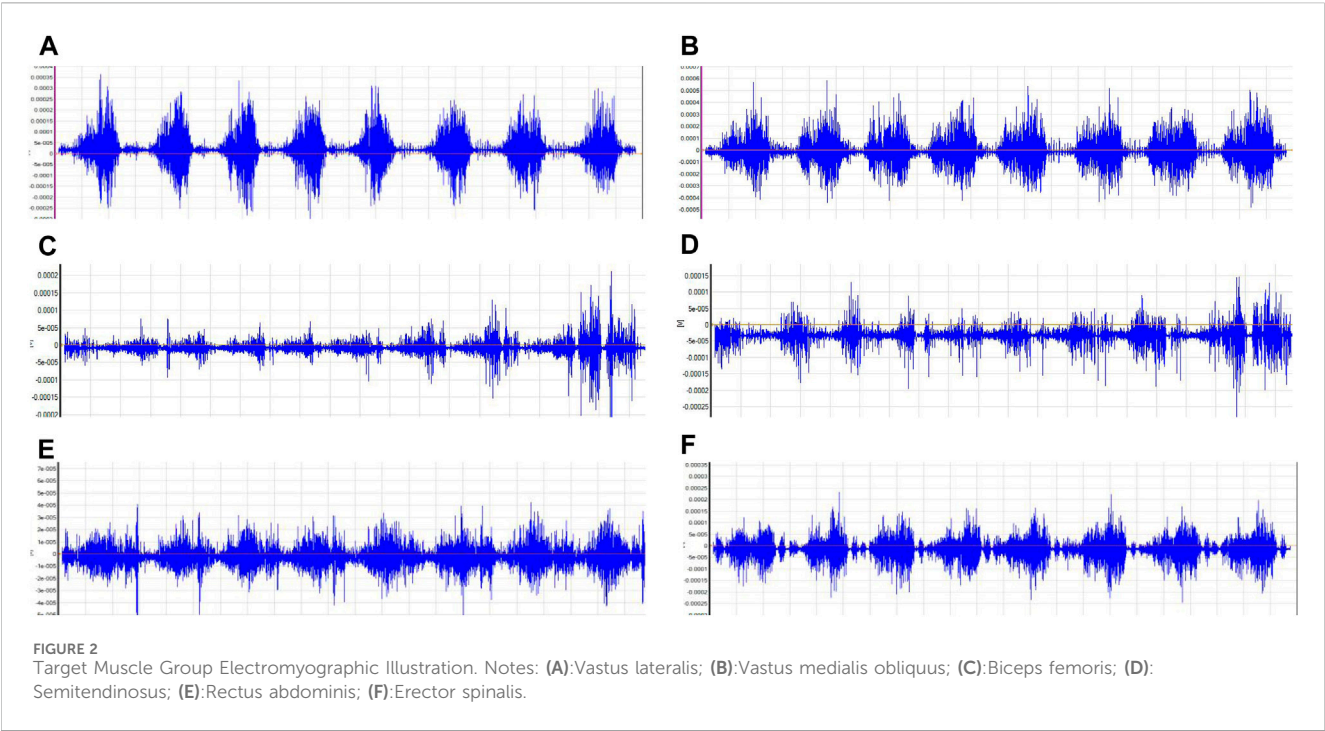


TABLE 3 Effects of BFR mode, exercise group and their interaction on target muscle %MVC.

	Anterior thigh muscles				Back side muscles in the thigh				Core muscle			
	VL		VMO		BF		ST		RA		ES	
	F	P	F	P	F	P	F	P	F	P	F	P
BFR mode	20.175	0.000*	9.267	0.000*	4.021	0.041*	8.437	0.000*	0.424	0.608	5.593	0.002*
Exercise sets	4.956	0.018*	6.430	0.006*	3.001	0.083	0.028	0.973	0.360	0.659	3.155	0.063
Interaction groups	2.242	0.071	0.487	0.816	1.260	0.301	0.667	0.667	0.037	0.992	0.391	0.883

Notes: VL: vastus lateralis; VMO: vastus medialis obliquus; BF: biceps femoris; ST: semitendinosus; RA: rectus abdominis; ES: Erector spinalis.* indicates a significant difference, with a p-value <0.05.

degree of muscle activation change during high-intensity squat training. The specific results are shown in Table 3.

Changes in the muscle %MVC value in each group of squat training under different compression modes

In the anterior thigh muscles, the %MVC of vastus lateralis percentages for groups T1, T2, and T3 were significantly higher than group C during the first squat ($p < 0.05$), with T3 showing a higher value than T1 ($p = 0.032$). During the second squat, the MVC percentages for T1, T2, and T3 remained significantly higher than C. In the third squat, only groups T1 and T2 maintained a significant MVC percentage increase over C. Additionally, T1 showed a significant increase in MVC percentage from the first to the second squat ($p = 0.047$), while T3 showed significant increases in MVC percentage during the first and second squats compared to the third ($p = 0.014$ and $p = 0.009$, respectively). For the vastus

medialis, in the first squat, the MVC percentages for T1, T2, and T3 were significantly higher than C ($p < 0.05$). In the second squat, the MVC percentages for T1, T2, and T3 continued to be significantly higher than C. Furthermore, T1 showed significant increases in MVC percentage during the third squat compared to both the first ($p = 0.025$) and second squats ($p = 0.027$).

In the posterior thigh muscles, specifically the biceps femoris, T3 had a significantly higher MVC percentage than C in the first squat ($p = 0.039$), and T1 in the second squat ($p = 0.040$). In the third squat, both T1 and T2 had significantly higher MVC percentages than C, with T2 also showing a significant difference compared to T3 ($p = 0.020$). Moreover, T2 showed significant increases in MVC percentage during the third squat compared to the first ($p = 0.005$) and second squats ($p = 0.006$). For the semitendinosus, T1 and T3 had significantly higher MVC percentages than C in the first squat ($p < 0.05$), with only T1 maintaining this difference in the second and third squats ($p < 0.05$). Additionally, T3 showed significant increases in MVC percentage during the first squat compared to the second ($p = 0.039$) and third squats ($p = 0.009$).

TABLE 4 Different BFR modes squats targeted muscle in training the MVC %.

Muscle	BFR	Three sets squat (8 + 8+8)			ES (95%CI)			F	η^2
		First set	Second set	Third set	T1	T2	T3		
VL	C	51.29 ± 8.44	55.45 ± 11.67	56.47 ± 12.26	−0.73[−1.0, −0.47]	0.13[−0.11,0.38]	0.82[0.51,1.2]	20.175	0.428
	T1	60.91 ± 14.25* [△]	65.59 ± 15.42* [§]	64.46 ± 13.41*	~	0.24[−0.01,0.51]	0.13[−0.11,0.38]		
	T2	66.70 ± 11.40*	71.06 ± 13.47*	63.72 ± 18.89*	~	~	−0.1[−0.32,0.12]		
	T3	67.34 ± 14.20* [§]	70.26 ± 13.68* [§]	59.27 ± 18.34	~	~	~		
VMO	C	53.12 ± 9.47	50.29 ± 13.81	50.71 ± 13.06	1.14[0.72, 1.65]	0.70[0.28, 1.17]	0.67[0.35, 1.05]	9.267	0.256
	T1	68.22 ± 8.68* [§]	65.53 ± 8.82* [§]	57.79 ± 8.87	~	−0.17[−0.59,0.24]	−0.26[−0.64,0.09]		
	T2	64.37 ± 17.18*	65.72 ± 17.26*	56.65 ± 15.79	~	~	−0.07[−0.33,0.19]		
	T3	63.01 ± 14.26*	60.39 ± 13.44*	58.15 ± 17.33	~	~	~		
BF	C	21.91 ± 7.88	23.38 ± 12.13	24.40 ± 10.03	0.62[0.29, 1.00]	0.54[0.16, 0.96]	0.44[0.04, 0.88]	4.021	0.13
	T1	26.97 ± 12.97	31.29 ± 14.44*	33.82 ± 13.76*	~	0.25[−0.02,0.54]	−0.31[−0.77, 0.13]		
	T2	29.86 ± 16.21 [§]	31.88 ± 20.14 [§]	51.35 ± 56.67*	~	~	0.40[0.01,0.82]		
	T3	29.90 ± 9.62*	27.32 ± 6.78	25.41 ± 7041 [†]	~	~	~		
ST	C	24.68 ± 10.04	25.02 ± 9.55	26.32 ± 9.45	1.06[0.63,1.57]	0.59[0.15,1.07]	0.75[0.39,1.16]	8.437	0.238
	T1	39.37 ± 19.34*	44.54 ± 22.81*	43.14 ± 20.76*	~	−0.44[−0.81, −0.1]	−0.29[−0.62,0.03]		
	T2	31.19 ± 17.06	34.21 ± 21.85	35.98 ± 15.57			−0.15[−0.61,0.29]		
	T3	42.01 ± 20.86*	34.81 ± 20.21 [§]	33.16 ± 16.25 [§]	~	~	~		
RA	C	13.77 ± 3.06	14.72 ± 3.06	12.96 ± 3.06	−0.04[−0.46,0.38]	0.08[−0.17,0.34]	0.15[−0.08,0.4]	0.424	0.015
	T1	12.65 ± 3.67	14.75 ± 3.67	12.87 ± 3.67	~	0.1[−0.27,0.48]	0.17[−0.21,0.57]		
	T2	15.92 ± 7.15	15.02 ± 7.15	14.70 ± 7.15	~	~	0.05[−0.04,0.13]		
	T3	16.13 ± 6.21	16.54 ± 6.21	15.80 ± 6.21	~	~	~		
ES	C	29.04 ± 4.23	32.66 ± 4.23	35.26 ± 4.23	0.03[−0.24,0.3]	0.16[−0.15,0.47]	0.51[0.25,0.8]	5.593	0.172
	T1	31.35 ± 4.83	33.94 ± 4.83	33.70 ± 4.83	~	0.12[−0.15,0.4]	0.46[0.19,0.76]		
	T2	35.28 ± 4.91	33.03 ± 4.91 [#]	35.22 ± 4.91	~	~	−0.33[−0.57, x−0.11]		
	T3	38.03 ± 4.87*	40.13 ± 4.87*	40.11 ± 4.87	~	~	~		

Core muscle group: Rectus abdominis: There were no significant differences in the %MVC value of rectus abdominis among all groups ($P > 0.05$). Erector spinae muscle: In the first squat, the % MVC value of the group T3 ($p = 0.011$) was significantly higher than that of the C group ($P < 0.05$). In the second squat, the %MVC value of the group T3 ($p = 0.030$) was also significantly higher than that of the C group ($P < 0.05$), at the same time, the activation degree of group T3 ($p = 0.023$) was significantly higher than that of group T2 ($p < 0.05$). The specific results are shown in [Table 4](#) and [Figure 3](#).

Changes in thigh circumference in each group of squat training under different BFR modes

The left leg circumference of the group C ($p = 0.007$), group T1 ($p = 0.001$), group T2 ($p = 0.001$) and group T3 ($p = 0.005$) after training was significantly higher than that measured before training.

The right leg girth of the C group ($p = 0.001$), group T1 ($p = 0.002$), group T2 ($p = 0.003$) and group T3 ($p = 0.000$) after training was significantly higher than that of the right leg girth measured before training ($P < 0.05$). The results are shown in [Table 5](#).

Test results of subjective fatigue after squat training in each group under different BFR modes

The RPE value of the group T2 after the first squat ($p = 0.013$), the second squat ($p = 0.005$) and the third squat ($p = 0.007$) was significantly higher than that of the blank control group C. The RPE value of the group T1 during the third squat ($p = 0.011$) was significantly higher than that of the blank control group C ($P < 0.05$). At the same time, the RPE value of the nonpressurized blank control group C during the second ($p = 0.024$) and third ($p = 0.004$) squats was significantly higher than that of the first squats. The RPE value of the

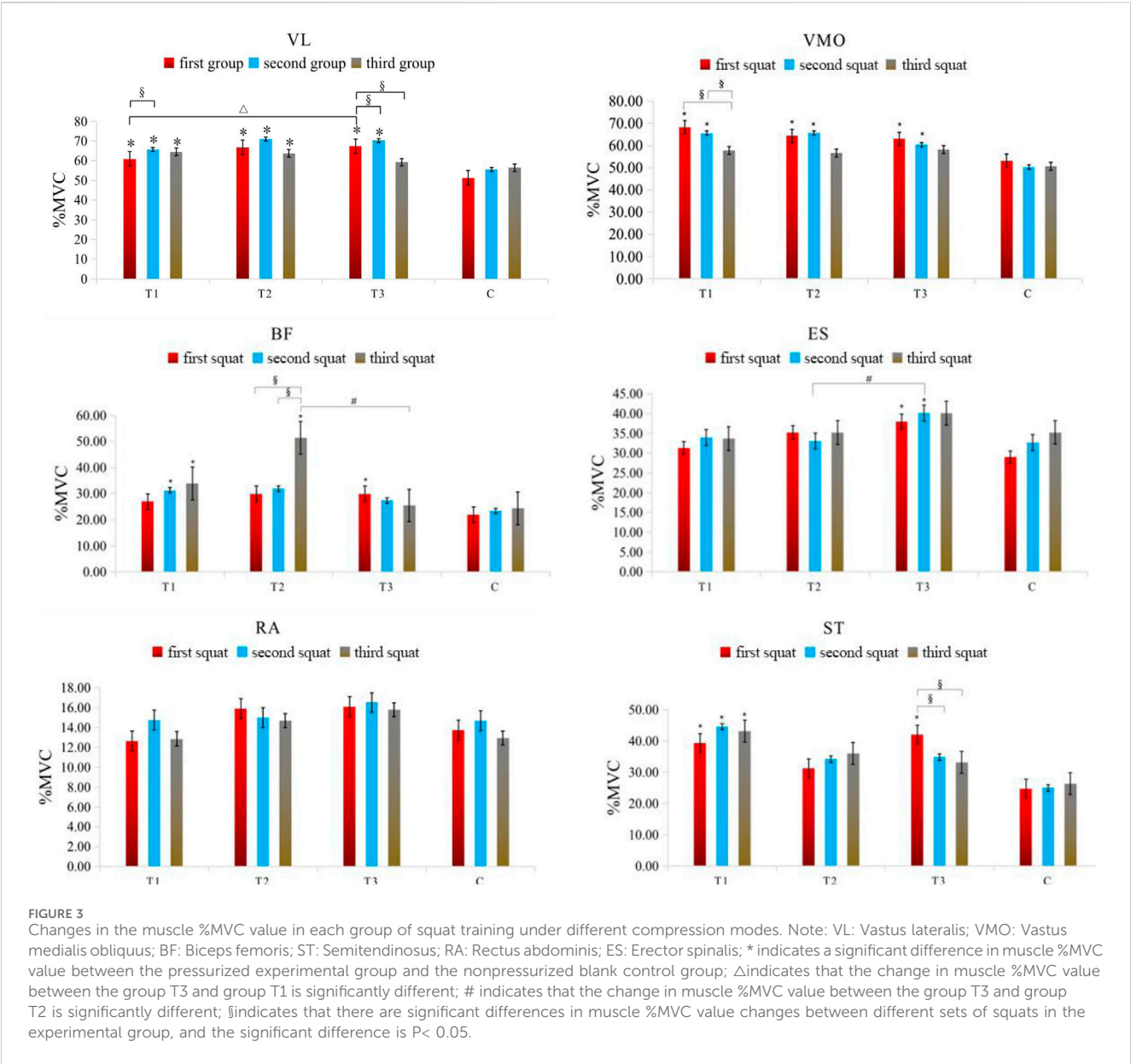


TABLE 5 Changes in hindleg circumference in each group of squat training under different pressure modes.

		Left leg circumference (cm)	Right leg circumference (cm)
pre-test	C/T1/T2/T3	56.06 ± 1.77	55.75 ± 2.29
post-test	C	56.73 ± 1.70*	56.67 ± 2.25*
	T1	57.24 ± 2.12*	57.17 ± 1.90*
	T2	57.49 ± 2.27*	57.45 ± 2.27*
	T3	57.17 ± 2.57*	57.06 ± 2.39*

Note: * indicates that there is a significant difference in the changes in leg circumference between the post-test and pre-test leg circumference after exercise intervention with different pressure resistance modes; The significant difference is $P < 0.05$.

third squat in the group T1 was significantly higher than that of the second squat ($p = 0.037$) and the first squat ($p = 0.001$), and the RPE value of the second squat was also significantly higher than that of the first squat ($p = 0.005$). The RPE values of the second ($p = 0.005$) and third squats ($p = 0.011$) in the group T2 were also significantly higher than those of the first squats ($P < 0.05$). In contrast, there was no

TABLE 6 List of changes in subjective fatigue after squat training in each group under different pressure modes.

	BFR mode	Exercise sets		
		First	second	Third
RPE value	C	5.2 ± 1.14	5.8 ± 1.40 [§]	6.1 ± 1.50 [§]
	T1	5.9 ± 1.28	6.5 ± 1.51 [§]	6.9 ± 1.29* ^{§#}
	T2	6.4 ± 1.78*	7.0 ± 1.89* [§]	7.2 ± 1.55* [§]
	T3	6.1 ± 1.10	6.6 ± 1.17	6.9 ± 1.37

Note: * indicates a significant difference in RPE, values between the pressurized experimental group and the non-pressurized blank group C; § indicates that there are significant differences in RPE, values between the first squats and the second and third squats in each experimental group; # indicates that there are significant differences in RPE, values between the second squat and the third squat; the significant difference is $p < 0.05$.

significant difference in RPE values in the group T3 during all three squats ($P > 0.05$). The specific results are shown in Table 6.

Discussion

Analysis of changes in the degree of muscle activation in the anterior thigh muscles

The present study’s findings demonstrate that the percentage of %MVC for anterior thigh muscles during the initial two sets of squats under diverse compression conditions was markedly elevated in comparison to the non-compressed state, corroborating the outcomes of prior investigations. In a 2021 study, Che Tongtong et al. facilitated four sessions of half-squat compression training for female wrestlers at a pressure of 180 mmHg and an exercise intensity of 30% 1RM, noting a significantly higher muscle activation level in the experimental group subjected to compression compared to the non-compressed control group (Tong-Tong et al., 2021). Consistent with these findings, Li Zhiyuan et al. (2021) observed that both continuous and intermittent pressures, ranging from 40% to 60% AOP, significantly augmented the activation level of Vastus lateralis and Vastus medialis in handball players during low-intensity (30% 1RM) squat training (Zhiyuan et al., 2021). It has been established that pressure stimulation can enhance the reliance on anaerobic metabolism for energy provision (Manini et al., 2011), causing rapid exhaustion of type I muscle fibers and a subsequent increase in the recruitment of type II muscle fibers with higher activation thresholds. Furthermore, the application of pressure during muscle contractions induces intramuscular hypoxia due to restricted blood flow, resulting in the accumulation of metabolic byproducts, such as lactic acid. This accumulation leads to an “overload” effect, triggering a cascade of stress responses that activate the III and IV afferent nerve centers (Yasuda et al., 2006). These responses, in turn, stimulate muscle fiber recruitment and may potentially inhibit α motor neurons (Yasuda et al., 2006). As a result, a greater number of muscle fibers are recruited to sustain equivalent force and mechanical power output, as evidenced by the observed significant increase in muscle activation (Gizzi et al., 2021).

Significantly, the activation level of the lateral femoral muscle during the initial squat session was observed to be higher under high-pressure conditions group T3 than under medium-pressure conditions group T2, a result that aligns with prior research. Zhang

and Yi (2022) conducted high-intensity (75% 1RM) squat training with subjects under very high pressure and reported increased muscle activation during the eccentric phase of squats under high pressure (350 mmHg) as opposed to low pressure (250 mmHg). A synthesis of the dose-response relationship in BFR (Lin et al., 2023) suggests an “inverted U-shaped” correlation between pressure and muscle functional performance. Within an optimal pressure range, incremental occlusion pressure intensifies the blood flow blockade and augments the stimulation effect, culminating in peak stress and adaptation responses at a critical pressure value, which is conducive to achieving the best training outcomes. Conversely, pressures surpassing this threshold can lead to a decline in training efficacy due to excessive metabolic load that overwhelms the body’s adaptive capacity. The findings imply that a 60% arterial occlusion pressure during high-intensity BFR squat training is within the effective pressure range for positive stimulation. Additionally, the activation level of the lateral femoris muscle during the second squat under continuous low-pressure conditions group T1 was notably higher than during the first, potentially attributable to the post-activation potentiation (PAP) effect. Moore et al. (2004) and Wilk et al. (2020) have successively demonstrated that unilateral elbow flexion training and bench press training of the upper limbs, combined with BFR intervention, can induce the occurrence of PAP effects in the upper limbs, significantly enhancing the activation capacity of the upper limb muscles and promoting exercise performance. Furthermore, Doma et al. (2020) and Hongwen and Xiang (2022) have found in subsequent studies that lower limb training combined with BFR intervention can also significantly induce PAP effects in the lower limbs, significantly improving lower limb explosive power and enhancing the performance of lower limb muscle movements. This effect is mediated by the recruitment of high-threshold motor units, which elevates the phosphorylation of light chains, enhances the sensitivity of calcium ions (Ca^{2+}) within muscle cells, and thus improves muscle fiber contractility, leading to enhanced exercise performance. As a result, the power output and motor performance in subsequent training movements surpass those of the initial movements (Lin et al., 2023).

Analysis of changes in the degree of muscle activation in the thigh posterior group

The study’s findings indicate that under the continuous low-pressure BFR mode group T1, there was a significant increase in the

activation levels of the biceps femoris and semitendinosus muscles during the second and third sets of squats. In comparison, the intermittent medium-pressure BFR mode group T2 showed an increase in activation only during the third set, and the high-pressure BFR mode group T3 demonstrated an increase solely in the first set. Biomechanically, a complete squat is a complex, multi-joint movement that requires the coordinated action of muscles across the hip, knee, and ankle joints, with different muscle groups assuming specific roles in force transfer and joint movement (Trujillo et al., 2011). The muscles in question are antagonists, and their increased activation under the group T1 condition in the latter sets suggests a progressive recruitment pattern. According to the principles of motor unit recruitment, as the load intensity and volume of an exercise reach certain thresholds, there is a corresponding increase in the activation of both agonist and antagonist muscle groups involved in the movement. Thus, the study's outcomes are consistent with established muscle recruitment dynamics.

Among the BFR modes tested, only the continuous low-pressure mode group T1 was found to consistently and effectively enhance the activation of the posterior muscle group. The other two intermittent pressure modes showed variable effects on muscle activation levels. Upon comparison, the intermittent release of pressure likely reduced the tissue hypoxia and excessive metabolic stress associated with continuous BFR, leading to less pronounced muscle activation increases. The intermittent modes' approach to pressure application may not have provided sufficient metabolic stress to further enhance muscle activation beyond a certain point. However, the continuous low-pressure mode allows for a cumulative metabolic stress that improves muscle fiber recruitment efficiency and contractile capacity, which can be advantageous for squat performance. Consequently, the study suggests that utilizing a continuous low-pressure BFR mode may be more beneficial for squat training outcomes.

Analysis of changes in the muscle activation degree of the core muscle group

According to the study (Sha and Luo, 2015), the effective activation of a series of core muscles, such as the rectus abdominis muscle and erector spinae muscle, is crucial for maintaining movement stability during squats, enhancing the overall success of the subjects' transition from squatting to standing. The findings of this study indicated that there was no significant difference in the activation level of the rectus abdominis muscle regardless of whether compression intervention was applied, which contrasts with previous research. Tong-Tong et al. (2021) suggested that continuous compression intervention during low-intensity half-squat training could lead to the transfer of training effects, with the activation level of the rectus abdominis in the non-compression area also significantly improved (Tong-Tong et al., 2021). This discrepancy in results can be attributed to differences in training intensity, training movements, and individual characteristics. In this study, college students were recruited to participate in the experiment, and the resistance

training protocol involved compressive squat training at 75% 1RM exercise intensity. The mechanical load pressure and metabolic pressure of this training far exceeded those in the previous study, while the training level of the college students may have been lower, resulting in a higher overall difficulty in completing the movements. During the training process, the activation level of the rectus abdominis muscle consistently peaked during exercise, so there was no significant change in the activation level of this muscle regardless of whether pressure intervention was applied.

The activation level of the erector spinae muscle during the first and second squats was significantly higher in the group T3 condition compared to the control group; that is, the intermittent high-pressure compression mode more effectively enhanced the activation level of the erector spinae muscle compared to the other modes. This may be related to the higher blocking pressure and sufficient intermittent rest. On one hand, higher blocking pressure can induce greater metabolic stress stimulation and promote the recruitment of fast muscle fibers (Gizzi et al., 2021). On the other hand, after pressure relief, some metabolic pressure can be reduced to ensure rapid synthesis of phosphocreatine, thereby minimizing acidosis caused by the accumulation of metabolic products and muscle fatigue resulting from insufficient energy supply. This allows for the avoidance of exercise fatigue (Zhiyuan et al., 2021) after the release of the pressure cuff and restoration of neural regulation of muscle function.

Analysis of changes in leg circumference test results

The study's results demonstrate that squat training, irrespective of pressure stimulation, led to a significant increase in leg circumference. Post-training measurements revealed that the circumference of both the left and right legs was notably higher than pre-training measurements across all groups. Prior research (Joyner and Casey, 2015) has established that exercise is a potent activator of the autonomic nervous system, potentially causing muscle congestion. The muscular contractions during exercise elevate central hemodynamics, triggering a cascade of autonomic responses. This includes increased cardiac output, ventilation, vascular sympathetic tone, and blood flow to the active muscles, which collectively contribute to muscle congestion. This physiological response may account for the observed increase in leg circumference following acute exercise, regardless of whether compression was applied.

However, a divergence from some scholarly observations (Loenneke and Pujol, 2009) was noted; typically, a more pronounced increase in limb circumference is expected in the pressurized group post-exercise compared to the non-pressurized group. The rationale provided is that pressurization may impede venous return, leading to limb effusion and subsequent reactive congestion. The pressure gradient created by pressurization may facilitate the shift of effusion from the plasma into muscle cells. Contrary to these findings, the current study's results suggest that the muscle congestion may have already peaked due to the high-intensity nature of the resistance training, implying that additional

pressure stimulation did not surpass the threshold for further increasing muscle congestion.

Analysis of changes in subjective fatigue test results

RPE, as utilized by the Center for Sports Training, serves as a critical tool for gauging mental fatigue during athletic endeavors and for adjusting exercise intensity in real-time (Tong-Tong et al., 2021). The current study's findings suggest that both continuous low-pressure and intermittent medium-pressure BFR can lead to increased psychological fatigue among participants. However, the intermittent high-pressure BFR mode was associated with a more favorable subjective fatigue perception. Previous research by Vieiea et al. (2015), Schwiete et al. (2021), and Tong-Tong et al. (2021) has consistently shown that in controlled trials, the RPE is significantly higher in the pressurized group compared to the non-pressurized group, likely due to the elevated metabolic stress from pressurization. Notably, the present study observed no significant difference in RPE values across the different BFR modes.

This discrepancy may stem from the fact that previous studies altered only the BFR mode while maintaining a constant pressure level. In contrast, the current study featured variations in both the BFR mode and the pressure intensity, with the continuous mode at a low pressure and the intermittent modes at medium and high pressures. It is hypothesized that the intermittent mode, despite allowing for recovery periods, may not reduce the RPE value significantly due to the higher metabolic demand, resulting in similar RPE values to the continuous mode. Strikingly, the intermittent high-pressure BFR mode yielded the lowest RPE values, suggesting that the asynchronous application of mechanical load and metabolic stress during intermittent pressurization does not accumulate fatigue to the same extent as continuous pressure. Consequently, participants reported a lower subjective fatigue level, indicating that this mode may be particularly suitable for high-resistance training in a collegiate setting. Furthermore, the study observed a progressive increase in RPE values across the three sets of squat training, with significant elevations from the first to the second and third sets. This increase can be attributed to the physiological consequences of continuous resistance training, including muscular and nervous fatigue, depletion of energy resources, and the accumulation of metabolic byproducts, all of which contribute to a heightened sense of physical and psychological fatigue.

Conclusion

In the realm of BFR and squat exercises, all BFR modalities have been found to significantly enhance the activation of the anterior thigh muscles. Continuous low-pressure BFR uniquely and reliably boosts the activation of the posterior thigh muscles, while intermittent modes show variable effects. Although BFR does not alter leg circumference, it does elevate subjective fatigue levels. Notably, the intermittent high-pressure BFR mode is particularly effective at enhancing erector spinae activation and produces the least subjective fatigue during squats. Given the overall effectiveness

and practicality of training, the intermittent high-pressure BFR mode stands out as advantageous for improving muscle activation and fostering neuromuscular adaptations, which are key objectives in training regimens.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Zhengzhou University's School of Basic Medical Sciences. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Writing—original draft. YZ: Conceptualization, Data curation, Investigation, Validation, Writing—original draft. JZ: Methodology, Supervision, Visualization, Writing—review and editing. SL: Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing—original draft. XW: Formal Analysis, Resources, Supervision, Validation, Visualization, Writing—original draft.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

Thanks for the support of the subjects recruited in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 17 June 2024

ACCEPTED 24 July 2024

PUBLISHED 09 August 2024

CITATION

Sun F, Williams CA, Sun Q, Hu F and Zhang T
(2024), Effect of eight-week high-intensity
interval training versus moderate-intensity
continuous training programme on body
composition, cardiometabolic risk factors in
sedentary adolescents.
Front. Physiol. 15:1450341.
doi: 10.3389/fphys.2024.1450341

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Effect of eight-week high-intensity interval training versus moderate-intensity continuous training programme on body composition, cardiometabolic risk factors in sedentary adolescents

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Objectives: This study aimed to assess and compare the effect of an 8-week high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT) programme on body composition and cardiovascular metabolic outcomes of sedentary adolescents in China.

Methods: Eighteen sedentary normal-weight adolescents (age: 18.5 ± 0.3 years, 11 females) were randomized into three groups. HIIT group protocol consisted of three sessions/week for 8-week of "all out" sprints to reach 85%–95% of HR_{max} , and MICT group protocol undertook three sessions/week for 8-week of continuous running to reach 65%–75% of HR_{max} . The control group resumed normal daily activities without any intervention. Blood pressure and body composition were measured, and fasting blood samples were obtained at baseline and 48 h post-trial. Mixed-design ANOVA analysis was employed followed by *post hoc* t-tests and Bonferroni alpha-correction was used to evaluate interaction, between-group, and within-group differences, respectively.

Results: Results indicated that HIIT and MICT similarly affected body fat mass ($p = 0.021$, $ES = 0.19$; $p = 0.016$, $ES = 0.30$, respectively), body fat percentage ($p = 0.037$, $ES = 0.17$; $p = 0.041$, $ES = 0.28$, respectively), visceral fat area ($p = 0.001$, $ES = 0.35$; $p = 0.003$, $ES = 0.49$, respectively) of body composition. A positive outcome was observed for waist/hip ratio ($p = 0.033$, $ES = 0.43$) in HIIT, but not MICT ($p = 0.163$, $ES = 0.33$). No significant differences were found between groups for any clinical biomarkers. However, pairwise comparison within the group showed a significant decrease in systolic blood pressure ($p = 0.018$, $ES = 0.84$), diastolic blood pressure ($p = 0.008$, $ES = 1.76$), and triglyceride ($p = 0.004$, $ES = 1.33$) in HIIT, but no significant differences were found in the MICT and Control group.

Conclusion: Both 8-week HIIT and MICT programmes have similar positive effects on reducing body fat mass, fat percentage, and visceral fat area.

However, sedentary adolescents may have limited scope to decrease insulin resistance after these 8-week interventions. Notably, the 8-week HIIT intervention was highly effective in increasing cardiometabolic health compared to the MICT. The exercise intensity threshold value and metabolic outcomes of high-intensity interval sprints should be explored further to extend the long-term benefit in this cohort.

KEYWORDS

sprint interval training, adolescent, cardiovascular disease, visceral fat, waist circumference, clinical biomarker

Introduction

Cardiovascular-related chronic diseases, e.g., obesity, type 2 diabetes, and induced arterial stiffness are a major global health concern. The cause of chronic diseases is multifactorial but ultimately results from a chronic imbalance of metabolism (Sharifi-Rad et al., 2020; Raut and Khullar, 2023). Waist circumference, fasting glucose, triglycerides, low-density lipoprotein cholesterol, insulin resistance, and chronic inflammation have all been implicated in the development of these diseases and are components of the metabolic syndrome (Kessler et al., 2012; Gottsäter et al., 2015; Palombo and Kozakova, 2016). The origins of these chronic diseases often lie in childhood or adolescence (Cockcroft et al., 2018), whereas active lifestyle and habits developed very early in childhood and adolescence often lead into positive habits in adulthood (Telama et al., 2014). Furthermore, if obesity becomes established in adolescence, a later spontaneous reversal of the weight gain is uncommon (Thompson et al., 2007; Patton et al., 2011; Brisbois et al., 2012), and highlights the importance of physical activity intervention for this young cohort. Furthermore, a considerable number of the investigated health outcomes had a significant association with overweight or obese children and adolescent cohorts (Delgado-Floody et al., 2018; Dias et al., 2018; Espinoza-Silva et al., 2019), and whilst the majority of children and adolescents are typically normal-weight, yet still exposed to potential risk factors, e.g., increased lipid levels and high weight category, they are still likely to demonstrate cardiovascular disease later in adulthood (Rosengren et al., 2017; Cooper and Radom-Aizik, 2020; Agbaje, 2024). Therefore, considering that cohorts of 18-year-olds will regularly spend several years in a constant environment, i.e., college or university, this presents a perfect opportunity to intervene effectively before later adult years set in. Hence, strategies that can modify glucose metabolism, insulin resistance, and visceral adiposity during youth may play an important role in disease prevention in later life (Esquivel Zuniga and DeBoer, 2021; Calcaterra et al., 2022; Polidori et al., 2022).

Higher levels of exercise are regularly prescribed for the prevention and treatment of several cardiovascular diseases, e.g., type 2 diabetes (De Nardi et al., 2018; Syeda et al., 2023), arterial stiffness (Way et al., 2019; Sequi-Dominguez et al., 2023), and stroke (Boehme et al., 2017; Gjellesvik et al., 2021). Children and adolescents are currently recommended to undertake at least 60 min of moderate-to-vigorous physical activity on a daily basis, but 81% of adolescents do not meet the WHO global recommendations on physical activity for health (Chaput et al.,

2020; Farooq et al., 2020; Marzi et al., 2022). Moderate-intensity continuous training (MICT) has been shown to provide numerous health benefits, including improved cardiorespiratory fitness (Faria et al., 2020), reduced body fat (Mendonça et al., 2022), and enhanced cardiovascular function (Collins et al., 2023), making it a cornerstone of many physical activity guidelines for children and adolescents. However, MICT requires a longer duration of continuous exercise, which may pose challenges for adherence among children and adolescents with busy schedules or lower motivation levels, potentially affecting their long-term commitment to regular physical activity. Consequently, researchers have focused on alternative forms of exercise, i.e., high-intensity interval training (HIIT), which is considered time-efficient and enjoyable. HIIT may induce similar or greater potential benefits compared with MICT in children and adolescents (Corte de Araujo et al., 2012; Martin-Smith et al., 2020). As a result, HIIT has emerged as a promising alternative, potentially more appealing and practical for youth due to its shorter duration and varied intensity. The mechanisms by which HIIT produces beneficial effects are complex. However, physiologists generally agree that, compared to MICT, HIIT effectively enhances lipid metabolism (Gripp et al., 2021), levels of anti-inflammatory factors (Khalafi and Symonds, 2020), insulin signaling pathways (Islam and Gillen, 2023), and endothelial nitric oxide synthase expression (Khalafi et al., 2022). These improvements contribute to the prevention of metabolic disorders and the amelioration of adverse cardiometabolic outcomes. In particular, HIIT has been shown to improve body composition, aerobic fitness, and vascular function during supervised lab-based studies (Barker et al., 2014; Dias et al., 2018; Ingul et al., 2018), which is associated with a reduced risk of cardiovascular events in later life. However, adherence, enjoyment, and health benefits of school-based field HIIT performed independently are yet to be fully understood, e.g., the enjoyment levels observed in supervised, structured settings may not necessarily translate to independent exercise sessions, and the health benefits may not be fully replicated in real-world environments (Lubans et al., 2021; Sun, 2024). In addition, previous studies have focused more on the relationship between overweight and metabolic health in adolescents (Racil et al., 2016; Miguët et al., 2020). Although laboratory-based HIIT studies have played an important role in consultation and clinical treatment, the current situation is that sedentary behavior and a lack of exercise are prevalent in young people (Arundell et al., 2016; Reilly et al., 2022).

Characterizing the effects of two different intervention protocols (i.e., HIIT vs. MICT) may help individuals to find the most

appropriate approach for targeting academic performance and future well-being. The research in this cohort may also improve the comprehension of school/college administrators about the optimal exercise strategies to implement. Therefore, the purpose of this study was two-fold: i) to analyze the within-group variations of HIIT and MICT interventions lasting 8 weeks on body composition and cardiovascular metabolic of sedentary adolescents; and ii) to analyze the between-group differences of both training interventions on body composition and cardiovascular metabolic outcomes of sedentary adolescents. The primary hypothesis of this study was that HIIT and MICT will significantly improve body composition in terms of body mass index, fat mass percentage, visceral fat area, and waist/hip ratio in sedentary youth compared to a control group performing regular physical activity intervention. The secondary hypothesis was that cardiovascular metabolic outcomes benefits in terms of blood pressure, total cholesterol, triglyceride, low-density lipoprotein cholesterol, and HOMA-IR would decrease in both intervention groups compared to the control group.

Methods

Study participants

A power analysis completed with G*Power (ver. 3.1.9.7; Heinrich-Heine-Universität, Düsseldorf, Germany) using a medium effect size revealed that 24 participants would be necessary to detect a significant medium effect ($d = 0.5$) for the outcome in a within-between interaction with alpha error probability set at 0.05 and power adjusted to 0.80. However, the COVID-19 pandemic made enrollment and fidelity to aspects of study protocols challenging (McDermott and Newman, 2020). In this trial, pandemic-related restrictions that limited in-person visits resulted in unprecedented obstacles to interviewing potential participants, trial enrollment, data collection, and intervention delivery for this trial. Eventually, twenty-three healthy normal-weight youth were recruited from Nanjing Agricultural University, Nanjing, China. While a disappointing result for the exceptional time and effort placed into enrollment, this trial also remains a helpful guide to Chinese sedentary adolescents towards finding ways of engaging appropriate protocols and improving cardiometabolic health outcomes. Importantly, it should be noted that the sample was highly representative of the study population, as exclusion criteria included any known disease or contraindications to exercise and the use of medications or substances known to influence blood pressure, cholesterol, and carbohydrate metabolism. Participants were inactive as assessed by participating in television, computer, smartphone, video games viewing, and sitting socializing for over 8 h daily in the past 5 months. Furthermore, participants took China's college entrance exam before recruitment, had no regular physical activity during the last 3 years, and performed only activities of daily living. After participants underwent an initial health-related behavior questionnaire testing and electrocardiogram examination, one female participant was excluded because of bradycardia, and two male participants failed to join the project because of their enrolment in more than 3 h per week structured program of physical activity recently, one male and one female

participant withdrew from the project due to personal reasons unrelated to the experiment. Thus, a total of 18 participants (age: 18.5 ± 0.3 years, 11 females) completed this investigation from November 2021 to January 2022 (Figure 1). This investigation was conducted following the recommendations of the Declaration of Helsinki for Human Studies and the university ethical approval (number: RT-2023-01). Written informed consent was obtained from all participants.

Body composition and blood pressure measurements

Multifrequency bioelectrical impedance analysis of body composition is regarded as an appropriate alternative to dual-energy x-ray absorptiometry, and is widely used in clinical practice (Anderson et al., 2012). Body mass index (BMI), body fat mass (BF), body fat percentage (BFP), visceral fat area (VFA), waist/hip ratio (WHR) were evaluated before and after intervention using multifrequency bioelectrical impedance analysis (Multifrequency bioelectric impedance analyzer InBody 720, Biospace Co. Ltd., Seoul, Korea). All assessments were conducted between 9 a.m. and 12 p.m. to minimize variability due to time-of-day effects or physical activity. Measurements are performed on five segments (right upper limb, left upper limb, trunk, right lower limb, and left lower limb) using six bioimpedance frequencies (including 1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz, and 1,000 kHz). Participants wore everyday indoor clothing and were required to stand barefooted in an upright position with their feet on the feet electrodes of the machine platform and their arms abducted with hands gripping onto the hand electrodes of the handles during the test.

Blood pressure was determined using an auto-inflating cuff (Omron, HEM-1020, Dalian, China). To ensure consistency, all measurements were taken from 9 a.m. to 12 p.m. on the same day, following a 10-min rest period. Participants were seated, with their back supported, feet on the ground, and arms supported at heart level. Measurements were taken on the right arm, and the average of three consecutive readings was recorded. This standardized protocol helped to minimize variability due to time-of-day effects and participant posture. Participants were also advised to remain silent throughout the measurement.

Biochemical measurements

Blood samples were collected from the participants' peripheral veins in the morning after a fasting period of 12 h. The whole blood samples were centrifuged at 3,500 revolutions per min for 8 min. Plasma was separated and stored at -80°C until assayed. The automated biochemical analyzer (ARCHITECT c16000, Abbott, Singapore) and the automated chemiluminescence immunoassay analyzer (i 2000SR, Abbott, Singapore) were used to detect the biochemical indicators. Plasma glucose (PG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined by Hexokinase Method, COD-PAP, GPO-PAP, CAT-Assay, and Surfactant Assay respectively, using Biosino Kits (Biosino Bio-

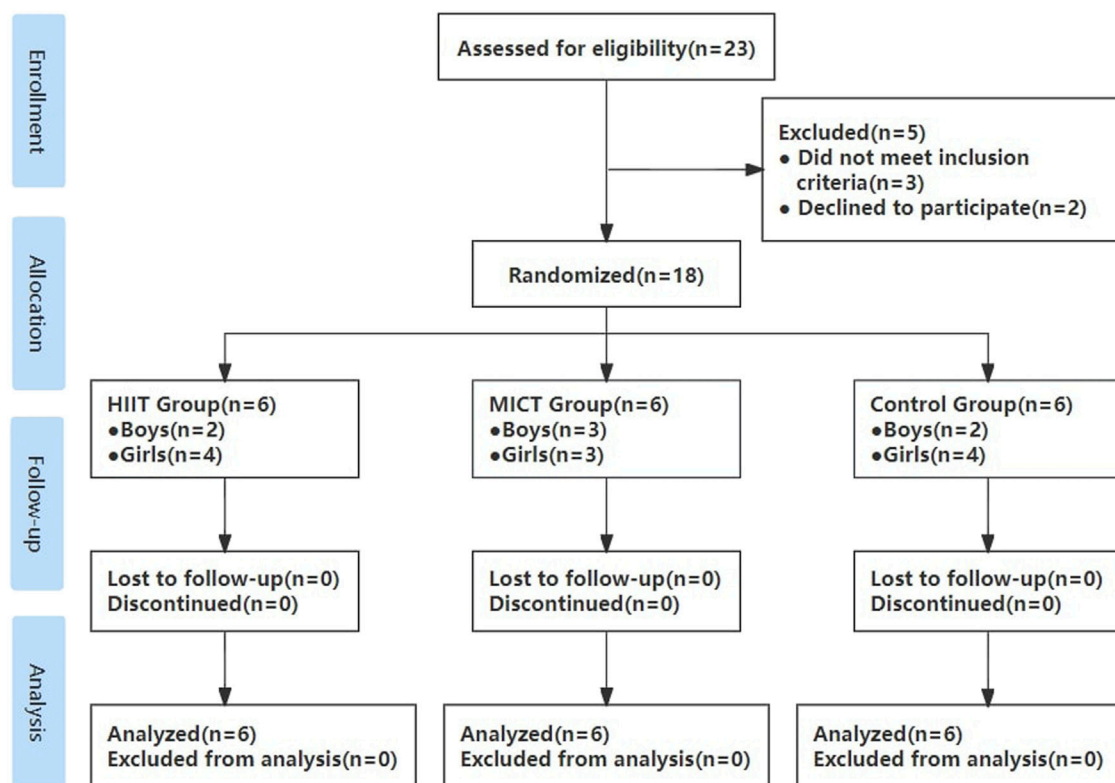


FIGURE 1
Flow diagram throughout the course of the study. HIIT indicates high-intensity interval training; MICT indicates moderate-intensity continuous training; Control indicates no intervention.

technology and science INC., Beijing, China). Plasma insulin was determined by CLIA, using an Abbott Insulin Reagent Kit (DENKA SEIKEN Co., LTD., Tokyo, Japan). Hypersensitive C-reactive protein (Hs-CRP) was determined by Immunoturbidimetry, using an Abbott CPR Vario (DENKA SEIKEN Co., LTD., Tokyo, Japan). All detection steps and operations were carried out in accordance with the kit instructions. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) according to the formula: $HOMA-IR = \text{fasting plasma insulin} \times \text{fasting plasma glucose} / 22.5$ (Invitti et al., 2003).

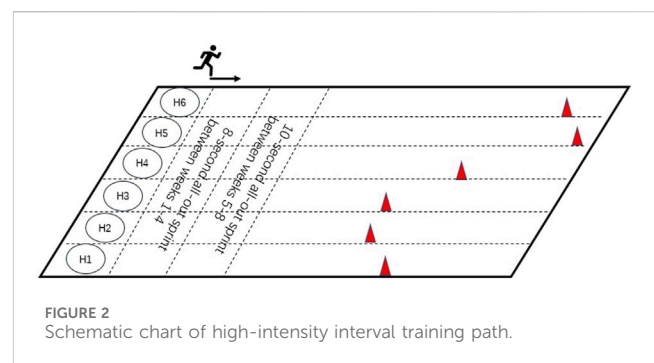
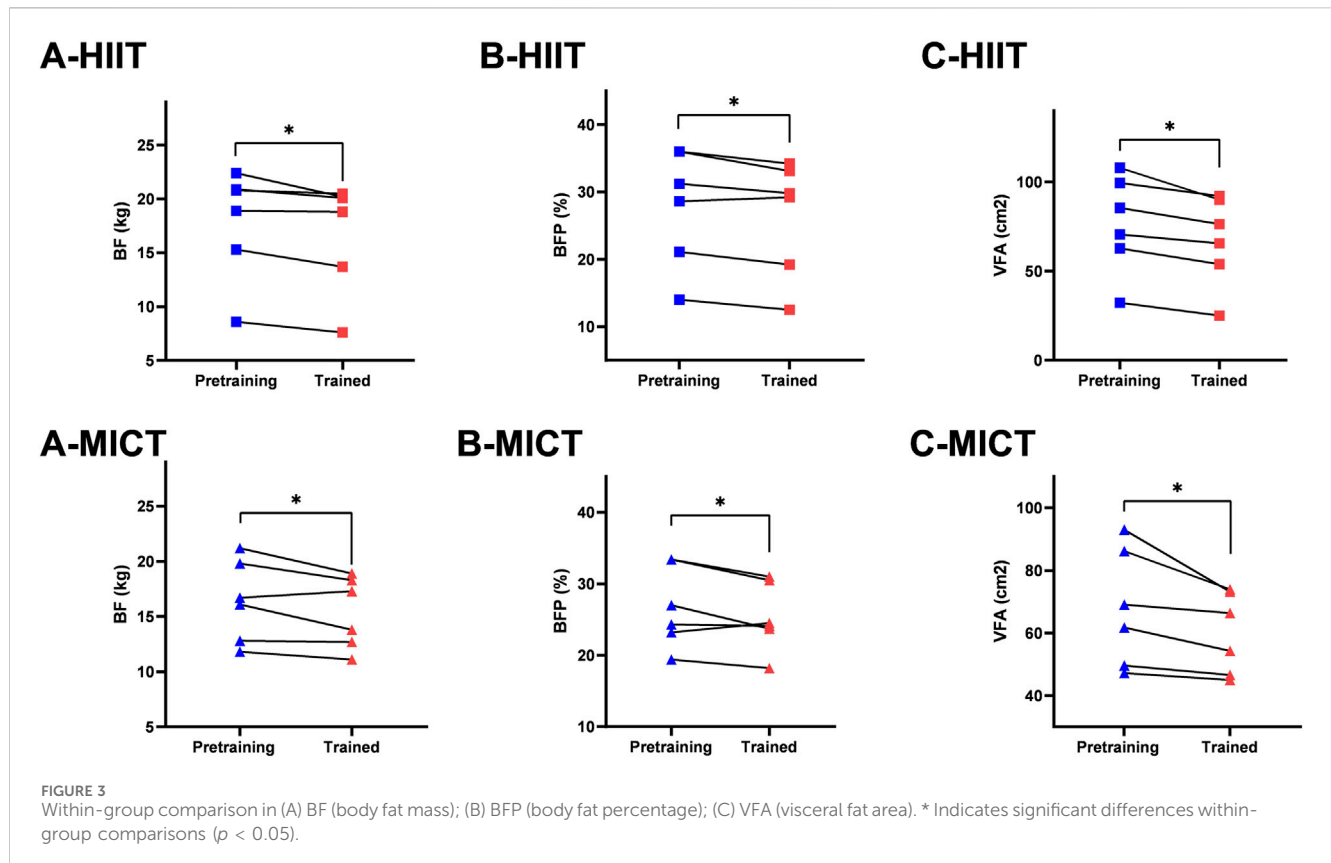


FIGURE 2
Schematic chart of high-intensity interval training path.

Interventions

Participants were randomly allocated into three groups: HIIT ($n = 6$), MICT ($n = 6$), and Control ($n = 6$). The intervention of HIIT group was conducted three times per week, 30 min per session (including a 5-min warm-up, 20-min sprint interval training, and a 5-min cooldown and stretching) for 8 weeks, whilst the MICT group received three times per week, 30 min per session (including a 5-min warm-up, and 20-min continuous running, and a 5-min cooldown and stretching) for 8 weeks, Control group was advised to maintain their current physical activity levels and dietary habits during the study. All participants were recommended to maintain a daily calorie intake appropriate for late adolescents, depending on body size and activity level, and to avoid unhealthy snacks. HIIT

group consisted of three sessions/week for 8 weeks of “all out” sprint, 8-s sprint, and 24-s active recover/during 1–4 weeks and 10-s sprint and 30-s active recover/during 5–8 weeks. HIIT group participants were required to turn-back and walk after every all-out sprint running and repeat the exercise. Participants were required to try to reach the location of the cone within the specified time. HIIT group intensity was set at 85%–95% of HR_{max} with active recovery periods of walking between bouts, MICT group intensity was set at 65%–75% of HR_{max} . HR_{max} was determined through a baseline VO_{2max} test using the Bruce protocol until the point of voluntary exhaustion. This test was performed on a treadmill (H/P/Cosmos para graphics, H/P/COSMOS Sports and Medical, Germany). Simultaneously, respiration and heart rate were monitored using a wearable metabolic system (K5 Wearable



Metabolic System, COSMED, Italy). HIIT group exercise interventions were performed on a short track of Nanjing Agricultural University Athletics Field (Figure 2), MICT group interventions were performed on the circle track of the same athletics field. The HIIT group completed between 21–24 all-out sprint running within a 20-min session. The total distance of the HIIT group in each session was 4.20 ± 0.56 km, and the total distance of the MICT group was 3.98 ± 0.26 km. The intensity of all the intervention groups was monitored by the POLAR OH1 MODEL 2L (Patented WR3, Malaysia). All interventions were also supervised by professionals, with intensity feedback and speed adjustments using Polar monitors with GPS tracking, recording heart rate every second. Researchers also encouraged the participants to elicit the running at all-out intensity during HIIT programs, the heart rate response during HIIT programs ranged from 96 to 197 bpm (113 ± 8.62 bpm– 189 ± 5.38 bpm), including the lowest heart rates recorded during the warm-up phase. Participants were encouraged to run and avoid any walking during MICT programs, the heart rate response during MICT programs ranged from 90 to 188 bpm (103 ± 8.23 bpm– 180 ± 6.79 bpm), also accounting for warm-up periods. The heart rates generally corresponded to the target intensity during the main exercise bouts.

As shown in Figure 2, the location of the cones is determined by each participant, e.g., 8-s all-out sprint distance between weeks 1–4, and 10-s all-out sprint distance between weeks 5–8. The cones were repositioned by a professional supervisor at the first training session of each week to avoid deviation due to training fitness increase.

Statistical analysis

The descriptive characteristics of the participants are presented as mean and SD using the IBM SPSS Statistics version 26.0 (Chicago, IL, United States). Normality and homogeneity of the sample were preliminary tested using Shapiro-Wilk test and Levene's test, respectively (Razali and Wah, 2011). The normality of the outcomes was explored individually and for each group revealing a $p > 0.05$ for all the outcomes considered. The homogeneity of the outcomes was tested using Levene's presenting values above $p > 0.05$. One-way ANOVA and Bonferroni's *post hoc* tests were used for the evaluation baseline data of three groups. A mixed model ANOVA with group (CON, MICT, HIIT) and time (baseline and after intervention) analyzed the effects of body composition and cardiometabolic outcomes after the intervention. Interaction was reported in the format of p -value and partial eta squared (η^2_p). An η^2_p of 0.01 indicated a small effect, 0.06 a medium effect, and 0.14 a large effect (Lakens, 2013). A simple effect test was conducted after confirmation of interaction (group \times time) to avoid misleading analyses. Magnitude of standardized differences between two means were evaluated using the standardized effect size of Cohen's d (ES), with the equation (mean post–mean pre)/SD pooled. An ES of 0.20, 0.50, and 0.80 was considered to represent a small, moderate, and large change between means (Kazis et al., 1989). A level of $p < 0.05$ was set *a priori* to establish statistical significance.

TABLE 1 Body composition before and after training for HIIT, MICT, and Control groups.

Outcomes	Group	Pretraining	Trained	ANOVA (F, p, η^2_p)								
				Time effect			Group effect			Time x group effect		
BM (kg)	HIIT	64.4 ± 5.1	63.8 ± 4.5	2.21	0.158	0.13	4.25	0.035*	0.36	0.45	0.648	0.06
	MICT	61.3 ± 6.4	60.6 ± 5.9									
	Control	53.5 ± 8.5	53.4 ± 8.0									
SM (kg)	HIIT	25.7 ± 4.6	26.3 ± 4.3	3.59	0.078	0.19	1.76	0.206	0.19	0.54	0.593	0.07
	MICT	24.6 ± 3.7	24.9 ± 3.2									
	Control	21.5 ± 4.8	21.7 ± 4.3									
BF (kg)	HIIT	17.8 ± 5.1	16.8 ± 5.2	11.35	0.004*	0.43	0.93	0.418	0.11	1.45	0.266	0.16
	MICT	16.4 ± 3.7	15.4 ± 3.2									
	Control	13.9 ± 5.1	13.7 ± 4.7									
BMI (kg/m ²)	HIIT	23.5 ± 2.3	23.4 ± 2.3	1.99	0.179	0.18	4.36	0.032*	0.37	0.50	0.618	0.06
	MICT	22.5 ± 1.7	22.2 ± 1.6									
	Control	20.3 ± 1.7	20.3 ± 1.7									
BFP (%)	HIIT	27.8 ± 8.7	26.3 ± 8.6	9.05	0.009*	0.38	0.05	0.949	0.01	0.82	0.460	0.10
	MICT	26.8 ± 5.7	25.3 ± 4.8									
	Control	26.0 ± 8.2	25.6 ± 7.1									
VFA (cm ²)	HIIT	76.4 ± 27.5	67.1 ± 25.2	23.92	<0.001*	0.62	0.65	0.538	0.08	3.65	0.051	0.33
	MICT	67.8 ± 18.8	59.9 ± 13.0									
	Control	57.1 ± 27.9	55.7 ± 25.1									
WHR	HIIT	0.84 ± 0.03	0.83 ± 0.04	1.84	0.195	0.11	0.14	0.869	0.02	3.99	0.041*	0.35
	MICT	0.83 ± 0.03	0.82 ± 0.02									
	Control	0.82 ± 0.02	0.83 ± 0.02									

Results shown as mean ± SD.

Abbreviations: HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; BM, body mass; SM, skeletal muscle; BF, body fat mass; BMI, body mass index; BFP, body fat percentage; VFA, visceral fat area; WHR, waist/hip ratio. * $p < 0.05$, # $p < 0.01$.

Results

A total of 18 adolescents (age = 18.5 ± 0.3 years) performed the baseline assessments. The baseline characteristics of the study participants are shown in Table 1. There was a significant difference between HIIT and CON in BM and BMI ($p < 0.05$), but no other significant differences were observed between the groups before exercise intervention ($p > 0.05$). Furthermore, no testing or training-related severe injuries occurred over the trial period, and the attendance rates were 100% for the two intervention groups, i.e., HIIT and MICT.

Descriptive statistics of pre- and post-intervention values of body composition can be found in Table 1. There was time main effect of BF ($p = 0.004$, $\eta^2_p = 0.43$), BFP ($p = 0.009$, $\eta^2_p = 0.38$), VFA ($p < 0.001$, $\eta^2_p = 0.62$), no significant differences across the two-time point in BM ($p = 0.158$, $\eta^2_p = 0.13$), SM ($p = 0.078$, $\eta^2_p = 0.19$), BMI ($p = 0.179$, $\eta^2_p = 0.12$), and WHR ($p = 0.195$, $\eta^2_p = 0.11$). There was group main effect of BM ($p = 0.035$, $\eta^2_p = 0.36$), BMI ($p = 0.032$, $\eta^2_p = 0.37$), no significant differences between groups in SM ($p = 0.206$, $\eta^2_p = 0.19$), BF ($p = 0.418$, $\eta^2_p = 0.11$), BFP ($p = 0.949$, $\eta^2_p = 0.01$), VFA ($p = 0.538$, $\eta^2_p = 0.08$), and WHR ($p = 0.869$, $\eta^2_p = 0.02$). Figure 3 presents the within-and between-group variations for the body composition. The within-group changes revealed a significant decrease in BF in HIIT ($p = 0.021$, ES = 0.19) and MICT ($p = 0.016$, ES = 0.30), whereas no significant changes occurred in CON ($p = 0.585$, ES = 0.04). Significant decrease in BFP in HIIT ($p = 0.037$, ES = 0.17) and MICT ($p = 0.041$, ES = 0.28), whereas no significant changes occurred in CON ($p = 0.499$, ES = 0.06). Significant decrease in

VFA in HIIT ($p = 0.001$, ES = 0.35) and MICT ($p = 0.003$, ES = 0.49), whereas no significant changes occurred in CON ($p = 0.527$, ES = 0.06). Between-group variations revealed a significant difference in post-interventions between HIIT and CON ($p = 0.036$, ES = 1.60) in BM and between HIIT and CON ($p = 0.037$, ES = 1.53) in BMI, however considering that there were also significant differences of the baseline between HIIT and CON ($p = 0.041$, ES = 1.56) in BM and between HIIT and CON ($p = 0.031$, ES = 1.62) in BMI, the statistically significant is severely limited. There was also a group × time significant interactions effect in WHR ($p = 0.041$, $\eta^2_p = 0.35$), and a trend of significant interactions effect in VFA ($p = 0.051$, $\eta^2_p = 0.33$), although the result was not significant. However, no significant interaction effects were found in BM ($p = 0.648$, $\eta^2_p = 0.06$), SM ($p = 0.593$, $\eta^2_p = 0.07$), BF ($p = 0.266$, $\eta^2_p = 0.16$), BMI ($p = 0.618$, $\eta^2_p = 0.06$), and BFP ($p = 0.460$, $\eta^2_p = 0.10$). Follow up for this interaction indicated that there were no significant differences between groups at baseline and post-intervention in WHR. However, a pairwise comparison within the group showed that HIIT group had a significant decrease ($p = 0.033$, ES = 0.43) in WHR, and no significant difference was found in MICT ($p = 0.163$, ES = 0.33) and Control group ($p = 0.163$, ES = 0.37) (Figure 4).

Descriptive statistics of pre- and post-intervention values of clinical biomarkers can be found in Table 2. No significant differences in clinical biomarkers were found between groups in baseline and post-intervention ($p > 0.05$). There were no significant differences in clinical biomarkers within groups in baseline and post-intervention in HIIT and MICT. However, PG had a time main effect in CON ($p = 0.008$, $\eta^2_p = 0.39$). The within-

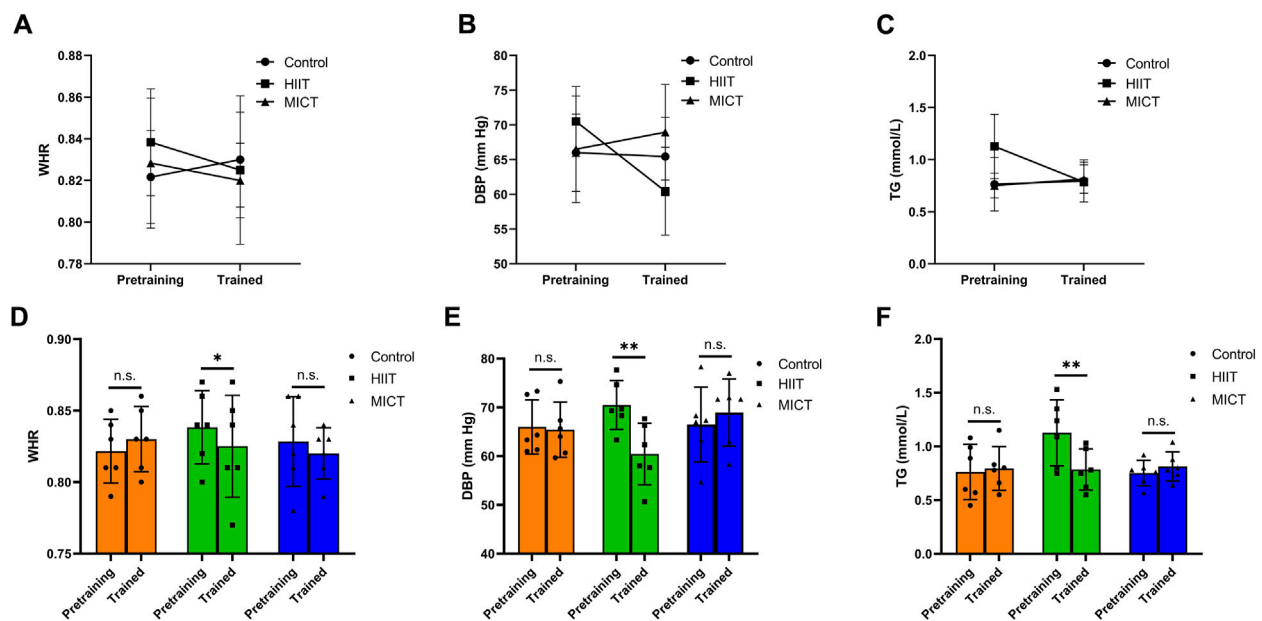


FIGURE 4 Group \times time significant interactions effect in (A) WHR (waist/hip ratio); (B) DBP (diastolic blood pressure); (C) TG (triglyceride). Pre- and post-training values [Mean (\pm SD)] for (D) WHR, (E) DBP, and (F) TG in HIIT (High-intensity interval training), MICT (Moderate-intensity continuous training), and Control groups. * Indicates significant differences within-group comparisons ($p < 0.05$), ** Indicates significant differences within-group comparisons ($p < 0.01$).

group changes revealed a significant increase in PG in CON ($p = 0.024$, $ES = 0.64$), whereas no significant changes occurred in HIIT ($p = 0.059$, $ES = 0.63$) and MICT ($p = 0.443$, $ES = 0.08$). Significant interactions were found between groups and time (pre-post) in the mixed ANOVA conducted for DBP ($p = 0.041$; $\eta^2_p = 0.35$) and TG ($p = 0.023$; $\eta^2_p = 0.40$) (Figure 4). No significant interactions were found in mixed ANOVA for the case of SBP ($p = 0.134$; $\eta^2_p = 0.24$), PG ($p = 0.472$, $\eta^2_p = 0.10$), Insulin ($p = 0.095$, $\eta^2_p = 0.27$), TC ($p = 0.423$, $\eta^2_p = 0.11$), HDL-C ($p = 0.115$, $\eta^2_p = 0.25$), LDL-C ($p = 0.347$, $\eta^2_p = 0.13$), hs-CRP ($p = 0.900$, $\eta^2_p = 0.01$), and HOMA-IR ($p = 0.141$, $\eta^2_p = 0.23$). PG in three groups increased (3.91%, 1.40%, and 2.20%, respectively), insulin in HIIT decreased (10.80%), but insulin in MICT and control both increased (19.65% and 49.54%, respectively). HOMA-IR also showed a similar trend among the three groups, HOMA-IR decreased in HIIT (5.63%), increased in MICT and Control (21.28% and 55.17%, respectively). Following up this interaction indicated that there was no significant difference between groups at baseline and post-intervention in DBP and TG. However, pairwise comparison within the group showed HIIT group, there was a significant decrease in SBP ($p = 0.018$, $ES = 0.84$), DBP ($p = 0.008$, $ES = 1.76$), and TG ($p = 0.004$, $ES = 1.33$), respectively. No significant difference was found in MICT ($p = 0.932$, $ES = 0.02$) and Control group ($p = 0.983$, $ES = 0.01$) for SBP, MICT ($p = 0.466$, $ES = 0.34$) and Control group ($p = 0.867$, $ES = 0.10$) for DBP, and MICT ($p = 0.552$, $ES = 0.48$) and Control group ($p = 0.759$, $ES = 0.14$) for TG.

Discussion

The primary goal of this study was to evaluate and compare the effects of HIIT and MICT intervention programmes on body

composition and cardiometabolic health in sedentary adolescents. It was hypothesized that HIIT and MICT would significantly improve body composition in terms of body mass index, fat mass percentage, visceral fat area, and waist/hip ratio in sedentary youth compared to a control group. Our secondary hypothesis was that cardiovascular metabolic outcomes benefits in terms of blood pressure, total cholesterol, triglyceride, low-density lipoprotein cholesterol, and HOMA-IR would decrease in HIIT group and MICT group compared to the control group. The present experiment demonstrated the primary hypothesis that HIIT and MICT have similar effects on body composition in sedentary Chinese adolescents. Additionally, the secondary hypothesis was partially confirmed, while both intervention programmes improved cardiometabolic health, the 8-week HIIT was highly effective in increasing cardiometabolic health compared to MICT.

Our findings indicated that the BMI of all groups did not significantly change after the 8-week exercise intervention ($p > 0.05$). Previous studies have presented positive conclusions regarding the effect of HIIT on BMI. 12-week HIIT or 8-week HIIT combined nutrition intervention significantly decreased BMI in obese adolescent boys (age 11.2 ± 0.7 years) (Meng et al., 2022) and overweight adolescent girls (age 15.5 ± 0.7 years) (Bogataj et al., 2021). To our knowledge, however, there was little evidence to confirm the positive findings of BMI after HIIT intervention in normal-weight adolescents. Our data confirm the conclusion of the previous meta-regression analysis, which found that having an entire population classified as overweight or obese significantly moderated the results for BMI ($n = 9$, $\beta = -1.38$, $p < 0.0001$) and waist circumference ($n = 7$, $\beta = -0.56$, $p = 0.009$) (Duncombe et al., 2022). Adolescents who are overweight and obese are associated with significantly increased risk of later

TABLE 2 Clinical biomarkers before and after training for HIIT, MICT, and Control groups.

Outcomes	Group	Pretraining	Trained	ANOVA (F, p, η^2_p)								
				Time effect			Group effect			Time x group effect		
SBP (mmHg)	HIIT MICT Control	114 ± 6.3 115 ± 9.5 109 ± 8.8	108 ± 9.6 115 ± 7.5 109 ± 10.3	2.48	0.136	0.14	0.80	0.470	0.10	2.31	0.134	0.24
DBP (mmHg)	HIIT MICT Control	71 ± 5.0 67 ± 7.7 66 ± 0.6	60 ± 6.3 69 ± 6.9 65 ± 5.7	2.08	0.170	0.12	0.40	0.680	0.05	3.99	0.041*	0.35
PG (mmol/L)	HIIT MICT Control	4.85 ± 0.32 5.22 ± 0.92 4.87 ± 0.38	5.04 ± 0.29 5.29 ± 0.82 5.11 ± 0.35	9.48	0.008#	0.39	0.54	0.591	0.07	0.79	0.472	0.10
Insulin (uU/mL)	HIIT MICT Control	9.54 ± 3.37 8.18 ± 2.31 6.09 ± 1.90	8.51 ± 2.41 9.79 ± 2.99 9.11 ± 4.21	2.82	0.114	0.16	0.60	0.561	0.07	2.77	0.095	0.27
TC (mmol/L)	HIIT MICT Control	3.31 ± 0.41 4.06 ± 0.77 3.71 ± 0.44	3.32 ± 0.74 3.99 ± 1.09 3.98 ± 0.48	0.44	0.517	0.03	1.90	0.185	0.20	0.91	0.423	0.11
TG (mmol/L)	HIIT MICT Control	1.13 ± 0.31 0.75 ± 0.12 0.76 ± 0.26	0.79 ± 0.19 0.81 ± 0.14 0.80 ± 0.21	2.00	0.178	0.12	2.06	0.162	0.22	4.91	0.023*	0.40
HDL-C (mmol/L)	HIIT MICT Control	1.17 ± 0.14 1.49 ± 0.31 1.51 ± 0.29	1.29 ± 0.19 1.42 ± 0.38 1.59 ± 0.30	1.33	0.267	0.08	2.22	0.143	0.23	2.51	0.115	0.25
LDL-C (mmol/L)	HIIT MICT Control	1.66 ± 0.51 2.16 ± 0.46 1.81 ± 0.18	1.61 ± 0.62 2.10 ± 0.66 1.96 ± 0.33	0.04	0.849	<0.01	1.67	0.221	0.18	1.14	0.347	0.13
Hs-CRP (ug/mL)	HIIT MICT Control	0.90 ± 0.45 0.49 ± 0.22 0.75 ± 0.45	0.70 ± 0.27 0.40 ± 0.46 0.56 ± 0.54	2.49	0.135	0.14	1.50	0.255	0.17	0.11	0.900	0.01
HOMA-IR	HIIT MICT Control	2.03 ± 0.64 1.92 ± 0.64 1.33 ± 0.45	1.92 ± 0.61 2.32 ± 0.82 2.10 ± 1.09	4.27	0.057	0.22	0.63	0.548	0.08	2.24	0.141	0.23

Results shown as mean ± SD.
Abbreviations: HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; SBP, systolic blood pressure; DBP, diastolic blood pressure; PG, plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Hs-CRP, hypersensitive C-reactive protein; HOMA-IR: homeostatic model of insulin resistance. **p* < 0.05, #*p* < 0.01.

cardiometabolic morbidity, such as hypertension, ischaemic heart disease, and stroke in adulthood (Reilly and Kelly, 2011). BMI and WHR are useful measurable indices for assessing obesity and overweight. It should be noted that although the correlation of BMI is associated with increased mortality from cardiovascular disease (CVD), the validity of BMI in predicting CVD is controversial (Westphal, 2008). This disparity of risk may relate to different factors, e.g., body fat and visceral adipose tissue. For example, visceral adipose tissue can predict the development of obesity-related cardiometabolic disease and is an independent predictor of all-cause mortality in men independently of age, race, and sex (Kuk et al., 2006). Body fat mass and visceral adipose will be discussed in a subsequent section of this article. A significant interaction effect between group and time was observed for WHR (*p* < 0.05, effect size 0.35). Follow up for this interaction indicated WHR significantly decreased in HIIT group (*p* < 0.05, effect size 0.43). However, no significant difference was found in MICT group (*p* > 0.05, effect size 0.33) and Control group (*p* > 0.05, effect size 0.37) between baseline and post-intervention. In

line with our results, Ahmadi et al. found that 8-week HIIT with nutritional recommendations significantly reduced WHR in obese adolescents (Ahmadi et al., 2020). In contrast, Chuensiri et al. reported that a 12-week HIIT did not affect WHR, whereas novel cardiovascular factors, i.e., carotid intima-media thickness and endothelium-dependent vasodilation improved significantly (*p* < 0.05) in obese preadolescent boys (Chuensiri et al., 2018). Except for the heterogeneity of the samples, we assumed that HIIT combined with the nutritional recommendation was associated with favorable observation in WHR. Future studies are needed to validate these findings, i.e., the effect of HIIT protocol combined with nutritional recommendations on the reduction of lipid profile and other CVD risk factors in children and adolescents.

Body composition, especially fat mass index, is associated with wide range of cardiovascular conditions (Larsson et al., 2020). Our findings demonstrated that HIIT and MICT have small to medium effects on BF (*p* < 0.05, effect size 0.19 and 0.30, respectively), BFP (*p* < 0.05, effect size 0.17 and 0.28, respectively), and VFA (*p* < 0.01, effect size 0.35 and 0.49, respectively) in sedentary Chinese

adolescents. In line with our results, previous investigation demonstrated that a 12-week school-based HIIT protocol effectively reduced BF, BFP, and VFA of obese children (age 11.0 ± 0.6 years; BMI 23.6 ± 1.5) (Cao et al., 2022). It is inconsistent with the results of Camacho-Cardenosa et al. (2016) who reported that HIIT group (age 11.0 ± 0.2 years; BMI 18.4 ± 2.8 kg) and MICT group (age 11.2 ± 0.4 years; BMI 20.0 ± 3.3 kg) did not significantly change in total and trunk fat mass after an 8-week high-intensity program developed in adolescents during physical education classes (Camacho-Cardenosa et al., 2016). Furthermore, Lambrick et al. (2016) found that a 6-week high-intensity games intervention induced positive changes in waist circumference for obese participants (age 11.6 ± 0.8 years; BMI 49.3 ± 8.9 kg), however, with no significant difference for normal-weight participants (age 12.3 ± 0.9 years; BMI 32.5 ± 8.9 kg). According to the above findings, we inferred that at least 8-week intervention duration was essential factor to elicit metabolic outcomes in normal-weight adolescents.

Favorable metabolic outcomes were observed in SBP ($p = 0.018$, effect size 0.84), DBP ($p < 0.01$, effect size 1.76), and TG ($p < 0.01$, effect size 1.33) after the 8-week HIIT sessions. Our findings indicated consistency with previous investigation that demonstrated HIIT attenuated SBP and DBP (Alvarez et al., 2017; Ketelhut et al., 2020), TG (Racil et al., 2013; Khammassi et al., 2018). Interestingly, Khammassi et al. (2018) found that variables of findings depend on the patients' initial obesity degree. So, further studies are then needed to explore this potential implication of HIIT programme in normal-weight adolescents. In contrast, McDaniel et al. demonstrated that 5-week aquatic HIIT induced a trend ($p = 0.053$) for a reduction in SBP, whereas there was no change in DBP (McDaniel et al., 2020). Popowczak et al. (2022) reported that a 10-week HIIT significantly reduced SBP, whereas no impact on DBP. Furthermore, Dias et al. reported that a 12-week HIIT was highly effective in increasing cardiorespiratory fitness, there were no concomitant reductions in blood biomarkers (Dias et al., 2018). Although the blood pressure success change is partly explained by traditional CVD risk factors. However, the release and bioactivity of endothelium-derived nitric oxide induced by shear stress associated with HIIT regulate vascular tone by stimulating guanylate cyclase in the underlying smooth muscle, which may explain the favorable outcome of the blood pressure parameter (Bond et al., 2015; da Silva et al., 2020; Paniagua et al., 2001). No significant differences were observed in TC, HDL-C, and LDL-C in this trial between baseline and post-intervention ($p > 0.05$). Given that SBP, DBP, and TG decreased in HIIT group, there was no significant change in MICT group and Control group in the present study. Our finding demonstrated that the 8-week HIIT intervention programme was highly effective in increasing cardiometabolic health compared to MICT.

Various methods are used to assess insulin sensitivity. However, validity, reproducibility, cost, and degree of subject burden are important factors for researchers to consider when weighing the merits of a particular method (Patarrão et al., 2014). In this study, simple surrogate indexes for insulin resistance are assessed that are derived from blood insulin and glucose concentrations under fasting conditions. Compared with some previous research, e.g., a single bout of HIIT (Cockcroft et al., 2018), 6 weeks of HIIT (Alvarez et al., 2017), and 12 weeks of HIIT (Ryan et al., 2020) both can induce improvements in insulin sensitivity. In this 8-week HIIT trial,

statistical results did not confirm an effective change, although there was a slight decrease in insulin resistance (5.63%) in the HIIT group after 8-week intervention, but an increase in the MICT (21.28%) and Control (55.17%). Insulin resistance is a hallmark of obesity and cardiovascular diseases, especially as insulin resistance precedes and contributes to the development of many metabolic disorders, e.g., stroke and atherosclerotic (Nigro et al., 2006; Ago et al., 2018). Furthermore, the evidence showed that insulin resistance was relevant to an increase in different inflammatory markers (Dandona et al., 2004). The increase of inflammatory factors and the associated alterations of oxidative stress seem to play a crucial role in the early stages of atherogenesis (Giannini et al., 2008). It is believed to be involved throughout the atherogenic process, facilitating everything from the initial recruitment of leukocytes to the arterial wall to the eventual rupture of the plaque (Clearfield, 2005). Thus, hs-CRP is a potential adjunct for global risk assessment in the primary prevention of CVD. Our findings indicated that the hs-CRP decreased in the HIIT group (22.44%) and MICT group (19.32%) after the 8-week HIIT intervention. However, a similar decrease was observed in Control group (24.60%). Our findings are not in accordance with previous observations. Paahoo et al. (2021) reported that a 12-week HIIT had more positive effects than aerobic exercise on hs-CRP in obese adolescent boys. Furthermore, a significant hs-CRP decrease was observed in adolescent girls with obesity after 12-week HIIT combined with diet intervention (Playsic et al., 2020). Overall, our results refute the widely reported increase in insulin sensitivity and decrease in hs-CRP demonstrated with HIIT, which is likely due to day-to-day variability in determinations of plasma biomarkers, as well as the heterogeneity of our sample. However, considering that the HIIT intervention programme only marginally reduced insulin resistance, and insulin resistance of MICT group and control group increased, although the MICT group underwent regular training. These results demonstrated that adolescents who experience chronic sedentary behavior, such as taking China's college entrance exam, remain at high risk of metabolic syndrome after entering university, and these findings reinforce the need to develop programs for the effective prevention of chronic disease in this cohort.

Effective protocols to improve the metabolic profile in adolescents include 10-s sprints interspersed with 10-s recovery (Baquet et al., 2001), 30-s sprints interspersed with 30-s rest bouts (Buchan et al., 2012), and 60-s sprints with 180-s of active recovery between bouts (Corte de Araujo et al., 2012). These protocols are usually practised at 90% maximum heart rate or at 100% peak velocity. Our research adopts multiple times and low-volume in a ratio of 1:3 for all-out sprints and intervals, mainly to reflect better enjoyment and feasibility, so that it is easier for the participants to persist in HIIT for long-term exercise and ensure a high-speed running performance (Williams et al., 2000; Corte de Araujo et al., 2012; Malone et al., 2019). Furthermore, this experiment was conducted strictly to ensure the accuracy and feasibility of the HIIT and MICT programs, and the participants' subject feelings were good. The heart rate monitoring in this experiment found that the heart rate during the active intervals in the HIIT group and the heart rate during the stabilization period in the MICT group were similar with previous trial delivered in real-world (Camacho-Cardenosa et al., 2016; Logan et al., 2016). Our study enhanced

the understanding for confirming the heart rate range for HIIT or MICT delivered on an athletics field.

Despite evidence indicating that HIIT is a time-efficient strategy to improve cardiometabolic risk factors, HIIT appears to promote superior improvements in some cardiometabolic risk factors when performed by healthy participants for at least 8–12 weeks (Kessler et al., 2012). This is consistent with only parts of the cardiometabolic risk factors being examined in our trial. Previous studies also confirmed the inference of sufficient duration (Hottenrott et al., 2012; Racil et al., 2013; Abassi et al., 2020; Playsic et al., 2020). Therefore, it is possible that our intervention was of insufficient duration to improve some health outcomes. Future studies and practitioners may choose to adapt these effective protocols or create new training programs for youth (Chmura et al., 2023; Liu et al., 2024). However, it should be noted that the majority of studies originate from laboratories rather than using a field-based approach, thus in the context of a school-based HIIT experimental studies are highly recommended and needed (Leahy et al., 2019; Kennedy et al., 2020). Our study does highlight the possibility and feasibility of sprint interval training to induce favorable outcomes in a university setting.

The study has several limitations. The study does not provide enough evidence to interpret the mechanism of sedentary causes and potential increased risk of insulin resistance, including whether it is related to gut health caused by food intake. Additionally, despite the favorable metabolic outcomes observed, a small sample limits this study's generalization ability to a broader population. Follow up research should refer to sex, nutritional control, and gut bacterial monitoring when designing the study. Therefore, the potential impact of multiple factors able to influence blood parameters was minimized.

Conclusion

This study examined the physiological efficacy of HIIT compared with MICT in sedentary adolescents, and our findings demonstrated that 8-week HIIT and MICT have similarly favorable outcomes in body fat mass, body fat percentage, and visceral fat area. HIIT can decrease waist/hip ratio, systolic blood pressure, diastolic blood pressure, and triglyceride, but not MICT. We provide an update on body composition and blood biochemical parameters responses after HIIT and MICT in sedentary Chinese adolescents; we propose that HIIT is an efficacious part of this cohort management. Furthermore, an 8-week HIIT and MICT may have limited scope to decrease insulin resistance in sedentary normal-weight adolescents. The precise determination of the dose-response between different protocols and health-related outcomes is worth further exploring so as to optimize cardiometabolic benefits.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Nanjing Sport Institute Human Experiment Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

FS: Writing–review and editing, Writing–original draft. CW: Writing–review and editing, Writing–original draft. QS: Writing–review and editing. FH: Writing–review and editing. TZ: Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by Jiangsu Province Social Science Foundation (21TYD003), Jiangsu Province Education Science “14th Five-Year Plan Period” Key Project (T-a/2021/09), and Jiangsu Province Social Science Foundation (22TYD008).

Acknowledgments

The author is grateful for the generous help from Professor Zhuoying Wang for her research method consultation, Associated Professor Quanfu Zhou for his body composition testing, Clinician Ping Ji for her ECG Examination and Monitoring, Experimentalist Zhibang Wang for his exercise density supervision, and participants at Nanjing Agricultural University, Jiangsu Province, China, for their help and participation in this project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 28 August 2024

ACCEPTED 29 October 2024

PUBLISHED 15 November 2024

CITATION

Jovanović R, Živković M, Stanković M,
Zoretić D and Trajković N (2024) Effects of
school-based high-intensity interval training
on health-related fitness in adolescents.
Front. Physiol. 15:1487572.
doi: 10.3389/fphys.2024.1487572

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Effects of school-based high-intensity interval training on health-related fitness in adolescents

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Background: High-intensity interval training (HIIT) in school settings has been much less studied in adolescents. The aim of this study was to examine the impact of HIIT on health-related fitness in adolescents.

Methods: The total sample consisted of 60 adolescents (age 16.33 ± 0.62 years) from secondary Grammar school, randomly divided into two groups: the experimental (EG) (30) and the control group (CG) (30). The experimental program (12-weeks; 2 times per week) involved two Tabata sessions during one physical education class lasting 4 min each. Participants were tested for health-related fitness components-cardiorespiratory fitness (The Shuttle Run Test (SRT) and strength, hand grip test, standing long jump (SLJ) and counter movement jump (CMJ).

Results: Both the EG and the CG experienced significant positive changes in SRT (meters) and VO_{2max} values compared to baseline value ($p < 0.05$), however, the increase in the EG was significantly higher than that in the CG ($SRT - \eta_p^2 = 0.111$; $VO_{2max} - \eta_p^2 = 0.111$, $p < 0.01$). The EG showed significant improvement in SRT (meters) and VO_{2max} values compared to the CG ($p < 0.01$). Regarding the hand grip test results, a significant time \times group interaction was found only for right hand ($p < 0.01$). Moreover, the improvements in SLJ and CMJ values was greater in EG than that in the CG group ($SLJ - \eta_p^2 = 0.182$; $CMJ - \eta_p^2 = 0.112$, $p < 0.01$).

Conclusion: Findings indicate that HIIT implemented into physical education classes can result in significant improvements in selected health related fitness components in adolescents.

KEYWORDS

HIIT, youth, class, cardiorespiratory fitness, intervention

1 Introduction

Childhood and adolescence are key stages in the development and adoption of healthy habits. Insufficient physical activity (PA) (Kipping et al., 2008) is associated with all risks for developing cardiovascular diseases (Skinner et al., 2015; McMurray and Andersen, 2010), thus increasing the risk of premature mortality (Freedman et al., 2007; Franks et al., 2010). Furthermore, overweight and obesity, poor nutrition, reduced cardiorespiratory

fitness, hypertension, chronic infections, and dyslipidaemia are evident in youth and become persistent health problems in adulthood (Simmonds et al., 2016).

Even though regular physical activity protects against the development of many diseases, studies showed that adolescents failed to meet the recommendations for physical activity in the past decade (Logan et al., 2014; Tapia-Serrano et al., 2022). Physical education (PE) classes often face structural limitations that hinder their ability to meet the recommended 60 min of moderate-to-vigorous physical activity (MVPA) per day for children and adolescents. As highlighted (Costigan et al., 2015b; Jurić et al., 2023), PE classes are typically constrained by limited time, with only a portion of the session dedicated to active movement, while the rest involves instruction, organization, or low-intensity activities. Moreover, Deutsch et al. (2022) emphasize that traditional warm-ups and activities in PE do not consistently engage students at the intensity levels required to promote cardiovascular fitness and muscular strength effectively. These limitations reduce opportunities for students to accumulate sufficient MVPA, underscoring the need for innovative approaches, which can maximize the use of available time through short bursts of high-intensity effort.

There is a strong reason to study how high-intensity interval training (HIIT) affects the quality of life and health of adolescents, as an important predictor of cardiometabolic health of the young (Segovia and Gutiérrez, 2020; Weston et al., 2020). Given that lack of time is cited as a major barrier for regular exercise, high intensity exercise of short duration is an excellent way to improve health in a short period of time (Costigan et al., 2015a). Moreover, it reduces the risk of cardiovascular diseases, in healthy and obese children and adolescents (Eddolls et al., 2017). Also, such a high intensity exercise in a short time with short rest intervals is a more suitable way of exercise for adolescents (Crisp et al., 2012; Buchan et al., 2013). However, in order to have an impact on cardiorespiratory fitness and health, it is recommended that an exercise program last a minimum of seven to 12 weeks (Steene Johannessen et al., 2013). Duncombe et al. (2022) showed in recent meta-analysis the effectiveness of HIIT training in school, aiming to promote the health of children and adolescents compared to a control group or another exercise modality. The authors found significant improvements in anthropometric characteristics, cardiorespiratory fitness, in children and adolescents who practiced HIIT compared to the control group. However, Domaradzki et al. (2023) stated that the HIIT program introduced to a typical PE lesson can be considered partially effective and the programs should be designed specifically for males and females.

Tabata training has been considered one of the high-intensity “interval or intermittent” training (HIIT) methods (Tabata, 2019). It could vary considerably as regards of the characteristics of the training exercise, i.e., the exercise mode, intensity, and durations of exercise and rest. Weston et al. (2014) defined HIIT as “near maximal” effort performed at an intensity that elicits >80% (often 85%–95%) of the maximal heart rate. HIIT has been much less studied in children and adolescents in school settings, especially during the PE classes. Nevertheless, the results of HIIT studies in children and adolescent athletes have confirmed the improvement of cardiorespiratory fitness (shuttle run test - (SRT) (Buchheit et al., 2009), sprint (Sperlich et al., 2011), as well as 60 s sprint (Buchheit et al., 2010) and vertical jump (Tønnessen et al.,

2011). The results of the study by Costigan et al. (2016) suggest that current physical activity and fitness levels among adolescents are low, which increases the risk of developing chronic diseases, and that it is necessary to include HIIT in mandatory Physical Education (PE) classes to improve cardiorespiratory function and body composition among adolescents. The results of several studies demonstrated improvement in health-related fitness in boys following HIIT (Meng et al., 2022; Corte de Araujo et al., 2012; Camacho-Cardenosa et al., 2016). Additionally, Petrušič et al. (2022) showed that only two additional training sessions a week are sufficient to bring about significant changes in physical fitness among adolescent girls.

Despite the growing body of literature examining the effects of HIIT on health-related fitness parameters across various age groups, there remains a notable gap in research specifically focusing on adolescents aged 15–17 years. While studies have investigated HIIT’s impact on adults and younger children, the unique physiological and develop mental characteristics of adolescents warrant dedicated attention. Understanding how HIIT influences health-related fitness components such as cardiovascular endurance and muscular strength in this age group is essential for designing effective and age appropriate exercise interventions. Moreover, there is a lack of studies investigating the effects of HIIT interventions as a part of physical education classes. Such research could contribute to evidence based strategies aimed at promoting optimal health and fitness during this critical period of physical development. Therefore, the aim of this study was to examine the effects of HIIT on health-related fitness in adolescents aged 15–17 years. It was hypothesized that the high-intensity program will contribute to better effects on the physical fitness of adolescents compared to the control group.

2 Materials and methods

2.1 Study design

The study employed a two-groups with pre post design, with participants divided into an experimental group and a control group. The intervention aimed to assess the impact of HIIT on health-related fitness (shuttle run performance, and strength outcomes, including standing long jump, countermovement jump (CMJ), and handgrip strength test) in adolescents. The experimental group replaced the traditional warm-up in physical education (PE) classes with a Tabata-based HIIT protocol, consisting of varied exercises performed twice a week for 12 weeks. Each session followed the standard Tabata format of 20 s of intense activity followed by 10 s of rest.

2.2 Participants

The total sample consisted of 60 adolescents (aged 15–17) from a secondary grammar school. Basic descriptive characteristics can be seen in Table 1. These participants were randomly assigned to two groups: an experimental group (EG) and a control group (CG), with 30 participants in each. The selection process involved adolescents from the first- and second-year classes (six classes in

TABLE 1 Basic descriptive characteristics in the experimental and control group in the initial and final measurements.

Experimental group (n = 30)			Control group (n = 30)	
Outcome	Initial	Final	Initial	Final
BH (cm)	176.66 ± 8.77	177.26 ± 7.57	175.56 ± 5.71	176.66 ± 5.65
BM (kg)	66.73 ± 14.41	67.26 ± 13.38	71.09 ± 13.61	72.50 ± 13.63
BMI	21.51 ± 3.67	21.66 ± 3.41	23.01 ± 3.99	23.34 ± 3.97

Descriptive data are given as mean values ± standard deviations; BH- body height, BM -body mass, BMI -body mass index.

total) at the beginning of the school year. We used simple, non-returnable group randomization, conducted with the tool available at www.randomization.com, to ensure an unbiased allocation of participants. Biological maturity was calculated for each participant using the formula established by Mirwald et al. (2002). It is a non-invasive method that evaluates the time of greatest increase in height (PHV) taking into account anthropometric characteristics (body height, sitting height, leg length) and chronological age. The inclusion criteria for participation in the study were the following: male adolescents aged 15–17 years, not involved in any organized training processes beside school PE classes. At the time of the study, the participants had a medical certificate confirming they were healthy and able to meet the requirements expected of them. The exclusion criteria included adolescents with respiratory and cardiovascular diseases, developmental disabilities, chronic diseases, and those recovering from injuries or diseases, as well as active athletes. Prior to the onset of the study, the participants parents and the grammar school principal gave their written approval, which is in accordance with the Declaration of Helsinki. This research has been improved by the Ethical Committee, as the authors wrote in the end of the manuscript: Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and results of this study resulted for the purposes of the doctoral dissertation, the topic of which was adopted at the University of Niš, Serbia (decision No. 8/18-01-007/23-031, approval date 06.11.2023). Furthermore, each parent gave written consent on behalf of their child to voluntarily participate in the study. The participants were allowed to withdraw from the experimental treatment at any time. In addition, the participants and their parents were acquainted with the importance and advantages of this study.

2.3 Testing procedures

Testing was conducted in two stages: the initial measurement, performed before the start of the experimental program, and the final measurement, conducted 12 weeks after the experimental program. Participants were tested for the basic anthropometric parameters and health related fitness components - cardiorespiratory fitness and strength. The measurements occurred in early October, following the start of the school year, and in late December. The testing as well as the experimental program were performed within a school athletic facility. For the purposes of all measurements and the experimental program, the same researchers were involved. Also, the measurements were carried out at the same

time of day with the same schedule of tests for both measurements. Prior to the testing protocol, all participants underwent an introductory session where they were instructed on the correct form and technique for each fitness test. Since it is known that the type of warm-up can influence on performance (Patti et al., 2022), a standardized 15-min warm-up including dynamic excersises was administrated before testing.

In the morning, body composition was first measured, then tests of general and explosive strength, and finally, after a break, an aerobic endurance test, i.e. a shuttle run test.

2.3.1 Body composition

Body height (BH) was measured with an anthropometer (SECA 214, Hamburg, Germany) according to standard procedures. The subject stands on a flat surface, with weight distributed equally on both legs. The shoulders are relaxed, the heels are gathered, and the head is placed in the position of the socalled. Frankfurt plane, which means that the imaginary line connecting the lower edge of the left orbit and the tragus helix of the left ear is horizontal. During the measurement, the subject rested his back on the anthropometer, maximally stretched.

Body mass (BM) - The Omron bf511 (Omron BF511, Kyoto, Japan) was used to obtain body mass values (Brtková et al., 2014).

Body mass index (BMI) - Height-weight indicator of an individual's nutrition, which is valid for people over 20 years old and shows the ratio of body weight to height. According to the World Health Organization, a BMI less than 18.5 is considered underweight, while a BMI greater than 25 is considered overweight, and a value greater than 30 is considered obesity. It was obtained by formula ($BMI = \text{weight}/\text{height}^2$).

2.3.2 Hand grip test

This is a test used to measure the static strength of the hand flexor muscles. The Takei T.K.K.5401 GRIP-D (Takei Scientific Instruments Co., Ltd., Tokyo, Japan) hand grip dynamometer was used for all data collection. This was performed with the participants in standing, arm by their side with full elbow extension. The test taker has the right to two attempts, and the better one is entered. The hand on the dial must return to zero after the first attempt. The result of the test is the maximum muscle force of the hand grip Fmax expressed in kilograms (kg). The validity of this test was proved by Le-Ngoc and Janssen (2012).

2.3.3 Standing long jump

The standing long jump test protocol involves the participant standing with feet shoulder width apart behind a marked line, bending the knees, and swinging the arms to generate momentum. The participants then jump forward as far as possible, landing on both feet. The distance from the starting line to the nearest point of contact on the landing (the back of the heels) is measured in cm. This process is repeated three times, with the longest jump recorded as the final result.

2.3.4 Countermovement jump (CMJ)

The starting position of the participants is an upright position with a straight torso. The knee angle is 180°. The feet should be shoulder width apart. The participants should be in that position for at least 2 s. After that, they lower themselves until the knees are approximately bent at an angle of 90°. This is followed by a maximal effort, i.e., an explosive jump with legs extended and knees at an angle of 180° (Petrigna et al., 2019). Optojump (Optojump, Microgate, Bolzano, Italy) was used to assess the jump height, and the test result is the jump height expressed in cm. The test was repeated three times with a 30-s break between runs. The best achieved result was taken into further analysis. The validity of this test was proven by Markovic et al. (2004).

2.3.5 Cardiorespiratory fitness

The Shuttle Run Test (SRT) also known as the 20-m shuttle run test or the beep test, is a commonly used assessment of cardiorespiratory fitness. It involves running back and forth continuously between two parallel 20 m lines. It consists of several stages (also called levels) each lasting about 1 min, with each stage consisting of several 20 m laps (also called shuttles). In each phase, the running speed is increased until the subject can no longer run 20 m in the given time with a sound signal (on two consecutive occasions) or when the subject stops due to fatigue. The result of this test is the maximum number of levels that participants complete before time runs out. The obtained result was used to calculate the maximum oxygen consumption according to the equation $VO_{2max} = 31.025 + 3.238 X - 3.248A + 0.1536 AX$, in which X represents the speed in the last station in km/h and A the age. The required data (X) is read from existing tables. The validity of this test was proven by Matsuzaka et al. (2004).

2.4 Experimental program

Before the beginning of the experimental program, in agreement with the school director and professors, and with the written consent of the students parents, the participants were divided by randomly selection into two groups, experimental and control, with the included and excluded factors explained below. In addition to regular PE classes (45 min, twice a week), the experimental group underwent HIIT. The experimental part of the study was organized through a 12-week HIIT exercise program adapted to high school students and conducted in the preparatory phase of the class, twice a week, for a total duration of 9 min per class. First, all students did a 5 min exercise to warm up the muscles. In the preparatory phase of the class, the control group followed the PE lesson plan and program and traditional warm up, whereas the experimental group

started with the Tabata protocol. A total of 8 exercises (running, burpees, split jump, jumping jack, push-ups, crunches, frog jump, Russian twist etc.) were performed within 4 minutes. The duration of the exercises was 20 s, with a break of 10 s, while at the end of an entire Tabata there was a break of 1 min. The exercise intensity was 80%–90% of the maximum heart rate (HRmax) as calculated by the shuttle run test (Ortega et al., 2023). The main and the final phase of the class were done together by the control and the experimental group following the PE lesson plan and program. The control group performed only their regular activities in PE classes.

2.5 Statistical analysis

We conducted an *a priori* power analysis using G*Power for a two-tailed t-test for dependent means (Faigenbaum and Myer, 2010). The input parameters included an effect size (d) of 0.55, an alpha level of 0.05, and a desired power (1-β) of 0.80. Based on these parameters, the total sample size required was 56 participants. Descriptive data are given as means standard deviations. Examination of the normality of the distribution of continuous variables, considering the size of the samples, was tested with the Kolmogorov-Smirnov test. The difference of the examined continuous variables within the examined groups, at the beginning and at the end of the experimental program, was determined by Paired samples t-tests in the case of normal distributions of the variables, or by the Wilcoxon Signed Ranks Test, in the case of deviations of the distribution's variables than normal. The effects of high intensity interval training (HIIT) on changes in the fitness component was determined by a two-way analysis of variance (ANOVA) with repeated measures (group × time). The effect sizes were calculated using partial eta squared (η_p^2). The value $p < 0.05$ was used as the threshold of statistical significance.

3 Results

All data passed the normality tests. General descriptive parameters for both groups can be seen in Table 1. In terms of age, range of adolescents was 15–17 years, but the average age was 16.33 ± 0.62 (Y-PHV, i.e., years to and from peak height velocity = 0.1 ± 0.8). The t-test showed that there were no significant differences between groups in health-related fitness indices at baseline (all $p > 0.05$). A significant time × group interaction effects were found in all tested variables ($p < 0.05$) except for LHG where there was no significant interaction ($p > 0.05$). Comparing the SRT values both in meters and VO_{2max} , it was determined that the values at the final measurement were statistically significantly higher compared to the initial measurement in both studied groups (Table 2; $p < 0.05$). However, the experimental group showed a significantly better increase in meters (SRT - $\eta_p^2 = 0.111$; $p < 0.01$) and in VO_{2max} performance (VO_{2max} - $\eta_p^2 = 0.111$, $p < 0.01$) after program (Table 2).

A significant group × time interaction was determined in right handgrip test ($\eta_p^2 = 0.102$; $p = 0.02$), with a significant increase ($p = 0.001$) in the EG but not in the CG ($p = 0.56$). The values of left handgrip test at the final measurement was higher in both groups compared to the initial measurement, but a statistically significant

TABLE 2 Differences between experimental and control group in terms of Shuttle run test (SRT), hand grip test and explosive strength.

Experimental group (n = 30)				Control group (n = 30)			F	η_p^2
Outcome	Initial	Final	p	Initial	Final	p		
SRT (m)*	335.33 ± 199.39	457.33 ± 205.22	0.001	308.67 ± 117.99	348.00 ± 136.92	0.02	7.223	.111
SRT (VO ₂ max)*	23.31 ± 2.52	25.79 ± 4.08	0.001	22.80 ± 2.52	23.63 ± 2.89	0.02	7.206	.111
RHG (kg)*	35.03 ± 7.19	36.80 ± 6.68	0.001	38.28 ± 9.09	38.93 ± 9.12	0.056	6.577	.102
LHG (kg)	33.45 ± 7.49	35.22 ± 7.15	0.01	35.60 ± 8.11	36.90 ± 8.10	0.09	.696	.012
SLJ (cm)*	175.73 ± 30.80	184.13 ± 26.89	0.001	182.10 ± 27.39	184.30 ± 26.41	0.83	12.931	.182
CMJ (cm)*	23.87 ± 6.92	25.94 ± 6.69	0.001	26.69 ± 4.26	27.36 ± 4.34	0.01	7.291	.112

Descriptive data are given as mean values ±SD; *significant difference between experimental and control group ($p < 0.05$); SRT- shuttle run test; RHG – right hand grip dynamometer test, LHG – left hand grip dynamometer test; SLJ, standing long jump; CMJ, Countermovement jump; η_p^2 -partial eta squared.

increase was found only in the experimental group (left hand $-p < 0.01$) (Table 2). Moreover, there was a significant group \times time interaction for SLJ and CMJ. The improvements in SLJ and CMJ values was greater in EG than that in the CG group (SLJ- $\eta_p^2 = 0.182$; CMJ- $\eta_p^2 = 0.112$, $p < 0.01$).

4 Discussion

The aim of this study was to examine the impact of HIIT on health-related fitness in adolescents aged 15–17 years. The main results of this study were that the experimental group showed a significant improvement in cardiorespiratory fitness and strength compared to control group after implementing HIIT as a substitute for traditional warm-ups during the PE classes over the school year. Specifically, HIIT induced significant improvement of SRT in meters and VO_{2max} compared to the control group. Additionally, the values of hand grip test as well as the values of standing long jump and CMJ were significantly higher compared to the control group. Having in mind there were obvious improvements in experimental group compared to the control group, the hypothesis was accepted.

The PA and fitness levels among adolescents are low during past decades (Costigan et al., 2016; Nevill et al., 2023; Aubert et al., 2021). In addition, recent review showed that most adolescents failed to meet the 24-Hour Movement Guidelines, which also include the 60 min of moderate to vigorous physical activity daily (Tapia-Serrano et al., 2022). HIIT training was proposed as intervention designed to increase moderate-to-vigorous PA in Physical Education classes (Muntaner-Mas and Palou, 2017). This research results are in line with above mentioned proposal. The HIIT implemented during mandatory PE classes, improved health related fitness in healthy adolescents.

Specifically, the 12-week HIIT program of this study showed better SLJ and CMJ results in the experimental group compared to the control group, which is in agreement with the results of several recent studies (da Silva et al., 2020; Li et al., 2023; Abassi et al., 2023). For example, Costigan et al. (2015a) highlight significant improvements in vertical jump performance following a school-based HIIT intervention, emphasizing the program's feasibility.

Similarly, Jurić et al. (2023) found that incorporating HIIT in physical education classes not only improved general fitness but also specifically enhanced standing long jump outcomes, suggesting the effectiveness of such protocols in fostering explosive leg power. These findings align with the results of Cvetković et al. (2018), who reported that a 12-week HIIT program led to substantial gains in both vertical jump and standing long jump, indicating that these activities can effectively promote explosive power among children and adolescents. Similar results for standing long jump and vertical jump ($p < 0.05$) were found following running-based and body-weight based HIIT in healthy adolescents (Li et al., 2023). Together, these studies demonstrate the potential of HIIT interventions to improve physical fitness outcomes, particularly those requiring lower-body explosive strength, when embedded within the school curriculum. On the other hand, some studies showed no significant improvement, especially for the vertical jump. After a 12-week school intervention based on small sided games (the program included futsal, basketball, handball, and volleyball), Petrušić et al. (2022) found no significant improvement for CMJ. Similar results were found by Trajković et al. (2020) after a small-sided games football intervention at school, which also showed small effects (3.5%). In contrast, recreational football training in obese school children showed significantly greater effects on CMJ (17.0%) (Cvetković et al., 2018). Differences in intervention duration among different studies, as well as differences in weight status and other characteristics, could be a possible explanation for this discrepancy in results between studies. In addition, different protocols might have been used to test vertical jump height, which could also add to the differences in results.

García-Hermoso et al. (2020) stated that early intervention that target CRF in children may be related with maintaining health parameters later in life. Recent systematic review and meta-analysis conducted by Martin-Smith et al. (2020) showed that HIIT is a sustainable and effective method for improving cardiorespiratory fitness in adolescents. The results of the current study confirm these conclusions, displaying significant improvements in the 20-m shuttle run test following 12 weeks of Tabata protocol. Studies such as Jurić et al. (2023) and Li et al. (2023) reported also a significant improvements in VO_{2max} and overall aerobic capacity

among participants who engaged in HIIT programs during physical education classes. These interventions, typically characterized by alternating high-intensity efforts with recovery periods, efficiently stimulate cardiovascular adaptations, even within relatively short timeframes. Similarly, [González-Gálvez et al. \(2024\)](#) found that HIIT protocols improved cardiorespiratory markers and metabolic health in adolescents. Furthermore, [Popowczak et al. \(2022\)](#) found that a 10 weeks HIIT Tabata exercise program in adolescents improved CRF, which is in agreement with the results of this study. However, some studies have also observed no significant improvements in cardiorespiratory fitness following HIIT interventions. [Costigan et al. \(2015b\)](#) noted variability in results, with some participants showing limited aerobic gains. Similarly, to our study, [Alonso-Fernández et al. \(2019\)](#) implementing the HIIT protocol in PE classes, did not find a significant difference compared to the control group in healthy adolescents. However, the author stated that findings suggest a promising outlook on how incorporating HIIT as a substitute for traditional warm-ups can improve students' physical fitness through the protocol of functional bodyweight exercises, all without impacting the remaining curriculum required in PE classes over the school year. Possible reasons for the lack of improvement include inadequate training intensity or volume, individual differences in baseline fitness levels, varying levels of motivation among participants, and the challenge of maintaining consistent effort throughout the sessions. Additionally, factors such as insufficient recovery between sessions and external lifestyle factors (e.g., diet, sleep) may influence the overall effectiveness of HIIT programs in improving aerobic fitness.

It is well documented that greater grip strength is related to longitudinal health maintenance and health improvements in adolescents ([Peterson et al., 2018](#)). Although HIIT is primarily designed to improve cardiovascular fitness, some studies have reported positive effects on muscular strength, including handgrip strength. For example, [González-Gálvez et al. \(2024\)](#) found improvements in upper-body strength, including handgrip, following a structured HIIT program, suggesting that high-intensity efforts can stimulate neuromuscular adaptations. Similarly, [Jurić et al. \(2023\)](#) observed that participation in HIIT during physical education classes positively influenced muscle strength, possibly due to the inclusion of bodyweight exercises and functional movements within the training protocol.

However, not all studies have shown significant improvements in handgrip strength. [Costigan et al. \(2015b\)](#) reported limited gains in muscular strength, including handgrip, which may reflect the nature of HIIT programs that focus more on cardiovascular demands rather than maximal strength development. The present results support the above mentioned inconsistent results for grip strength, with significant differences between groups only for right hand handgrip test. Reasons for improvement could include the indirect activation of upper-body muscles during explosive movements and improved neuromuscular coordination. On the other hand, the absence of improvement might result from insufficient specific strength training, low engagement of the upper limbs in HIIT exercises, or the relatively short duration of the intervention, which may not be enough to develop maximal strength in young populations.

Some possible limitations of the study should be mentioned. First of all, the number of participants is limited, and some participants declined to participate, which could introduce selection

bias into the study's findings. Larger sample sizes from school-based research are required in the future to confirm HIIT's usefulness as an intervention for adolescents. Second, because this study only involved males, we were unable to determine gender differences and the effects of exercise intervention. The absence of an objectively measured physical activity and strictly controlled diet may have influenced the results. However, all participants were advised to continue with their usual diet and they were not engaged in organized sport programs outside the school PE class. Nevertheless, a key strength of the study is that HIIT was implemented in a school setting during PE class warm-ups, ensuring feasibility, practicality, and seamless integration into students' existing routines.

5 Conclusion

Findings from the present study indicate that HIIT training can result in significant improvements in selected health related fitness components (SRT, SRTVO2max, RHG, LHG and CMJ) in adolescents. Having in mind that the intervention was formed as a part of the PE class, in which only the warm up phase (HIIT or traditional) differed, the results make a promising strategy for combating sedentary lifestyles in adolescents. Moreover, if we exert influence on adolescents to adopt physical activity as a daily routine, it will undoubtedly have a significant impact on reducing the risk of developing bad habits and a sedentary lifestyle in adulthood.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RJ: Conceptualization, Formal Analysis, Investigation, Writing—original draft. MŽ: Methodology, Validation, Writing—review and editing. MS: Data curation, Investigation, Visualization, Writing—review and editing. DZ: Project administration, Resources, Software, Writing—review and editing. NT: Conceptualization, Supervision, Validation, Writing—review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article. This research received no external funding.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 19 June 2024

ACCEPTED 11 November 2024

PUBLISHED 02 January 2025

CITATION

Ha M-S, Moon HY, Lee M and Yook JS (2025)
Exercise improves body composition, physical
fitness, and blood levels of C-peptide and
IGF-1 in 11- to 12-year-old boys with obesity.
Front. Physiol. 15:1451427.
doi: 10.3389/fphys.2024.1451427

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Exercise improves body composition, physical fitness, and blood levels of C-peptide and IGF-1 in 11- to 12-year-old boys with obesity

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Introduction: Exercise is vital in preventing and treating obesity. Despite its importance, the understanding of how exercise influences childhood obesity at the biochemical level is limited. In this study, we explore the effects of a 16-week exercise program (EP) on body composition, physical fitness, and the blood levels of hormones related to obesity.

Methods: Sixteen boys with obesity ($n = 16$) and seventeen boys without obesity ($n = 17$) took part in an EP comprising sports games and aerobic and resistance exercises. We examined alterations in body composition and physical fitness. In addition, we measured circulating hormone levels, including C-peptide, resistin, insulin-like growth factor 1 (IGF-1), and growth hormone (GH), in the blood.

Results: Body fat percentage (BFP) decreased from 37.61% at pre-EP to 29.16% at post-EP in the obese group, but not in the non-obese group. The EP decreased C-peptide (4.58 ng/mL vs. 2.96 ng/mL, $p < 0.001$) and resistin levels (14.05 ng/mL vs. 11.06 ng/mL, $p < 0.001$) in the obese group. After the EP, significant improvement in IGF-1 (non-obese: 265.56 ng/mL vs. 311.81 ng/mL, $p < 0.001$; obese: 224.74 ng/mL vs. 272.89 ng/mL, $p < 0.001$) and GH levels (non-obese: 3.91 ng/mL vs. 4.80 ng/mL, $p < 0.05$; obese: 1.76 ng/mL vs. 2.51 ng/mL, $p < 0.05$) were observed in both groups. Lower C-peptide levels were associated with BFP ($r = 0.447$, $p = 0.009$) and muscle mass ($r = -0.385$, $p = 0.02$), whereas enhanced IGF-1 levels correlated with increased muscle strength ($r = 0.343$, $p = 0.05$) and cardiovascular fitness ($r = 0.347$, $p = 0.04$). Multiple linear regression analysis revealed that cardiovascular fitness variability and BFP in the obese group were determined by C-peptide ($\beta = -0.054$, $p < 0.001$) and IGF-1 levels ($\beta = -2.936$, $p < 0.05$), respectively.

Discussion: Exercise may induce positive effects on improvements in body composition and physical fitness, as well as on blood levels of metabolic biochemicals such as C-peptide and IGF-1, in adolescent boys with obesity.

KEYWORDS

exercise, childhood obesity, physical fitness, C-peptide, IGF-1

1 Introduction

Childhood and adolescence overweight or obesity represent significant global health concerns, constituting a global public health challenge amid a shared obesogenic lifestyle and contributing to both physiological and psychological challenges throughout an individual's life (Lobstein et al., 2015; Cesare et al., 2019). The prevalence of childhood overweight and obesity has surged worldwide in recent years (Abarca-Gómez et al., 2017). As of 2016, a World Health Organization (WHO) report indicated that over 340 million children and adolescents, aged 5–19 years, were estimated to be overweight or obese. Moreover, childhood obesity is strongly linked to an elevated risk of mortality and the premature onset of chronic diseases, including type-2 diabetes, in adulthood (Abdullah et al., 2011; Sabin et al., 2015). Consequently, addressing excess weight during childhood and adolescence through lifestyle modifications is imperative for prevention and reversal (Salam et al., 2020).

Although the etiology of childhood obesity is multifaceted, it predominantly results from overconsumption and decreased energy expenditure (Woodruff et al., 2009). A high level of physical activity has been suggested as a significant lifestyle modification to positively affect the energy balance equation (Westerterp, 2017; Wysznińska et al., 2020). In addition, once obesity takes root in childhood, it tends to escalate by increasing the size and number of adipocytes; making overall body fat mass challenging to manage through exercise or diet alone (Raine et al., 2017; Zamora-Mendoza et al., 2018). Longitudinal studies have shown that adolescents who were physically active are less likely to become overweight in adulthood (Menschik et al., 2008). Notably, recent studies have indicated a further surge in childhood obesity rates since the onset of the 2019 coronavirus pandemic, owing to school closures and lockdowns that limited opportunities for physical activity (Jenssen et al., 2021). To address this global epidemic, the WHO has advocated for non-pharmacological interventions to mitigate physical inactivity (Waxman and Assembly, 2004). Furthermore, global guidelines published by the WHO suggest that children should engage in at least 60 min of moderate-to-vigorous physical activity daily (Chaput et al., 2020). Therefore, exercise intervention with increasing physical activity levels has been identified as a preventive and therapeutic measure for childhood overweight and obesity.

Numerous studies focusing on children and adolescents with overweight and obesity consistently highlight the positive impact of regular exercise on body composition, including body mass index (BMI) score, as well as improvements in metabolic abnormalities and biochemical variables, such as insulin resistance markers, inflammatory markers, and adipocytokines (Alberga et al., 2015; Kelley et al., 2015). Furthermore, our recent previous study identified that body composition is significantly correlated with physical fitness levels, including strength and agility, according to the degree of obesity classified based on BMI in children. This correlation further impacted the effects of exercise intervention depending on the degree of obesity (Lee et al., 2022). Consequently, expert consensus recommendations emphasize the importance of an appropriate exercise program (EP) for the effective prevention and improvement of obesity in children and adolescents.

Childhood obesity is typically categorized by an age-specific BMI for an appropriate reference population (Cole et al., 2000). However, relying solely on this anthropometric diagnosis, which is a rough measurement, may cause lack of precision in identifying individuals at a heightened risk of disease development. Consequently, an increasing body of literature proposes an alternative approach using obesity biomarkers, such as circulating hormones (e.g., adipokines, insulin, and insulin-like growth factor [IGF-1]), to delineate obesity phenotypes associated with an elevated risk of morbidity and mortality. These biomarkers also serve as valuable tools to monitor and implement intervention programs including physical activity (Nimptsch et al., 2018).

Clinical studies and experimental data indicate that C-peptide, a biological peptide (Wahren et al., 2007), has been traditionally viewed as an indicator of the pancreatic secretion ability or an indirect measure of β -cell function, making it a focal point in diabetes-related investigations (Polonsky et al., 1986). Furthermore, C-peptide exhibit physiological activity, functioning as peptides that act independently of insulin (Garcia-Serrano et al., 2015). In studies involving skeletal muscle, both insulin and C-peptide demonstrated equal effectiveness in mediating glucose uptake through insulin signaling pathways (Zierath et al., 1991). Moreover, C-peptide bind to the cellular membrane of adipocytes, influencing changes in adipocytokine secretion, thereby potentially playing a role in regulating adipose tissue within the context of human energy homeostasis (Garcia-Serrano et al., 2015). In addition, they contribute to the regulation of body composition and inflammatory biomarkers (Bo et al., 2005; Gishti et al., 2015). As a result, C-peptide has emerged as a potentially relevant biomarker of obesity.

In addition to exercise demonstrating positive effects on anthropometric indices of obesity progression, such as BMI (Kelley et al., 2015), its advantages extend to reducing insulin resistance and β -cell dysfunction, as evidenced by improvements in the disposition index among adolescents with overweight and obesity (Shih and Kwok, 2018). Furthermore, a longitudinal study has indicated a significant correlation between high physical activity and decreased β -cell function (assessed through serum C-peptide levels) in lean children (Huus et al., 2016). This suggests that exercise may regulate obesity-related biomarker hormones to mitigate childhood obesity.

A systematic review and meta-analysis revealed that the most effective approach to preventing childhood obesity in children aged 6–12 years is school-based interventions that incorporate both aerobic and resistance exercises (Podnar et al., 2021). Moreover, several previous studies have shown that combined resistance and aerobic exercise (CRAE) interventions, lasting 8–18 weeks, have a positive impact on body composition, physical fitness, and blood levels of metabolic hormones (leptin, adiponectin, and insulin) (Jeon et al., 2013; Lopes et al., 2016; Bharath et al., 2018). However, whether the improvement in body composition and physical fitness in children with obesity achieved by CRAE is regulated by such metabolic factors in the blood remains unclear. Therefore, the present study examined the effects of a 16-week EP, including CRAE, on body composition, physical fitness levels, and circulating hormones, including C-peptide, resistin, IGF-1, and growth hormone (GH), in adolescent boys with obesity. Additionally, to investigate a potential contributor of obesity-related circulating biomarker hormones response to

TABLE 1 Body composition of two groups before and after the 16-week exercise program.

Variables	Groups	Pre-EP	Post-EP	Effect size Cohen's <i>d</i>	Interaction	Main effect
		(Mean ± SD)	(Mean ± SD)			
Height (cm)	Non-obesity	144.55 ± 9.94	147.78 ± 9.05***	0.36 (small)	F = 3.445, <i>p</i> = 0.073	Time <i>F</i> = 139.157 *** <i>p</i> = 0.000
	Obesity	148.44 ± 7.53	152.88 ± 7.33***	0.59 (medium)		
	Difference	2.69% ± 8.63%	3.45% ± 7.88%	0.09 (no effect)		
Weight (kg)	Non-obesity	45.31 ± 7.02	46.94 ± 7.09	0.23 (small)	F = 0.519 <i>p</i> = 0.477	Group <i>F</i> = 26.132 *** <i>p</i> = 0.000
	Obesity	58.57 ± 6.93	59.31 ± 8.55	0.11 (no effect)		
	Difference	29.3% ± 22.2%	26.3% ± 24.0%	−0.14 (no effect)		
BMI (kg/m ²)	Non-obesity	21.62 ± 1.91	21.39 ± 1.67	−0.12 (no effect)	F = 1.538 <i>p</i> = 0.224	Group <i>F</i> = 50.291 *** <i>p</i> = 0.000
	Obesity	26.54 ± 1.61	25.56 ± 2.76	−0.61 (medium)		
	Difference	22.8% ± 11.7%	19.5% ± 15.2%	−0.28 (small)		
BFP (%)	Non-obesity	30.33 ± 6.23	29.08 ± 7.38	−0.20 (small)	F = 12.800 *** <i>p</i> = 0.001	Time <i>F</i> = 23.200 *** <i>p</i> = 0.000
	Obesity	37.61 ± 8.01	29.16 ± 5.77***	−1.06 (large)		
	Difference	24.0% ± 33.8%	0.28% ± 32.2%	−0.70 (large)		
Muscle mass (kg)	Non-obesity	21.28 ± 9.57	24.02 ± 10.02	0.29 (small)	F = 2.355 <i>p</i> = 0.135	Time <i>F</i> = 8.513 ** <i>p</i> = 0.007
	Obesity	22.12 ± 9.18	30.94 ± 12.15*	0.96 (large)		
	Difference	3.95% ± 62.3%	28.8% ± 66.7%	0.40 (small)		

Summary of repeated measures two-way repeated measures ANOVA, and Cohen's *d* effect size analysis for body composition data.
p* < 0.05, *p* < 0.01 vs. Pre-EP, ****p* < 0.001, *****p* < 0.0001.
Note: BMI, body mass index; BFP, body fat percentage; EP, exercise program; SD, standard deviation.
"Difference" represents a difference between the two groups in the column for each variable.
Cohen's *d* effect size range: no effect for *d* < |.20|; small for |.20| ≤ *d* < |.50|; medium for |.50| ≤ *d* < |.80|; large for |.80| ≤ *d*.

TABLE 2 Physical fitness levels of pre- and post-exercise program.

Variables	Group	Pre-EP	Post-EP	Effect size Cohen's <i>d</i>	Interaction	Main effect
		(mean ± SD)	(mean ± SD)			
Back-muscle strength (kg)	Non-obesity	32.34 ± 12.74	36.82 ± 17.08 [*]	0.35 (<i>small</i>)	<i>F</i> =1.446, <i>p</i> =0.238	Time <i>F</i> =14.999, ††† <i>p</i> =0.001
	Obesity	42.22 ± 12.21	50.75 ± 17.16 ^{**}	0.70 (<i>medium</i>)		Group <i>F</i> =5.806 † <i>p</i> =0.022
	Difference	30.56 ± 55.89 %	37.83 ± 68.06 %	0.13 (<i>no effect</i>)		
Handgrip strength (kg)	Non-obesity	17.13 ± 5.17	19.59 ± 4.48 ^{***}	0.48 (<i>small</i>)	<i>F</i> =1.447, <i>p</i> =0.238	Time <i>F</i> =38.083, ††† <i>p</i> =0.000
	Obesity	20.35 ± 3.68	24.01 ± 3.77 ^{***}	1.00 (<i>large</i>)		Group <i>F</i> =7.159, † <i>p</i> =0.012
	Difference	18.80 ± 37.48 %	22.56 ± 30.33 %	0.10 (<i>no effect</i>)		
Sit-up (times)	Non-obesity	20.35 ± 6.02	30.06 ± 10.90 ^{***}	1.61 (<i>large</i>)	<i>F</i> =0.008, <i>p</i> =0.224	Time <i>F</i> =65.683, ††† <i>p</i> =0.000
	Obesity	10.94 ± 6.08	24.69 ± 8.36 ^{***}	2.26 (<i>large</i>)		Group <i>F</i> =4.441 † <i>p</i> =0.043
	Difference	46.24 ± 44.21 %	17.86 ± 46.15 %	−0.64 (<i>large</i>)		
Flexibility (cm)	Non-obesity	10.18 ± 6.54	13.11 ± 8.57 ^{***}	0.45 (<i>small</i>)	<i>F</i> =10.166, <i>p</i> =0.263	Time <i>F</i> =29.130, ††† <i>p</i> =0.000
	Obesity	5.39 ± 5.20	9.89 ± 4.66 ^{***}	0.87 (<i>large</i>)		
	Difference	47.05 ± 87.47 %	24.56 ± 76.12 %	−0.26 (<i>small</i>)		
Cardiovascular fitness (times)	Non-obesity	20.76 ± 11.95	34.29 ± 23.12 ^{***}	1.13 (<i>large</i>)	<i>F</i> =2.576, <i>p</i> =0.119	Time <i>F</i> =50.372, ††† <i>p</i> =0.000
	Obesity	16.06 ± 7.58	37.50 ± 18.60 ^{***}	2.83 (<i>large</i>)		
	Difference	22.64 ± 69.40 %	9.36 ± 86.77 %	−0.19 (<i>no effect</i>)		
Balance (sec)	Non-obesity	47.25 ± 52.81	57.62 ± 36.25	0.20 (<i>small</i>)	<i>F</i> =2.765, <i>p</i> =0.106	Time <i>F</i> =17.544, ††† <i>p</i> =0.000
	Obesity	27.50 ± 26.92	51.52 ± 23.29 ^{***}	0.89 (<i>large</i>)		
	Difference	41.80 ± 133.87 %	89.41 ± 69.27 %	0.36 (<i>small</i>)		

Summary of repeated measures two-way ANOVA, and Cohen's *d* effect size analysis for physical fitness data.
p* < 0.05, **p* < 0.001 vs. Pre-EP, ††*p* < 0.01, †††*p* < 0.001.
Note: EP, exercise program; SD, standard deviation.
"Difference" represents a difference between the two groups in the column for each variable.
Cohen's *d* effect size range: *no effect* for *d* < |.20|; *small* for |.20| ≤ *d* < |.50|, *medium* for |.50| ≤ *d* < |.80|, *large* for |.80| ≤ *d*.

the EP, we assessed serum levels of these hormones and their changes in body composition and physical fitness levels after the EP intervention using correlation coefficients and stepwise multiple linear regression analysis.

2 Materials and method

2.1 Participants and study design

The ideal sample size was calculated using G-power software (version 3.1; Kiel University, Kiel, Germany), with parameters set

at an effect size of 0.25 (default), a significance level (alpha) of 0.05, and a power (1-beta) of 0.80. This computation indicated that 34 participants were needed. However, to account for potential dropouts, 42 participants were initially recruited. Owing to various reasons, nine participants withdrew, resulting in a final sample size of 33 individuals. A total of 33 elementary school students, aged 11–12 years (mean age 11.42 ± 1.12 years; all boys), actively participated in this study. Participants were stratified based on BMI scores with age- and sex-appropriate cutoff values, resulting in two groups: non-obese (BMI: 18–24; *n* = 17) and overweight and obese groups (BMI: 25–32; *n* = 16) (Onis et al., 2007). Comprehensive health assessments were conducted through a health questionnaire,

TABLE 3 Change of blood biomarkers after 16 weeks of the exercise training.

Variables	Group	Pre-test	Post-test	Effect size Cohen's <i>d</i>	Interaction	Main effect
		Mean ± SD	Mean ± SD			
C-peptide (ng/ml)	Non-obesity	2.36 ± 0.91	1.77 ± 0.76 [†]	-0.65	<i>F</i> =10.294, [†] <i>p</i> =0.003	Time <i>F</i> =48.101, ^{†††} <i>p</i> =0.000 Group <i>F</i> =15.333, ^{†††} <i>p</i> =0.000
	Obesity	4.58 ± 1.75	2.96 ± 1.67 ^{***}	-0.93		
Resistin (ng/ml)	Non-obesity	11.18 ± 8.07	10.76 ± 7.19	-0.05	<i>F</i> =15.481, ^{†††} <i>p</i> =0.000	Time <i>F</i> =27.291, ^{†††} <i>p</i> =0.000
	Obesity	14.05 ± 11.24	11.06 ± 9.69 ^{***}	-0.27		
IGF-1 (ng/ml)	Non-obesity	265.56 ± 128.11	311.81 ± 150.53 ^{***}	0.36	<i>F</i> =0.017, <i>p</i> =0.897	Time <i>F</i> =42.333, ^{†††} <i>p</i> =0.000
	Obesity	224.74 ± 114.70	272.89 ± 138.26 ^{***}	0.42		
GH (ng/ml)	Non-obesity	3.91 ± 3.09	4.80 ± 3.17 [†]	0.29	<i>F</i> =0.068, <i>p</i> =0.796	Time <i>F</i> =9.891, ^{††} <i>p</i> =0.004 Group <i>F</i> =5.739, [†] <i>p</i> =0.023
	Obesity	1.76 ± 2.25	2.51 ± 2.34 [†]	0.33		

Summary of repeated measures two-way repeated measures ANOVA and Cohen's *d* effect size analysis for blood biomarkers.

[†]*p* < 0.05, ^{***}*p* < 0.001 vs. Pre-EP, [†]*p* < 0.05, ^{††}*p* < 0.01, ^{†††}*p* < 0.001.

Note: IGF-1, Insulin Growth factor-1, GH, growth hormone; EP, exercise program; SD, standard deviation.

"Difference" represents a difference between the two groups in the column for each variable.

Cohen's *d* effect size range: no effect for *d* < |.20|; small for |.20| ≤ *d* < |.50|, medium for |.50| ≤ *d* < |.80|, large for |.80| ≤ *d*.

physical examination, and laboratory tests. Before participation, parents of the children willing to participate were given a detailed explanation of the objectives and intentions of the study, and all provided written informed consent. The exclusion criteria were having received drug therapy in the past 6 months, having engaged in regular exercise, or having had previous musculoskeletal disease. During the study period, the use of medications that could affect the experiment was restricted. Physical fitness test, body composition parameters, and blood samples were measured using the same methods under identical conditions. For the measurements, participants were required to maintain a minimum of 8 h of fasting, with no vitamin intake or vigorous exercise. The assessments were conducted twice for both the exercise and control groups (during the pre-16-week and post-16-week rest periods), following the procedures recommended by the American College of Sports Medicine (Thompson et al., 2013).

2.2 Exercise program

The EP was adapted into a structured and supervised after-school EP based on the exercise prescription program outlined by the American College of Sports Medicine (Thompson et al., 2013; Lee et al., 2022). The program was conducted three times per week during a 2-week adjustment period. Each session lasted 60 min, including a 5-min warm-up, a 50-min main exercise period (comprising sports games and aerobic and resistance exercises), and a 5-min cool-down. The intensity was gradually increased: from 50% to 60% of heart rate reserve (HRR)/11–12 on the rated perceived exertion (RPE) scale in weeks 1–4, to 60%–70% of HRR/12–13 of RPE in weeks 5–13, and finally to 70%–80% of HRR/13–14 of RPE in weeks 14–16. To ensure proper training intensity, each participant wore a heart rate monitor during the entire training session (V800; Polar Unk, Oy, Kempele, Finland).

The researchers supervised each training session. The researchers monitored heart rates during all exercise intervention sessions. The wearable heart rate monitors used in the study were set to sound an alarm if the heart rate fell below or exceeded the target HRmax range, allowing the researchers to ensure the intensity of exercise during all sessions. In addition, the wearable devices of participants were programmed to activate an alarm to maintain heart rates within the target HRmax range, and participants were pre-educated on the significance of these alarms.

2.3 Anthropometric assessments

Each participant's weight was measured automatically while comfortably standing with feet slightly apart on the instrument and wearing simple clothing. Barefoot stature was measured in centimeters without shoes, heels together, and with the back of the subject parallel to the stadiometer. Even with several limitations in accuracy, bioelectrical impedance analysis (BIA) still has high sensitivity and specificity in estimating body composition in children (Kyle et al., 2015). Body composition was determined using Inbody J10 (Biospace Corp., Seoul, Korea), evaluating body weight (kg), body fat percentage (BFP, %), and muscle mass (kg). The formula for BMI used was weight in kilograms divided by height in meters squared.

2.4 Physical fitness tests

Physical fitness tests were conducted before and after the exercise period. Back-muscle strength was measured using a back muscle dynamometer (TKK-5002, Takei Corp., Japan). The participants stood on the platform, bent their upper body forward approximately 30°, and then exerted maximum effort to straighten their upper

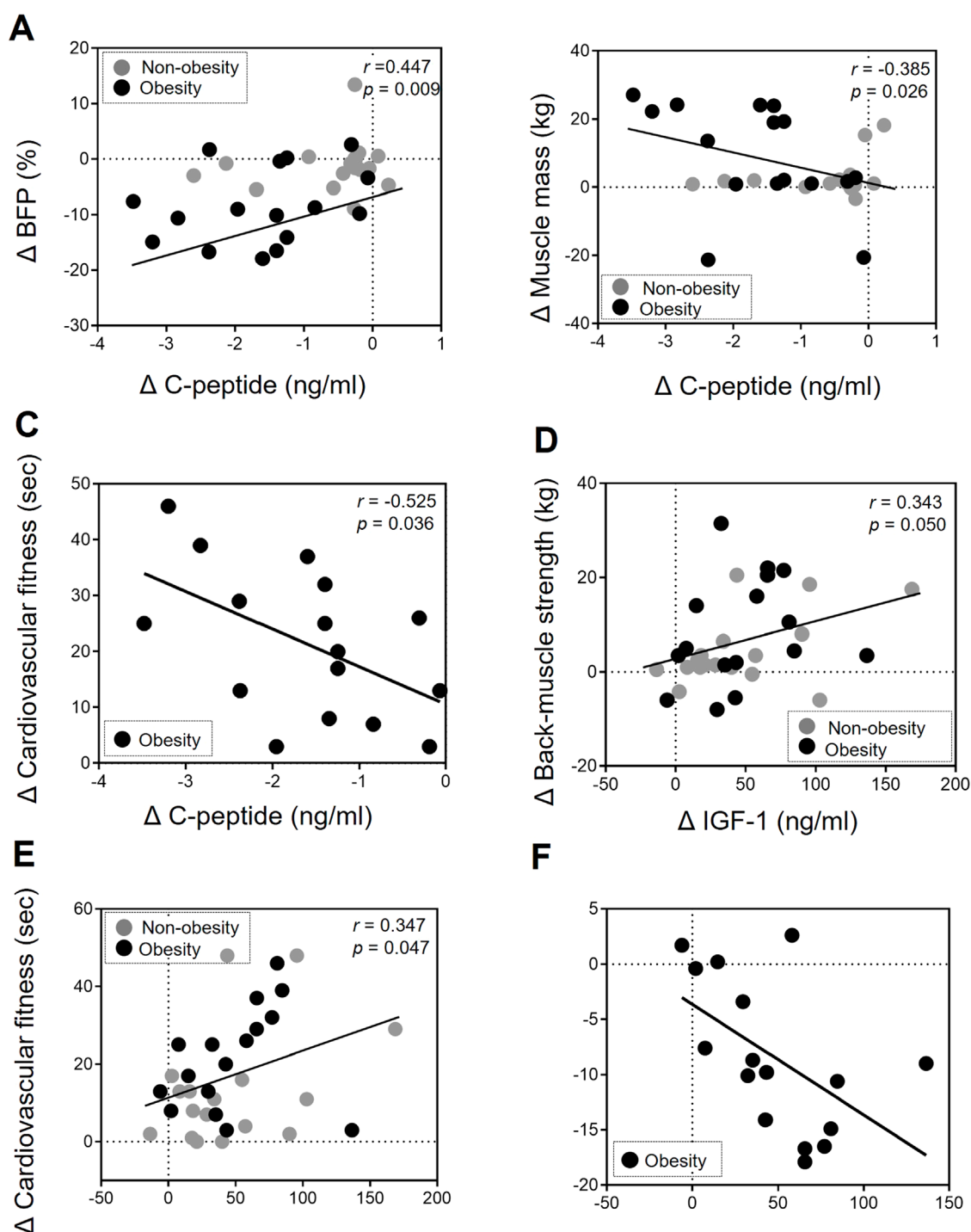


FIGURE 1

Association of changes in body composition and physical fitness with C-peptide and IGF-1 levels after exercise intervention. (A, B) The change in (Δ) C-peptide levels was correlated with the change (Δ) in BFP ($r = 0.447$, $p = 0.009$) and muscle mass ($r = -0.385$, $p = 0.026$) after undergoing the EP in the non-obesity and obesity group. (C) The change in (Δ) C-peptide levels was correlated with the change (Δ) in cardiovascular fitness ($r = -0.525$, $p = 0.036$) after undergoing the EP in the obesity group. (D, E) The change (Δ) in IGF-1 levels was correlated with the change (Δ) in back-muscle strength ($r = 0.343$, $p = 0.05$) and cardiovascular fitness levels ($r = 0.347$, $p = 0.047$) after undergoing the EP in the non-obesity and obesity group. (F) The change in (Δ) IGF-1 levels was correlated with the change (Δ) in BFP ($r = -0.542$, $p = 0.003$) after undergoing the EP in the obesity group. A correlation analysis was performed (Pearson's correlation coefficients). Each gray and black circle represents a participant in the non-obesity ($n = 17$) and obesity ($n = 16$) groups, respectively.

obese group. The inclusion and exclusion criteria for these analyses were based on the significance level of the F-value, set to 0.05. The best equation was selected based on the highest multiple correlation coefficient (R^2).

3 Results

3.1 Regulation of body composition balance through exercise

Body composition before and after the EP is shown in [Table 1](#). Before the EP, body weight ($F = 26.132$, $p = 0.000$), BMI scores ($F = 50.291$, $p = 0.000$), and BFP ($F = 12.800$, $p = 0.001$) were significantly higher in the obese group by 29.3%, 22.8%, and 24.0%, respectively, than those in the non-obese group. After the EP, the obese group showed significant reduction in BFP ($F = 23.200$, $p = 0.000$) and a significant increase in muscle mass ($F = 8.513$, $p = 0.007$). The difference in BFP showed a substantial reduction between the two groups, decreasing from a pre-EP BFP of 24.0% to a post-EP BFP of 0.28%. Another notable change was observed in muscle mass, transitioning from a pre-EP difference of 3.95% to a post-EP difference of 28.8%.

3.2 Physical fitness capacity improvement through exercise

Physical fitness before and after the EP is shown in [Table 2](#). No significant interactions between the time and group were observed for any physical fitness parameter. However, a significant group effect was detected in back-muscle strength ($F = 5.806$, $p = 0.022$), handgrip strength ($F = 7.159$, $p = 0.012$), and sit-ups ($F = 4.441$, $p = 0.043$), indicating differences in strength capacity between the two groups. Following the EP, a significant main effect of time was detected for all parameters, including back-muscle strength ($F = 14.999$, $p = 0.001$), handgrip strength ($F = 38.083$, $p = 0.000$), sit-ups ($F = 65.683$, $p = 0.000$), flexibility ($F = 29.130$, $p = 0.000$), and cardiovascular fitness ($F = 50.372$, $p = 0.000$), indicating an overall increase in physical fitness levels during the EP in both groups. The range of effect sizes for all physical fitness parameters in the obese group ($d = 0.70$ – 2.83) exceeded those in the non-obese group ($d = 0.20$ – 1.61). Particularly noteworthy was the balance level in the obese group ($d = 0.89$), which was 4.45-fold higher than that in the non-obese group ($d = 0.20$).

3.3 Improvement of C-Peptide, resistin, IGF-1, and GH levels in childhood obesity

Circulating hormone factors before and after the EP are shown in [Table 3](#). Before the EP, individuals with obesity exhibited significantly higher C-peptide levels than those without obesity ([Table 3](#)). Following the EP, C-peptide levels significantly decreased in both groups ($F = 48.101$, $p = 0.000$). While no significant main effect of group in resistin was observed, the EP significantly reduced resistin levels in the obese group ($F = 27.291$, $p = 0.000$), but not in the non-obese group. Baseline IGF-1 levels

were comparable between both groups and significantly increased after undergoing the EP ($F = 42.333$, $p = 0.000$). However, different responses were noted in GH levels between the non-obese and obese groups before and after undergoing the EP. GH levels in the obese group were significantly lower than those in the non-obese group ($F = 5.739$, $p = 0.023$), and the EP led to increased GH levels in both groups ($F = 9.891$, $p = 0.004$).

3.4 C-peptide and IGF-1 association with changes in body composition and physical fitness following exercise

To elucidate the impact of biochemical markers in the EP-improved body composition and physical fitness level, we conducted statistical correlations using delta values (difference) of all parameters pre- and post-EP. In both groups, changes in C-peptide levels were significantly associated with improvements in BFP ($r = 0.447$, $p = 0.009$; [Figure 1A](#)) and muscle mass ($r = -0.385$, $p = 0.026$; [Figure 1B](#)). A decrease in C-peptide levels in the obese group was associated with a change in cardiovascular fitness ($r = -0.525$, $p = 0.036$; [Figure 1C](#)). Changes in IGF-1 levels were significantly associated with EP-induced improvements in physical fitness, including back-muscle strength ($r = 0.343$, $p = 0.050$; [Figure 1D](#)) and cardiovascular fitness level ($r = 0.347$, $p = 0.047$; [Figure 1E](#)) in both groups. Regarding the association with body composition, a decrease in BFP was associated with a change in IGF-1 levels in the obese group ($r = -0.542$, $p = 0.003$; [Figure 1F](#)).

3.5 Factors affecting changes in obesity-related biochemical markers in the non-obesity and obesity groups

To analyze the factors associated with biochemical markers after undergoing the EP, we conducted four iterations of multiple linear regression analyses using the delta values in each group ([Table 4](#)). In the non-obese group, no factors affecting C-peptide and resistin levels were detected; however, back-muscle strength ($\beta = 3.045$, $p < 0.05$) influenced IGF-1, and height ($\beta = 0.45$, $p < 0.05$) affected GH levels. In the obese group, a significant association was observed between C-peptide levels and cardiovascular fitness level ($\beta = -0.054$, $p < 0.001$), as well as glucose levels ($\beta = 0.083$, $p < 0.001$). In addition, IGF-1 ($\beta = -2.936$, $p < 0.05$) had a reducing effect on BFP. These findings suggest that C-peptide and IGF-1 may contribute to improving body composition and physical fitness levels through regular exercise in adolescent with obesity.

4 Discussion

In this study, we explored the impact of a supervised exercise intervention on body composition and physical fitness in adolescent boys with obesity and the potential reduction in obesity-related circulating hormones. In addition, we examined the associations between biomarkers and exercise-induced variables. Our results demonstrated positive effects of the EP on body composition and physical fitness parameters. Moreover, this study is novel in

demonstrating the promising roles of blood levels of C-peptide and IGF-1 in contributing to exercise-induced improvements in body composition and physical fitness in early adolescent boys with obesity. This study specifically links biochemical changes to enhancements in muscle strength and cardiovascular fitness through a structured 16-week EP.

Body composition analysis is a cornerstone for identifying childhood obesity and assessing the clinical efficacy of interventions. Consistent with previous studies, our findings demonstrated that the EP intervention effectively reduced BMI scores and BFP in children with obesity. Conversely, no significant changes in body composition were observed among children without obesity (Tan et al., 2016; 2017). A recent randomized controlled trial compared the effect of aerobic exercise intensity on adolescent boys with obesity and found that reductions in BFP were more significant following continuous moderate-intensity training than high-intensity interval training (Meng et al., 2022). The EP training in our study may have similar effects on body fat reduction with moderate-intensity aerobic exercise. A notable finding was the increase in muscle mass among children with obesity participating in the EP, particularly those engaging in resistance training. This aligns with the established efficacy of a 16-week resistance exercise in enhancing lean body mass by approximately 7.4% in the context of obesity treatment in adolescent boys with obesity (Shaibi et al., 2006). However, in our study, obese children who participated in the EP increased their strength by approximately 28% during the EP. Although the exact mechanisms behind this strength gain cannot be determined, the higher frequency of exercise (3 days per week) may have contributed to the substantial differences in changes in lean body mass. This supports the notion that a higher volume of exercise intervention may have improved body composition, particularly muscle gain.

Previous research has established low childhood physical fitness as a critical determinant of health, with lower fitness levels linked to increased risks of metabolic and cardiovascular diseases (Ortega et al., 2008). Children with obesity and elevated high BMI scores often exhibit reduced physical capacity, including diminished endurance and muscular fitness (Musálek et al., 2020). Our findings corroborate this, revealing significantly lower muscle strength in children with obesity than in children without obesity. Aligned with a systematic review demonstrating improved muscular fitness following physical activity intervention in children with obesity (Thivel et al., 2016), our 16-week EP significantly enhances overall physical fitness, encompassing cardiovascular endurance, muscular strength, muscular endurance, flexibility, and balance. These findings suggest that the EP is an effective intervention for improving health outcomes in adolescent boys with obesity by targeting health-related physical fitness components through a balanced combination of aerobic, resistance, and sports activities.

Obesity-induced insulin resistance in children is considered to be an important pathophysiologic indicator linking obesity and metabolic derangement (Lee, 2006). In the context of monitoring insulin resistance and sensitivity, the measurement of C-peptide levels in the blood also serves as a significant biomarker of diabetes since it is secreted in equimolar amounts with insulin (Maddaloni et al., 2022). Previous studies consistently indicate that higher insulin production correlates with increased C-peptide levels (Steiner et al., 2009; Jones and Hattersley, 2013). Our study

found that the obese group exhibited higher C-peptide levels than the non-obese group, which is consistent with insulin-based indices. A recent systemic review and meta-analysis published in 2023 showed the positive effects of exercise interventions on insulin resistance in children and adolescents with overweight and obesity (Kazeminasab et al., 2023). Similar to the effects of exercise on insulin resistance, our study also observed that the EP intervention reduced C-peptide levels in obese children, with these declines correlating with changes in BFP and muscle mass. This study supports the notion that the positive effects of exercise on body composition and skeletal muscle metabolism beneficially improved insulin sensitivity and decreased insulin resistance in obese children, which subsequently resulted in lower insulin secretion.

Our study showed that changes in C-peptide levels were associated with improved physical fitness following the EP intervention. The relationship between C-peptide levels and physical fitness parameters requires further exploration. A recent meta-analysis and systematic review suggested that serum C-peptide is highly associated with an increased risk of cardiovascular disease (CVD) (Ahmadirad et al., 2023). Higher physical fitness, particularly cardiovascular fitness, has been associated with a favorable risk profile for CVD in children and adolescents (Ruiz et al., 2016). In our study, the reduction in C-peptide along with increased physical fitness may contribute to a reduction in obesity-related CVD risk.

Our study had several limitations. First, the relatively small sample size restricts the generalizability of our findings to larger populations of children with obesity. Future studies with larger sample sizes are essential to validate our results and provide more robust evidence. Second, the EP was limited to 16 weeks. Long-term follow-up studies are necessary to assess the sustainability and lasting effects of exercise on body composition and physical fitness in children with obesity. Third, our study focused on the effects of exercise on C-peptide and IGF-1 levels as mediators of the observed outcomes; however, other factors and mechanisms may contribute to the regulation of body composition and physical fitness in children with obesity. Further studies are required to identify additional biomarkers and pathways involved in these processes. Fourth, our study lacked sex-specific analysis, as we did not separately analyze data for girls. Future research should explore sex-specific effects of the EP to better understand its impact on body composition, physical fitness, and circulating hormones. Fifth, our study did not evaluate pubertal status of the participating boys. The causal relationship between pubertal timing and outcome measurements remains uncertain. Sixth, to estimate body composition, our study used the BIA method instead of a gold-standard measure like dual-energy X-ray absorptiometry. Finally, our study did not examine the potential effects of dietary factors or lifestyle changes on the outcomes. The effects of exercise on body composition and physical fitness may interact with other variables such as diet quality and adherence to EPs. Future studies should consider these factors when exploring the combined effects. Despite these limitations, our study provides valuable insights into the role of C-peptide and IGF-1 in the improvement of body composition and physical fitness through exercise intervention in early adolescent boys with obesity.

5 Conclusion

In conclusion, our study revealed that exercise is crucial in regulating body composition and physical fitness in early adolescent boy with obesity. As circulating biomarkers of obesity, C-peptide and IGF-1 were associated with the effects of exercise on these outcomes. Our findings support the development of targeted exercise interventions and reinforce the importance of exercise as an integral component of child and adolescent obesity management programs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the National Bioethics Committee and Institutional Review Board of Dongguk University (DUIRB-202206-18) (WS-2020-13). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

M-SH: Conceptualization, Funding acquisition, Investigation, Resources, Writing–original draft. HM: Funding acquisition, Writing–review and editing. ML: Formal Analysis, Supervision, Validation, Writing–original draft, Writing–review and editing. JY:

Conceptualization, Data curation, Formal Analysis, Investigation, Validation, Visualization, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2022R111A4053049; NRF-2024S1A5A8024477) and the 2023 Advanced Facility Fund of the University of Seoul. This study was supported by the JOMES Research Grant (Grant No. KSSO-J-2021005; KSSO-J-2023005) from the Korean Society for the Study of Obesity.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 27 September 2024

ACCEPTED 19 December 2024

PUBLISHED 08 January 2025

CITATION

Nowak M, Szymanek-Pilarczyk M, Stolarczyk A,
Oleksy Ł, Muracki J and Wąsik J (2025)
Normative and limit values of speed, endurance
and power tests results of young
football players.
Front. Physiol. 15:1502694.
doi: 10.3389/fphys.2024.1502694

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Normative and limit values of speed, endurance and power tests results of young football players

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Introduction: This study aimed to assess the development of speed, endurance and power in young football players and to create percentile charts and tables for standardized assessment.

Methods: Cross-sectional data were collected from 495 male players aged 12–16 years at RKS Raków Częstochowa Academy in 2018–2022. Players participated in a systematic training in which running time 5 m, 10 m, 30 m, lower limb power (standing long jump), and Maximum Aerobic Speed (MAS) were measured using the 30–15 Intermittent Fitness Test. All tests were performed under constant environmental conditions by qualified personnel. Statistical analysis included ANOVA and percentile distribution for P3, P10, P25, P50, P75, P90, P97.

Results: Results indicated that the most significant improvements occurred between the ages of 13 and 14, with increased speed over all distances and a significant increase in power. Percentile tables were developed, highlighting improvements in speed 5 m: 0.087–0.126 s; 10 m 0.162–0.215 s; 30 m: 0.438–0.719 s and power in the long jump test: 31–48 cm. Improvements in MAS ranged from 0.3 to 0.6 m/s across the percentiles.

Discussion: The results highlight the need for individual training programs tailored to the biological maturity of players. The developed percentile charts and tables offer a valuable tool for coaches and sports scientists to monitor progress, optimize training loads, and minimize the risk of injury, providing a frame of reference for assessing the physical development of young soccer players. Future research should focus on extending these charts and tables to other age groups and genders to refine training methodologies further.

KEYWORDS

endurance, speed, maximal aerobic speed, young soccer players, physical demands

Introduction

Football is one of the most popular sports played by children and teenagers worldwide. The development of this discipline affects many areas - social, scientific, educational, and economic, giving them an impulse for development. The involvement of young adepts and the popularity of football remains at a very high level (Toukabri and Toukabri, 2023). This, in turn, affects the work related to raising the level of skills of all people participating in systematic training processes (Chambers, 1991). The most important goal of the game of football is to score more goals than the opponent (Shan, 2023). An element that significantly affects this fact, apart from tactical and technical training, is motor preparation. It consists of, among other things, power, aerobic and anaerobic capacity - all at least at a high level (Clemente et al., 2020). Currently, the changing dynamics of the game force the preparation of training measures based on sub-maximum or maximum values, most often in total per week exceeding the total load values in relation to the actual conditions set by the opponent during the match competition.

Speed, motor coordination, or correct decision-making, as well as the intention (purposefulness) of using a given training measure (exercise), affect the development of a young player in the long run (Erikstad et al., 2018). Speed in football can be divided into several categories, e.g., the speed of movement of a player with the ball, without the ball, and the speed of decision-making. Each of these categories can be equally important in training processes. It is suggested that actions on the pitch based on speed directly affect the success or failure of the team (Milenkovic, 2011). In the literature, researchers most often analyze running speed over repetitive distances in the range of 5–55 m (Nicholson et al., 2021). In Poland's categories of children and adolescents, tests on very short distances, i.e., 5 m, 10 m, and 30 m, are accepted as the gold standard (Andrzejewski et al., 2009; 2013; Gardašević et al., 2016). Researchers indicate that the development process of a young athlete should be planned comprehensively, taking into account various motor skills (Duggan et al., 2022). Current requirements in soccer include multiple high-intensity efforts. One of the key objectives of the training process is to prepare players to maintain a state of readiness that allows them to effectively create activities characterized by high or very high intensity during the entire match (Duthie et al., 2018).

Scientists and coaches confirm the thesis that a high level of endurance is fundamental for achieving outstanding sports results (Clemente et al., 2021; 2024). This allows players to implement various advanced tactical concepts (Hoppe et al., 2013; Perroni et al., 2023). The speeds achieved by athletes in progressive tests with breaks, such as the Beep Test, Yo-Yo, or the 30–15 test, are correlated with the speeds at which the athletes achieve maximum oxygen consumption (VO₂max) (Magee et al., 2021; Quan et al., 2024). They also correlate with the level of intensity observed during real matches (Krustrup et al., 2003). This confirms the important role of specific endurance training, which not only affects performance but also the ability to regenerate quickly. Therefore, it is an integral part of modern football training programs (Clemente et al., 2024).

Another important feature in a footballer's training is to increase power by shaping, e.g., the explosive strength of the lower limbs (Szymanek-Pilarczyk et al., 2023; Wang et al., 2023). This can be observed in activities such as acceleration, changes of direction, and

performing repeated high-intensity efforts (De Salles et al., 2012). The results of the study indicate a negative correlation between the peak power and height of the Countermovement Jump (CMJ) and the sprint time obtained at different distances (Bartosz et al., 2024; Gísladóttir et al., 2024; Shan, 2023). In coaching practice, the importance of the development of explosive power of the lower limbs is emphasized in terms of direct impact on the effectiveness and efficiency of actions on the pitch and, consequently, on achieving the final success in football (Wang et al., 2023).

Training programs for children and adolescents should take into account the gradual and harmonious development of all motor skills. A modified wave periodization model was used in the training system at the RKS Raków Academy. A long-term plan for the development of the players' motor skills was developed. Systematic monitoring of selected motor parameters of players allowed us to create a new tool, growth charts. Growth charts are a statistical tool used to assess and monitor children's physical development by comparing their height, weight, and other parameters with those of their peers. They help specialists identify possible health problems and monitor the stability of the child's development. Their usefulness lies in enabling standardized assessment of development in different populations and comparing growth patterns at the global level. Regular use of growth charts makes it possible to detect deviations from the norm early and take appropriate medical interventions. They are a key tool in pediatrics that helps to provide children with optimal healthcare around the world. To the best of authors' knowledge there are no percentile charts for results of speed, endurance and power tests of youth elite and subelite soccer players described in scientific literature. In the case of our research, this is the first attempt to show that this type of data can also be used in work with young athletes.

The study's aim was to learn about the changes in the adaptive motor skills of children and adolescents participating in the RKS Raków Academy training program and to build tools for assessing the development of selected motor skills for young footballers. This allowed for the development of standardized reference curves, the so-called percentile charts, on the basis of data collected from 5-year studies for selected physical fitness indicators such as: running speed at 5 m, 10 m, 30 m, power and MAS index among boys training football at the RKS Raków Częstochowa Academy.

The application of this research could be to create a unified benchmark that will enable coaches and scientists to monitor and evaluate athletes' progress more accurately. Growth charts can be a tool that supports the optimization of the training process, improvement of sports performance, and reduction of the risk of injury, which in the long term will contribute to raising the sports level of young footballers. At the same time, placing individual results on the growth chart will allow you to estimate the potential for developing a given motor trait and give tips for working with the athlete.

Material and methods

Subjects

Cross-sectional studies of selected physical fitness indicators were carried out in 2018–2022 among football players of the RKS Raków Częstochowa Academy aged 12–16. The sporting level of the

TABLE 1 The characteristics of the study group are divided into age categories.

Age (years)	Number of players	Body height (cm) ± SD	Body weight (kg) ± SD	Body fat (%) ± SD	Muscle mass (kg) ± SD
16	98	178 ± 3.89	69.02 ± 5.17	15.15 ± 1.97	54.75 ± 4.18
15	102	178 ± 4.71	67.20 ± 5.42	15.22 ± 1.92	54.05 ± 4.42
14	101	172 ± 7.00	59.30 ± 6.78	14.99 ± 2.39	47.74 ± 4.73
13	97	165 ± 9.42	51.27 ± 8.91	15.15 ± 2.69	40.89 ± 6.82
12	97	154 ± 4.59	42.83 ± 7.11	19.24 ± 6.62	32.12 ± 4.71

SD, standard deviation.

examined players was Tier 3 according to McKay’s classification (McKay et al., 2022). The training experience of the respondents ranged from 4 to 8 years. During each of the analysed seasons the players competed in the highest possible junior league. The inclusion criteria were: i) age from 12 to 16 accordingly to the age groups; ii) being a male football player of the academy; iii) training experience over 4 years. The exclusion criteria were: i) serious injury or illness resulting in time loss over 1 month in the year period of the study; ii) illness or injury before or at the moment of testing. The analyzed group of players amounted to a total of 495 young healthy male football players. Considering age ranges, it was divided into five subgroups. The percentage distribution was: 16-year-olds – 19.8% of the respondents, 15-year-olds – 20.6%, 14-year-olds – 20.4%, 13-year-olds – 19.6% and 12-year-olds–also 19.6%. During the monitored period of 4 years there was a rotation of players, which is natural in the youth football academy, but it did not exceed 5% overall. The anthropometric characteristics of the respondents are presented in Table 1. The subjects participated in systematic sports training developed according to the wave periodization program developed following the tactical periodization work model (Szymanek-Pilarczyk et al., 2023; Szymanek-Pilarczyk et al., 2024a; Szymanek-Pilarczyk et al., 2024b).

The simplified training load scheme in each working week consisted of 6 training units on the pitch (4 team training x 90 min, 1 formation training including profiling of players in positions x 60 min, 1 match x 45–90 min), and 2 training sessions in the gym lasting 2 x 45 min (1 session—upper muscle parts and 2 sessions—lower muscle parts). All age categories in the described period were trained according to a uniform training plan. Every training session started with the warm-up, general development, specific preparation focused on selected motor abilities considering day-to-match and with accordance with the training plan. The methods and forms used in the training process were adjusted to the age and level of the participants (Szymanek-Pilarczyk et al., 2023; Szymanek-Pilarczyk et al., 2024a; Szymanek-Pilarczyk et al., 2024b).

Ethics

All participants were thoroughly informed about the content of the study, its objectives, possible risks, and benefits. When joining the club and renewing the declarations of participation in the academy every player signed an informed consent to participate in research analysing their monitoring data for scientific purposes and publication. This study’s tasks and tests were typically

performed during training (sprints, runs, and jumps). All participants had a federation license, based on which their parents signed a document at the beginning of the season authorizing them to participate in the club’s football activities. This type of intervention does not alter standard soccer training or involve motor activities different from regular training and matches. Therefore, the intervention has never posed an additional risk beyond the threat associated with ordinary football practice. Moreover, all participants underwent a medical examination before the start of the season, and the tests were carried out without injury or physical discomfort. The study was in line with the requirements of the Declaration of Helsinki. Approval was obtained from the bioethics committee at the District Medical Chamber in Krakow (approval number: No. 35/KBL/OIL/2024; approval date: 24 April 2024).

Procedures

The tests were always carried out in the same period and place—a sports hall with a synthetic surface and a temperature of about 20°C. The competitors performed the tests in sports shoes with an indoor sole. The measurements took place at the same times of the day in dedicated time intervals. 1 h per team. During this period, the measurements were taken by permanently qualified personnel, i.e., the training staff consisting of motor preparation trainers (qualifications confirmed by the international certificate of the Australian Strength and Conditioning Association (ASCA)). All players training at the Academy took part in the tests. The exclusion criterion was an injury, illness, or other infection that prevented the test from being performed at 100% of the athlete’s capacity. The tests were always carried out in the same microcycle on the day referred to in terminology as the third day after the match (MD+3). The training before the tests was of a low-intensity regenerative nature. The tests were always performed in June, after the end of the season in the same order: 1) warm-up, 2) speed test (1st attempt), rest 5 min, 3) long jump (1st attempt), rest 5 min, 4) speed test (2nd attempt), rest 5 min, 5) long jump (2nd attempt), rest 10 min, 6) endurance test.

RAMP warm-up

The warm-up lasted 12 min. The first part of the warm-up lasted 4 min and aimed to raise the body temperature Raise – (R) through running exercises performed at 15 m. In this part, the following were

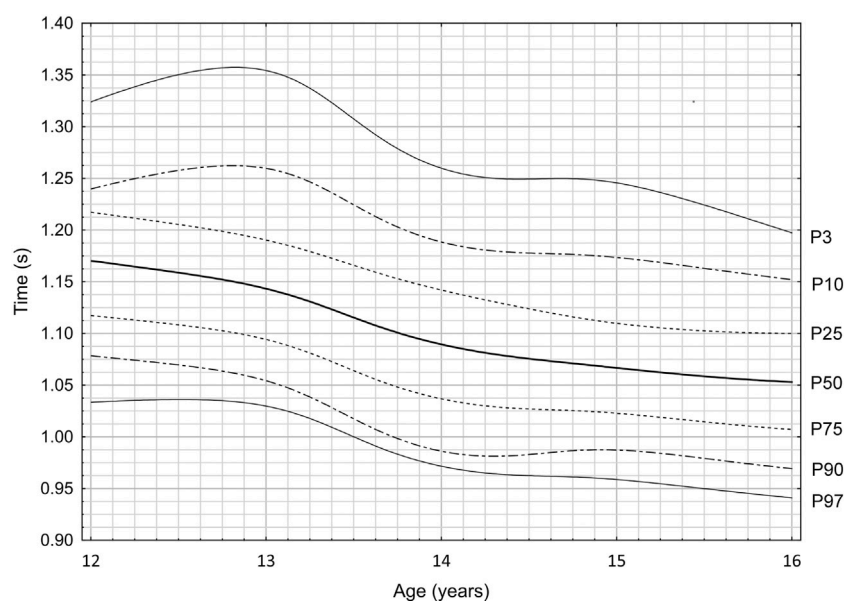


FIGURE 1
(Continued).

performed: jogging, running combined with swings/arm circles, stepping out-extended sideways and forward with a variable rhythm, abduction and adduction of legs in a free rhythm, skips A, B, C, D performed at a moderate speed. The second part of the warm-up was aimed at muscle activation and increasing the range of motion of both muscles and joints Activate – (A), Mobilise – (M). In this block, one series of 12 repetitions of exercises were used: Squat, Lunge forward, Lunge to the side, Hip Thrust, Single-Leg Romanian Deadlift (RDL SL), Push Up, Abdominal exercises, Back exercises - torso raises, and rowing. Exercises to increase the range of motion were carried out in the form of dynamic stretching of all muscle groups, taking into account many planes. The described part lasted 6 min. The last part of the warm-up was about increasing the speed potential of the Potentiate muscles – (P) (Jeffreys, 2017). Six runs were made on a 15 m section. Before the run, the competitors performed specific exercises (time = 3 s) with maximum speed and commitment. These were jumping jacks, fast feet, pogo jumps, and burpees. This part of the warm-up lasted 2 min.

Test protocols

Speed

The measurement of running speed took place in a straight line. The speed was recorded at distances of 5, 10, and 30 m. For this purpose, electronic measuring equipment was used—FITLIGHT® photocells (app version 3.2.6i, Ontario, Canada). The athlete's foot was 20 cm in front of the line of the first photocell. The start started from a high, passive position without torso rotation (swing of the arms). The photocells were placed 95 cm above the surface. The runner decided about the moment of the start himself. Each competitor made two attempts, and the best result was recorded with an accuracy of 0.001 s. The resting break between rehearsals was 3 min.

Power

The power test involved measuring the length of the long jump from a standstill using a tape measure with an accuracy of 0.01 m. The subjects performed a two-legged jump from the spot with an arms swing. The competitors made two jump attempts. The best result was entered into the sheet.

Endurance

The Velocity Intermittent Fitness Test (VIFT—the speed achieved by the subject at the end of the test) was measured using the Intermittent Fitness Test 30–15 according to Buchheit's assumptions (Buchheit et al., 2010). The competitors ran between lines 40 m apart, following the rhythm of sound signals. The participant's task was to reach the designated buffer zones at a specific sound signal. Between 30 s runs at increasing speed, there were 15 s pauses of active regeneration. The initial speed of the test was set at 10 km/h. When the athlete did not run to the line twice in a row at a specific sound signal or reported fatigue, the competitor (of his own volition) ended the test. The parameter used to create the charts was MAS. This is the speed at which the athlete reaches the VO_2 max. MAS is not directly measured in the 30–15 IFT test; VIFT is often used as a near-MAS indicator but overstated by 15%–25% due to the nature of the test. MAS is a key indicator of an athlete's aerobic endurance and is used to plan the intensity of endurance training. For the purposes of our study, a simple estimate of the MAS value, a simplified approach, was used according to the following formula: $\text{MAS} = \text{VIFT} \times 0.8$ (Buchheit et al., 2010).

Statistical analysis

The Shapiro-Wilk test checked the normality of the data distribution - equivalence of variance as Levene's test. The

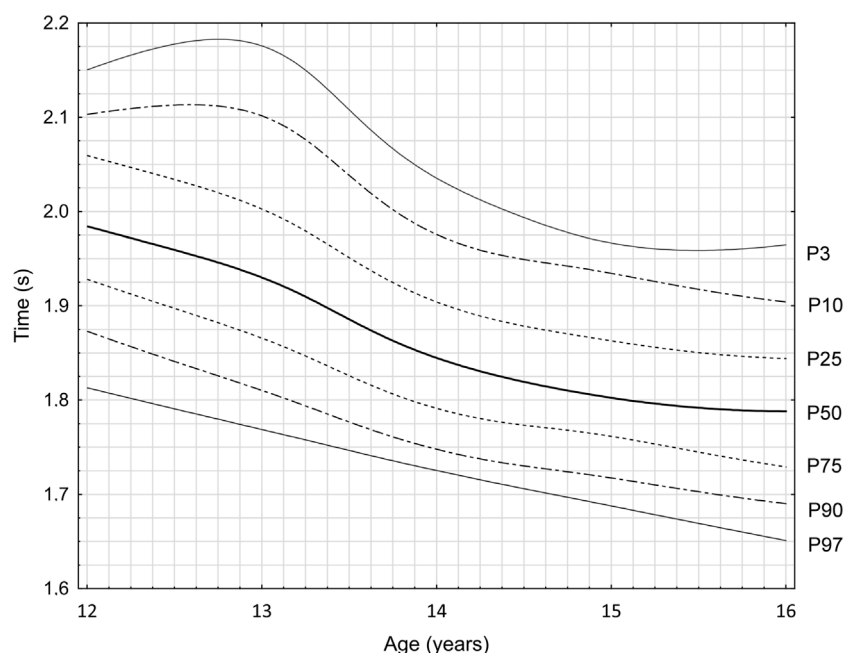


FIGURE 1
(Continued).

significance of differences between all age groups was examined with one-way ANOVA. Statistical significance was assumed at the level of $p < 0.05$. Percentile charts were created based on the division of the percentile range: P3, P10, P25, P50, P75, P90, P97. All statistical surveys were conducted using Statistica version 13.0 of TIBCO Software Inc. Results are mentioned as mean \pm SD. Sample size estimated using G*Power software (version 3.1.9.2; Kiel University, Kiel, Germany) (Faul et al., 2007) returned a minimum of 35 measurement positions, for $\alpha = 0.05$, effect size $f = 0.8$ and $\beta = 0.95$.

Results

Speed

Figures 1A–C shows the growth charts for motor skills in speed tests over running distances of 5, 10, and 30 m for young football players aged 12 to 16. The highest growth values in the scale of the entire study were: 0.126 s (P3) for 5 m; 0.215 s (P25) for 10 m; and 0.717 s (P3) for 30 m. The lowest values were: 0.087 s (P10) for 5 m; 0.162 s (P97) for 10 m; and 0.438 s (P97) for 30 m. Since the P97 value in the 5 m run is equal to 0.092 s, it can be assumed that the lowest values of positive changes were observed in the best-accelerating group of tested footballers. The intervals describing the magnitude of improvement in results at individual distances over the 5-year analysis period were as follows: for 5 m, $<0.087\text{--}0.126\text{s}>$; for 10 m, $<0.162\text{--}0.215\text{s}>$; and for 30 m, $<0.438\text{--}0.719\text{s}>$.

The most dynamic positive change in the value of velocity increase at all measurement distances was observed between 13 and 14 years of age, regardless of the studied percentile. The

maximum magnitude of these increases was: 0.117 s (P3) for 5 m; 0.164 s (P3) for 10 m; and 0.309 s (P25) for 30 m.

In total, 84 cases were analyzed, examining changes in individual percentiles over subsequent years. In 75 cases (89%), improvement was achieved; in 4 cases (5%), stabilization of results was observed; and in 5 cases (6%), regression occurred. Detailed data are presented in Table 2.

Power

Within the lower limb power measurements, the highest level of power increase is also visible between 13 and 14 years of age. It ranges from 14 to 22 cm. The exceptions are 12–13-year-old competitors placed at the P25 and P90 percentile level, where the results are also very high and amount to 16 and 18 cm, respectively. Analyzing the differences between the percentiles in the entire process of 5-year training, the highest positive value was observed at the P25 level—an improvement of 48 cm in the long jump, while the smallest differences were visible at the P3 level – 31 cm. The range describing the magnitude of the improvement in performance over the 5-year analysis period was 31–48 cm. A total of 28 cases were analyzed, examining changes in individual percentile in between subsequent years. In 24 (86%) cases, the following were obtained: in 2 (7%) - stabilization of the result; 2 (7%) regression. Comprehensive data are presented in Figure 2, and individual averages in Table 3.

Endurance

The analysis of the endurance results indicated a different trend. Clear incremental jumps were visible between the ages of 13 and 14 at the level of P50–0.3 m/s, P75–0.2 m/s, and P90–0.2 m/s. This constitutes 50% of the increase observed during the entire observation period. Between the ages of 14 and 15, this

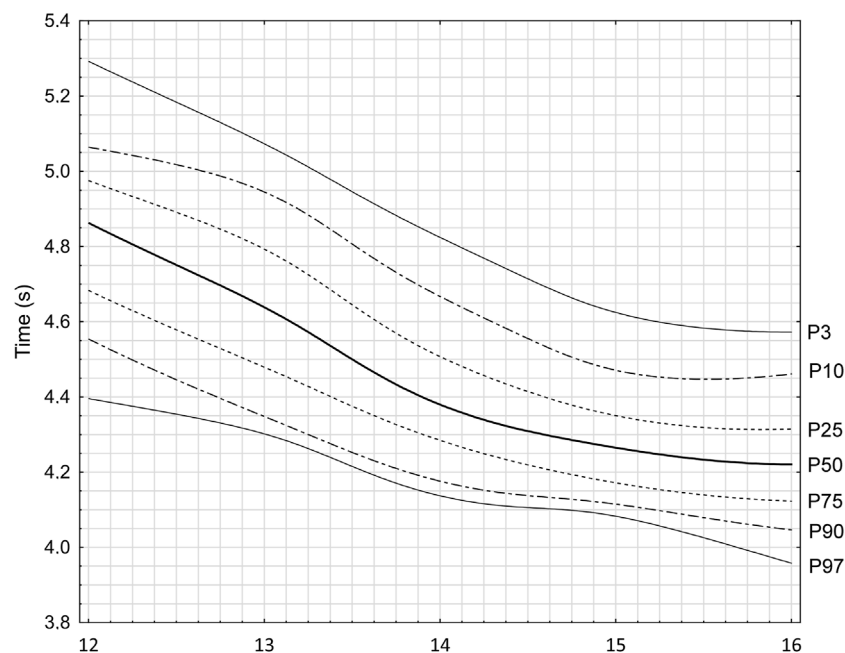


FIGURE 1

(Continued). Parameter growth chart sprint time at (A) 5 m ($F = 43.1$; $p < 0.001$) for football players. X-axis: age of competitors (years); Y-axis: time over 5 m (seconds); Lines on the graph: the individual lines represent percentiles in order (P3, P10, P25, P50, P75, P90, P97). The growth charts have been developed based on data from many years of research carried out at the RKS Raków Academy. Parameter growth chart sprint time at (B) 10 m ($F = 74.8$; $p < 0.001$) for football players. X-axis: age of competitors (years); Y-axis: time over 5 m (seconds); Lines on the graph: the individual lines represent percentiles in order (P3, P10, P25, P50, P75, P90, P97). The growth charts have been developed based on data from many years of research carried out at the RKS Raków Academy. Parameter growth chart sprint time at (C) 30 m ($F = 183.5$; $p < 0.001$) for football players. X-axis: age of competitors (years); Y-axis: time over 5 m (seconds); Lines on the graph: the individual lines represent percentiles in order (P3, P10, P25, P50, P75, P90, P97). The growth charts have been developed based on data from many years of research carried out at the RKS Raków Academy.

concerned P25 and P97–0.2 m/s—it was 40% and 33% of the entire increase, respectively. Between the ages of 15 and 16, at the level of P3 and P10–0.2 m/s – 66% of the entire increase. Considering the entire training cycle, the greatest differences were noted at the percentiles 50 and 97, with an improvement of 0.6 m/s. The lowest values are 0.3 m/s and concern percentile P3 and P10. Stagnation of the endurance parameter was also observed between the ages of 13 and 15 at the level of the two lowest percentiles, P3 and P10; the result was fixed at the level of 4.1 m/s and 4.2 m/s, respectively. The range describing the size of the improvement in the results in the perspective of 5 years of analysis was $<0.3\text{--}0.6\text{ m/s}>$. A total of 28 cases were analyzed, examining changes in individual percentiles between successive years. Progress was obtained in 22 (79%) cases; in 6 (21%), the result stabilized, and regression was not noted. The overall data are presented in Figure 3, and the individual averages are in Table 4.

Discussion

The observed adaptive changes in motor skills in young footballers participating in the RKS Raków Academy training program based on wave periodization indicate abrupt increases in speed, power, and endurance parameters in specific age groups. This suggests that the use of wave periodization could have contributed to these increases, which allowed the development of tools for assessing the magnitude and dynamics

of changes in performance parameters (Gil et al., 2007). The periodization of strength and speed training used in professional football academies is most visible at the turn of 12 and 13 years of age. Studies confirm the thesis that young footballers around 13 years of age achieve better results and more significant increase in speed and power than those who do not train in such a system (Gil et al., 2007; Malina et al., 2007).

Speed

The most dynamic speed gains were visible in the test results between 13 and 14 years of age at 5 m, 10 m, and 30 m, regardless of percentile. This suggests that the adaptation effect in this period is particularly significant and may be crucial for developing this motor feature. The analysis of the percentage share of these increases between the age of 13 and 14 in relation to the total increases between the age of 12 and 16 for different percentiles confirms that the wave periodization model functioned most effectively in this period in the context of running speed parameters. It was effective in 89% of the analyzed cases at individual percentiles on a 5-year scale. The development of speed and power is one of the most important tasks of motor preparation coaches in football. Studies confirm the significant influence of speed and power in key moments of match competition (Rampinini et al., 2007).

The analysis showed the occurrence of a certain anomaly in the results (4 cases) at the level of the lowest percentiles, P3 and P10, in the

TABLE 2 Values were obtained in a sprint time over 5, 10, 30 m of football players aged 12 to 16. Results are in (s) and include percentiles from P3 to P97.

Sprint time over a distance of 5 m (s)							
Percentile/Age (years)	P3	P10	P25	P50	P75	P90	P97
12	1.323	1.239	1.217	1.17	1.117	1.078	1.033
13	1.367	1.269	1.193	1.147	1.099	1.061	1.036
14	1.250	1.182	1.141	1.087	1.033	0.98	0.967
15	1.251	1.176	1.109	1.068	1.025	0.992	0.961
16	1.197	1.152	1.100	1.053	1.007	0.969	0.941
Sprint time over a distance of 10 m (s)							
Percentile/Age (years)	P3	P10	P25	P50	P75	P90	P97
12	2.149	2.102	2.059	1.984	1.928	1.873	1.813
13	2.192	2.115	2.009	1.935	1.869	1.812	1.769
14	2.028	1.967	1.900	1.842	1.788	1.746	1.725
15	1.964	1.937	1.864	1.803	1.764	1.719	1.688
16	1.965	1.904	1.844	1.788	1.729	1.690	1.651
Sprint time over a distance of 30 m (s)							
Percentile/Age (years)	P3	P10	P25	P50	P75	P90	P97
12	5.292	5.063	4.974	4.862	4.683	4.554	4.395
13	5.080	4.963	4.809	4.65	4.484	4.352	4.312
14	4.825	4.663	4.500	4.372	4.282	4.170	4.126
15	4.617	4.461	4.348	4.268	4.172	4.122	4.093
16	4.573	4.462	4.315	4.220	4.123	4.046	3.957

period between the age of 12 and 13 in the 5 and 10 m tests. The regression of the results could have resulted from a large increase in body mass and height. At this stage of training, a coordination problem could have occurred, consisting in an undeveloped mechanism for effectively using the additional muscle mass in the weakest group of trainees. The solution to this problem is a better-tailored training process of an individualized nature. The works of subsequent authors highlight the need for research on biological maturity and placing players in an appropriate training system to optimize sports results (González et al., 2021; Morris et al., 2018; Philippaerts et al., 2006). The training plan for motor preparation at the RKS Raków Academy is strongly focused on using adaptation periods, which is also applicable and translated into the work of coaches according to the Long-Term Athlete Development (LTAD) program (Lloyd and Oliver, 2012).

Power

In lower limb power measurements, the highest increases were observed between 13 and 14 years of age, ranging from 8 to 14 cm and 14–22 cm. In the remaining years, the increases are smaller, or the results stabilize at one level. Similar observations were made by Szymanek-Pilarczyk et al. (2023), where the minimum and maximum results in the long jump tests stabilized at a level from

about 190 cm to 287 cm in 15 and 16-year-olds. Although the increases in muscle mass were not directly analyzed, it can be assumed that the increased muscle mass during this period contributed to the increase in power. The increase in power is crucial for activities such as acceleration or changes in direction (De Salles et al., 2012). For coaches, this means that training explosive lower limb strength should be a priority at this age. From a scientific perspective, the greatest increases in power at the P25 percentile level suggest that average-level players have great potential for improvement with appropriate training. The research by Skratek et al. (2024) suggests that long-term, traditional strength training is effective and safe for young soccer players between the ages of 12 and 15 and should be included in long-term athletic development programs. This emphasizes the importance of individualizing the training process (Skratek et al., 2024; Szymanek-Pilarczyk et al., 2023).

Endurance

The MAS coefficient, which was one of the parameters studied in this study, is highly correlated with running efficiency, which is very important in football. This parameter allows for determining the speeds at which the player works using maximum oxygen consumption, thus determining the training load (Buchheit et al., 2021). A similar trend to that observed among the Częstochowa

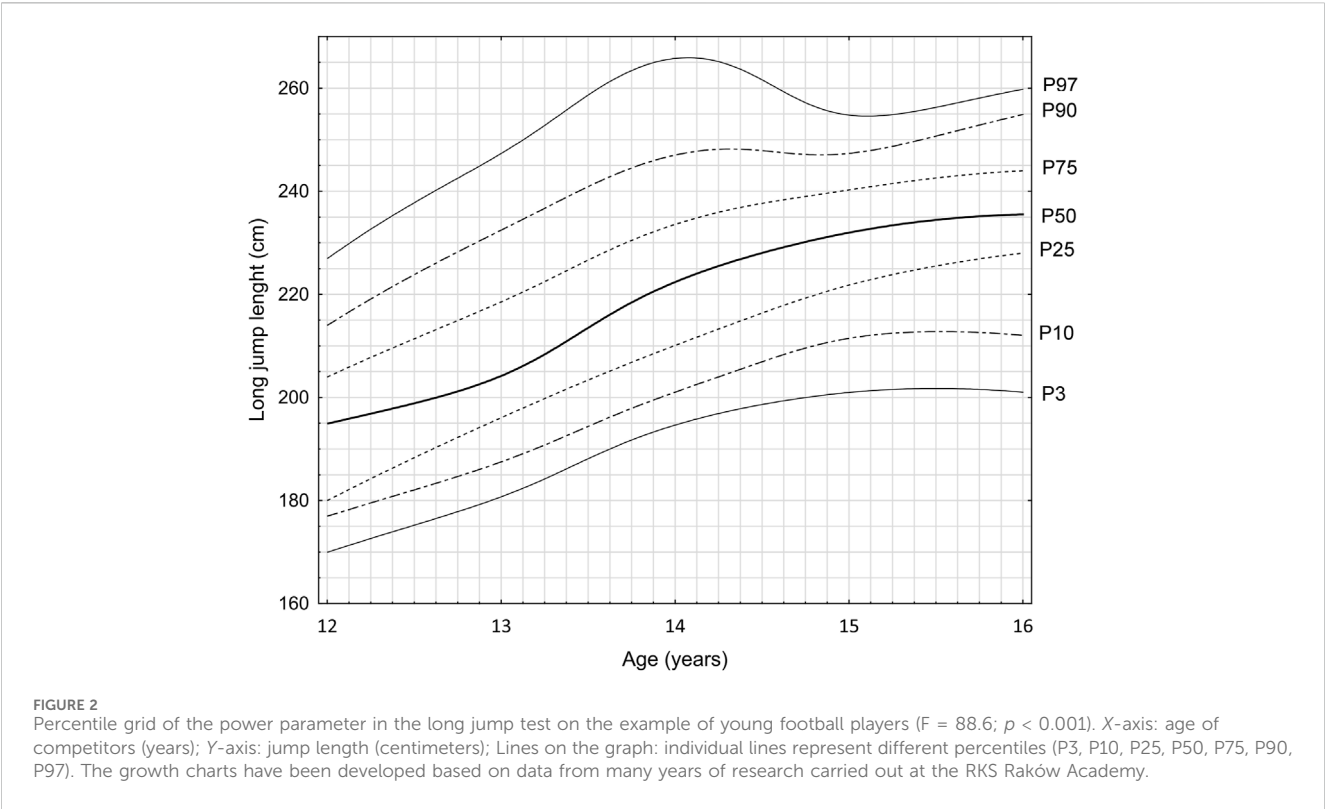


TABLE 3 Values obtained in the long jump test of young footballers between the ages of 12 and 16. Results are given in centimeters and include percentiles from P3 to P97.

Long jump (cm)							
Percentile/Age (years)	P3	P10	P25	P50	P75	P90	P97
12	170	177	180	195	204	214	227
13	180	187	196	203	218	232	246
14	195	201	210	223	234	248	268
15	201	212	222	232	240	246	252
16	201	212	228	235	244	255	260

Academy footballers can be observed in the studies conducted by [Deprez et al. \(2015a\)](#). Players who initially achieved very high results in the following years also achieved very high results (clear incremental jumps). This is confirmed by the observation of the behavior of this parameter at the level of the P97 percentile, where the increase in the MAS parameter value was 0.6 m/s between the ages of 12 and 16, as well as the difference in the values of the P25 and P97 percentile in individual years amounting to 0.6 m/s, and at the age of 16 even 0.7 m/s between P25 and P97.

Most of the analyzed subjects noted a positive adaptation to the proposed work system called wave periodization. The studies observed that there is a group of players from the P3 and P10 levels whose unplanned stability of results was observed - this concerns players aged 13 to 15. Their results remained at the level of 4.1 m/s and 4.2 m/s, respectively. It can be argued that this feature is strongly determined by genetics, and this type of training does not positively affect the development of aerobic capacity for

this group of players. However, no scientific studies show that this excludes them from being effective and useful football players. It also opens up space for the coach to manage such a group of players differently ([Deprez et al., 2015b](#)). Moreover, many variables can disturb or distort the linear development of MAS speed. Starting from a growth spurt and weight fluctuations to changes in position during the training process, which is associated with other values achieved on the morphocycle scale ([Abbott and Collins, 2002](#)). The research conducted by Nobari et al. confirms how the position on the pitch determines the development of such parameters as MAS and VO2max ([Nobari et al., 2021](#)).

It is very important to skillfully use the results in the percentile grids dedicated to the appropriate sports level ([Murtagh et al., 2018](#)). In youth football, the requirements for players in many areas are constantly changing. The position of the first teams in the table is related to their financing, which directly affects the development possibilities of the academy through the prism of multi-year

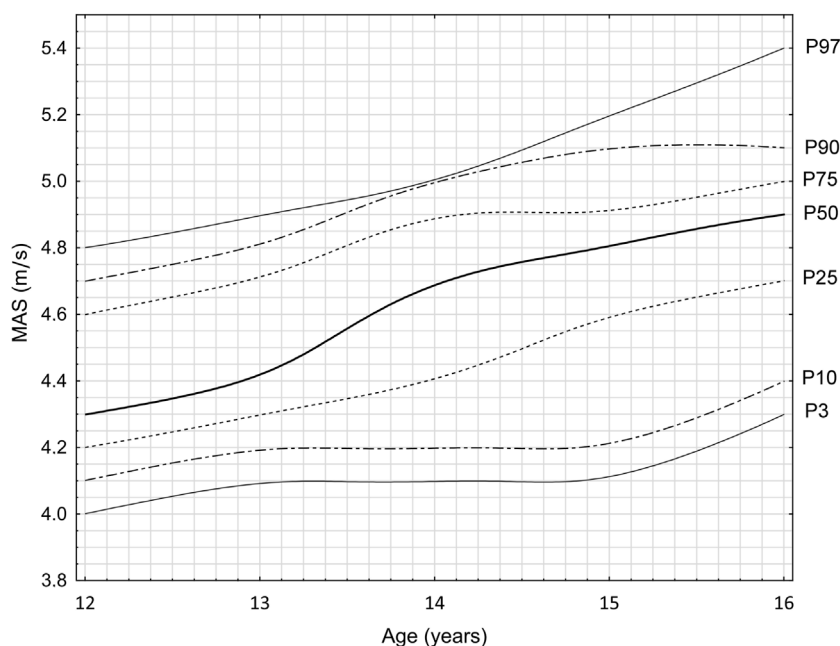


FIGURE 3

Endurance parameter growth chart in the 30–15 progressive test on the example of young football players ($F = 44.3$; $p < 0.001$). X-axis: age of competitors (years); Y-axis: value - Maximal Aerobic Speed (meters/seconds); Lines on the graph: individual lines represent different percentiles (P3, P10, P25, P50, P75, P90, P97). The growth charts have been developed based on data from many years of research carried out at the RKS Raków Academy.

TABLE 4 Values obtained in the 30–15 performance test in young footballers aged 12 to 16. Results are given in meters per second (estimated MAS value according to the formula $MAS = VIFT \times 0.8$) and include percentiles from P3 to P97.

Maximal aerobic speed (m/s)							
Percentile/Age (years)	P3	P10	P25	P50	P75	P90	P97
12	4.0	4.1	4.2	4.3	4.6	4.7	4.8
13	4.1	4.2	4.3	4.4	4.7	4.8	4.9
14	4.1	4.2	4.4	4.7	4.9	5.0	5.0
15	4.1	4.2	4.6	4.8	4.9	5.1	5.2
16	4.3	4.4	4.7	4.9	5.0	5.1	5.4

MAS, Maximum Aerobic Speed.

training. Regulations regarding the stability of such projects would have a positive impact on their harmonious development in the long term. Therefore, such a tool can help build stable foundations and programs for youth football. The task of the authors of the work is to ensure a calm, smooth development of players, without the so-called “disappearance” in the system, and to prepare patterns for introducing young, outstanding players to senior football. This research shows that players achieving low results can also catch up with potentially better ones if an appropriately selected, individualized training program is applied to them. The resulting percentile grids can facilitate the prediction of player development in many areas (Reilly et al., 2000). Using the information in the percentile charts developed during many years of research and considering the Peak Height Velocity (PHV) period may additionally help optimize the training

process, achieve training effects, and gradually reduce the risk of injury (Myer et al., 2011).

Practical application

Percentile charts, developed based on research data, constitute a useful tool for coaches and other specialists in the field of physical education sciences, such as physiotherapists and doctors, to monitor and assess the development of motor abilities in young football players. Their regular use allows for the individualization of the training process and the adjustment of loads and training methods to the needs of each athlete, which can contribute to the optimization of training outcomes and the reduction of injury risk (Myer et al., 2011).

Establishing normative value results enables precise identification of potential gaps in the sports specialization of athletes. Percentile charts can serve as a reference point for a given football level and be helpful in planning the return-to-sport stage after injuries. Such a tool facilitates planning work with youth, where the introduction of systematic monitoring and individualization is necessary, positively impacting the improvement of the training level.

Limitations

The main limitation is the lack of direct correlation analysis of muscle mass gains and other factors, such as changes in position on the pitch or individual differences in biological maturation—precise measurement of PHV. The age range does not include 17- and 18-year-olds. In addition, the study concerned only one football academy, which may limit the possibility of generalizing the results to a wider population of young footballers.

Future research directions

Future research directions include the creation of similar growth charts for males and females aged 8 to 18. This will provide a more comprehensive understanding of the development of motor skills in young athletes regardless of gender and age. This type of research will allow for even more precise adjustment of training programs and monitoring of the progress of players in different age and gender groups, which in the long term will contribute to raising the level of sports performance of all young football players. An intermediate goal is to create an open spreadsheet, updated every year and made available to coaches to compare the sports results of children and young people. In addition, the development of a publicly available tool will allow academies worldwide to use similar tests to compare the results of their players. This will enable global standardization of results, which will contribute to a better understanding and optimization of the training process of young football players around the world.

Players' physical attributes, such as height, weight, and speed, can affect their suitability for different positions on the pitch. Taller and stronger players may be better suited for defense, while faster and more agile players may be better suited for attacking. Further research into these relationships could provide valuable insights into talent identification, tactical planning, and improved positional matching.

Conclusion

1. The greatest increases in lower limb power and speed occur between the ages of 13 and 14, which indicates the need for intensified training of these features at this age.
2. Differences in motor skill gains between athletes at different percentiles emphasize the need for individualized training, especially for athletes at the highest and lowest levels.
3. The use of the wave periodization model can effectively support the development of motor skills when it is adapted to the individual pace of maturation of athletes.

4. Percentile charts are a valuable tool for coaches to monitor progress and plan individual training programs, which can lead to better sports results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Bioethics Committee at the District Medical Chamber in Krakow. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MN: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. MS-P: Writing—original draft, Writing—review and editing, Conceptualization, Data curation, Investigation, Methodology, Visualization. AS: Writing—original draft, Writing—review and editing, Formal Analysis, Project administration, Resources, Supervision, Validation. ŁO: Writing—original draft, Writing—review and editing, Conceptualization, Methodology. JM: Writing—original draft, Writing—review and editing, Conceptualization, Formal Analysis, Methodology, Project administration, Supervision. JW: Writing—original draft, Writing—review and editing, Formal Analysis, Project administration, Resources, Supervision, Validation.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We want to thank the Club's management board and the Academy Director Marek Śledź and Director Dariusz Grzegorzółka for their constant support and invaluable advice. Your guidance has been instrumental in our efforts, and your commitment is deeply appreciated. Thank you for being with us and looking for new solutions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2024.1502694/full#supplementary-material>

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OPEN ACCESS

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RECEIVED 26 September 2024

ACCEPTED 22 January 2025

PUBLISHED 14 February 2025

CITATION

Wu Q, Liu S, Wu C and Liu J (2025) The effect of transcranial pulse current stimulation on the accumulation of exercise-induced fatigue in college students after moderate intensity exercise evidence from central and peripheral sources. *Front. Physiol.* 16:1502418. doi: 10.3389/fphys.2025.1502418

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The effect of transcranial pulse current stimulation on the accumulation of exercise-induced fatigue in college students after moderate intensity exercise evidence from central and peripheral sources

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Objective: To investigate the intervention effect of cranial pulse current stimulator (tPCS) on fatigue accumulation after moderate-intensity exercise by using blood analysis and functional near-infrared spectroscopy, and to analyze the type and magnitude of the fatigue effect of tPCS on fatigue in combination with behavioral performance.

Methods: Ninety healthy college students were randomly and equally divided into an experimental group (Group A) and a control group (Group B), and both groups underwent moderate-intensity training for 7 days. Before and after the experiment, all subjects received physiological, biochemical, behavioral, and subjective fatigue indexes, followed by exercise training, and each day of exercise training was followed by tPCS intervention (stimulus intensity of 1.5 mA, stimulus duration of 20 min) and subjective fatigue scale (RPE) test.

Results: ① After the tPCS intervention, the daily RPE scores of group A were smaller than those of group B; ② The values of the indexes oxygenated hemoglobin concentration (Oxy-Hb), deoxyhemoglobin concentration (HHb), testosterone (T), and testosterone-to-cortisol ratio (T/C) of group A did not differ significantly from those of the pre-intervention period, and the values of all the indexes of group B were significantly different from those of the pre-intervention period. ③ After tPCS intervention, the values of Oxy-Hb, T, T/C, and on-attention decreased in Groups A and B, with Oxy-Hb decreasing the most; the values of HHb, total hemoglobin concentration (HbTot), hemoglobin concentration difference (HbDiff), cortisol (C), creatine kinase (CK), and reaction time (RT) increased, with the greatest increase in HbDiff; and the Group A The magnitude of change of each index was smaller than that of Group B. After tPCS intervention, the contribution of central fatigue to the effect of reaction time science was greater than that of peripheral fatigue.

Conclusion: ① tPCS can delay the development of central fatigue and peripheral fatigue. ② The effect of tPCS on central fatigue is greater than on peripheral fatigue. ③ The effect of tPCS on reaction timing is mainly realized by changing the state of central fatigue.

KEYWORDS

fatigue accumulation, transcranial pulse current stimulation, central fatigue, peripheral fatigue, behavioral indicators

1 Introduction

In sports competitions, fatigue is inevitable, and it can lead to a decline in athletic performance. With the increasing number of sports events, athletes are facing long-term competition challenges, which creates favorable conditions for the accumulation of fatigue. The accumulation of fatigue not only affects the physiological characteristics of individual muscles, such as reducing muscle strength and endurance (Ament and Verkerke, 2009), but also may trigger emotional fluctuations, anxiety, and depression symptoms, which may lead to a decrease in athletes' motivation and training investment (Pageaux and Lepers, 2018), ultimately inevitably affecting athletes' competitive performance. Accumulation of fatigue may interfere with neuromuscular control, increase the risk of injury (Jones et al., 2017), and shorten an athlete's career. Therefore, how to eliminate the accumulation of fatigue and keep athletes in a high-level competitive state is not only of great significance for achieving excellent results, but also has a positive effect on extending the professional life of athletes. Transcranial pulsed current stimulation (tPCS), as a new non-invasive brain stimulation technique, can modulate neuronal activity by delivering oscillatory currents to the cerebral cortex. Compared with transcranial direct current, continuous pulse stimulation of tPCS can cause repeated depolarization of cells, resulting in a cumulative effect of neural excitation and ultimately leading to greater cortical excitability changes (Datta et al., 2013; Ma et al., 2019). Due to its unique bipolar pulse characteristics, some scholars have used it in the fields of eliminating exercise fatigue and exercise cognition (Morales-Quezada et al., 2015; Wu et al., 2022). Therefore, in this study, tPCS was used as an intervention to combat fatigue accumulation. Previous studies have shown that most scholars have studied the immediate effects of tPCS intervention once, and have not yet paid attention to the cumulative effects of interventions on tPCS. Moreover, the occurrence of fatigue has a cumulative effect, and the Rating of Perceived Exercise (RPE) is more sensitive to measuring fatigue accumulation (Martin and Andersen, 2000). Therefore, studying the effect of tPCS on fatigue accumulation under long-term exercise can improve the application program of tPCS and expand its application scenarios, which has a positive significance for maintaining athletic performance (Kataoka et al., 2022).

According to the location of fatigue, it can be divided into central fatigue and peripheral fatigue; Its monitoring methods can be divided into behavioral, biochemical, and physiological categories, which measure the degree of fatigue from subjective and objective dimensions, respectively. The monitoring of behavioral indicators of fatigue refers to the completion of specific actions by subjects

according to established procedures (Li and Zhang, 2015; Qin, 2006), and the generation of actions is influenced by central fatigue - relying on neural activation to transmit signals; It is also affected by peripheral fatigue - relying on favorable external conditions of muscle tissue, such as sufficient energy supply or timely clearance of metabolites (Amann, 2011). This means that the completion of actions is influenced by both central fatigue and peripheral fatigue, therefore, the study analyzed the elimination effect of tPCS intervention on fatigue accumulation from both central and peripheral perspectives. Biochemical indicators are powerful evidence of peripheral fatigue (Qiu, 2022; Antunes et al., 2016), while testosterone (C), cortisol (T), and creatine kinase (CK) are often used in fatigue testing, and T/C is widely recognized as a sensitive indicator of fatigue status (Freitas et al., 2014; Selmi et al., 2022). Functional near-infrared spectroscopy (fNIRS) is a physiological fatigue monitoring method that uses non-invasive brain imaging technology to dynamically monitor brain blood oxygen signals to reflect the activation status of brain nerves. Insufficient oxygen delivery and/or low arterial oxygen pressure gradient in the brain can affect the diffusion of oxygen to the sarcomere and mitochondria, leading to central fatigue, FNIRS monitoring of cerebral blood oxygen can reflect central fatigue status (Amann and Calbet, 2008; Liu Jianxiu et al., 2016). Indeed, previous studies have shown a relationship between NIRS-related measures and measures of central fatigue (Ruggiero and McNeil, 2019). In behavioral indicators, reaction time and attention are commonly used to reflect fatigue status, and when fatigue occurs, reaction time becomes longer or attention decreases (Harrison et al., 2017). Previous studies have rarely analyzed the effects of tPCS on fatigue from three different fatigue monitoring perspectives, and the behavioral indicators are influenced by central fatigue or (and) peripheral fatigue (Liu T. et al., 2016; Carroll et al., 2017; Drummond et al., 2005). Therefore, we speculate that the changes in behavioral indicators induced by tPCS may be caused by peripheral fatigue or (and) central fatigue.

In summary, this study aims to explore the intervention effect of tPCS on fatigue accumulation after 7 days of moderate intensity training; And analyze from the perspective of fatigue generation mechanism which type of fatigue elimination effect tPCS has better, to guide the application of tPCS in practice; Simultaneously explore the fatigue pathway through which tPCS intervention promotes changes in behavioral indicators. Based on this, this study assumes that ① tPCS has a cumulative effect on the elimination of fatigue. ② tPCS has an eliminating effect on both types of fatigue, with a greater effect on central fatigue ③ The changes in behavioral indicators induced by tPCS are jointly caused by central fatigue and peripheral fatigue (Weng et al., 2016).

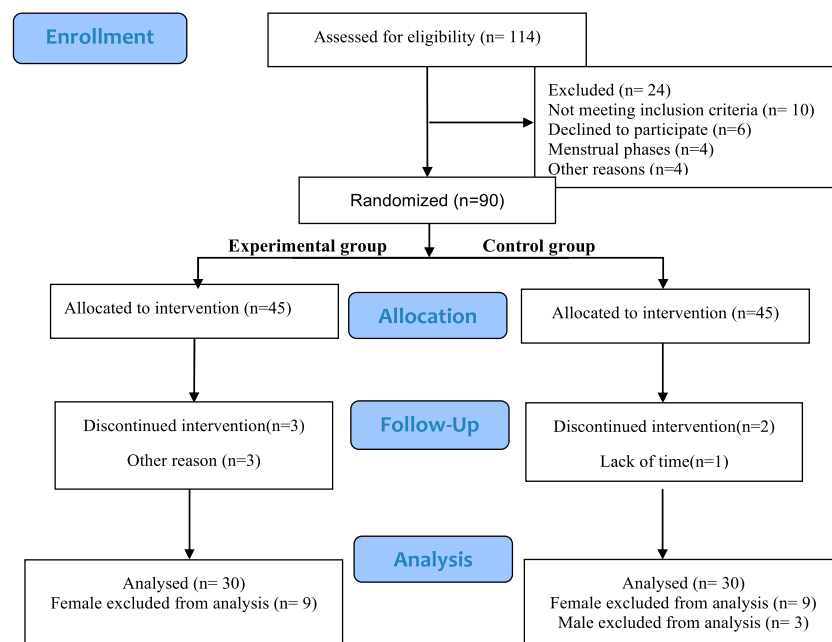


FIGURE 1
Inclusion subject flowchart.

2 Research object and methods

2.1 Ethics

All of the participants signed the informed consent form. The study was carried out in line with the Declaration of Helsinki and was approved by the Institutional Ethics Committee (16 November 2020 (no. 1) by Nantong University).

2.2 Research object

This study selected 90 healthy college athletes as participants, using a simple randomized scale method. Odd numbers were used as the experimental group, and even numbers were used as the control group. After multiple allocations, each group had 45 participants. After screening, 30 participants (all participants are male) from each group participated in the entire experimental process (Figure 1). The average age of the true stimulation group was 20.47 ± 0.72 years, with a training period of 4.12 ± 0.99 years and an average height of 177.89 ± 7.24 cm. The average weight is 73.97 ± 10.99 kg. The average age of the sham stimulation group was 20.63 ± 1.46 years, with a training period of 4.00 ± 0.75 years, an average height of 178.37 ± 6.40 cm, and an average weight of 70.58 ± 8.96 kg. All voluntary athletes participating in this study are ordinary college athletes with good physical condition and long-term running experience, without cardiovascular diseases or other illnesses, and have no injuries in the first 4 weeks. They did not engage in physical exercise in the week before the experiment and are all right-handed. During the experiment, the athletes did not participate in any activities that could lead to a decrease in physical fatigue (such as massage, or physical

therapy) or an increase (such as overtraining). Before the test, participants received explanations about transcranial pulse electrical stimulation, experimental objectives, and experimental procedures.

The sample size was calculated by G*Power 3.1. the ANOVA test method was selected. According to previous studies (Joundi et al., 2012; Pollok et al., 2015), the effect size of 0.6. With a error probability of 0.05 and power ($1-\beta$ error probability) of 0.8, the resulting sample size was 20. Considering potential dropouts, we recruited a bit more participants.

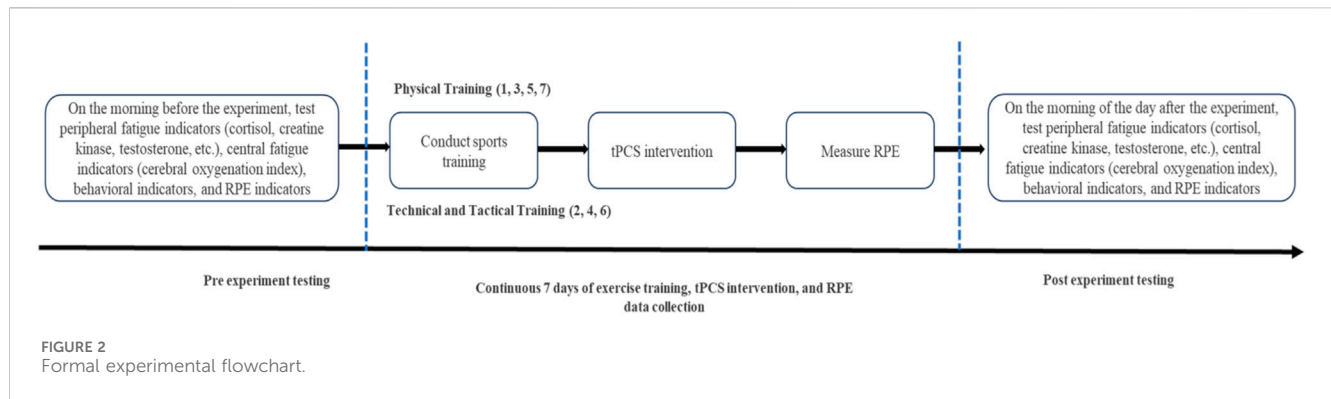
2.3 Experimental process

The 90 athletes in this study were randomly divided into two groups, Group A being the experimental group and Group B being the control group. All participants completed this study in two stages: the familiarization stage and the formal experiment stage. The familiarization stage requires participants to be familiar with all testing tasks, intervention processes, and indicator testing processes to reduce the impact of learning effects and physical discomfort on formal experiments.

The content of the familiarization section includes.

- 1) Familiarize oneself with sports training plans and grasp the key points during training;
- 2) Familiar with the instruments and testing indicators used in this research institute;
- 3) Finally, inform the subjects of precautions.

The flowchart of the formal experiment (Figure 2) shows that tPCS intervention is performed immediately after completing



exercise training every day, and RPE is measured after the intervention is completed.

2.4 Exercise plan

To fit the actual sports scenario, physical training and tactical training were alternated, and all participants used the moderate intensity training standard recommended by the American College of Sports Medicine (heart rate 140–150 beats per minute (ACSM., 2013). The athletes conducted a 1-week (7 days) training. The physical training tasks are conducted on the first day, third day, fifth day, and seventh day, the main training is for long-distance running. On the second, fourth day, and sixth days, technical and tactical training tasks were carried out, mainly for special technical action training. Group A and Group B are required to participate in training every day, with a training duration of approximately 3 h per day. During the main training period, the intensity was maintained at a heart rate of 140–150 beats per minute, and the RPE score exceeded eight points after training (Table 1).

The specific training plan is as follows:

Before training, the subjects are familiar with the RPE scale. The exercise trainer supervises the training and evaluates the exercise intensity of the subjects through the RPE scale and heart rate.

Every afternoon at 3 o'clock, the subjects start training. All participants wore Firstbeat Sports to monitor heart rate and collect RPE baseline before training. Subsequently, both Group A and Group B participants received warm-up runs and Pre-exercises preparation before starting formal training. The training content of Group A and Group B is shown in Figure 3.

2.5 Transcranial pulse current intervention plan

This study used a transcranial pulse current stimulation independently developed by the project team for intervention. To ensure the safety of all subjects, the intervention plan was based on the study by [Dissanayaka T et al. \(2020\)](#), with a stimulation intensity of 1.5 mA and a stimulation time of 20 min. Firstly, place a rectangular electrode piece with a size of (5 × 9) cm² in the center of the forehead, and place two rectangular electrode pieces with a size of (5 × 5) cm² at the bilateral papillae. Subsequently,

increase the current intensity to 1.5 mA within 30 s and continue for 20 min; During the stimulation process, participants are required to maintain an upright and static sitting position to avoid external interference; After the stimulation ends, adjust the current intensity to 0 mA within 30 s. All conditions for false stimulation are the same as true stimulation, except that after the initial acceleration reaches 1.5 mA, the operator readjusts the current to 0 mA. All conditions for false stimulation are the same as true stimulation, except that the operator readjusts the current to 0 mA after the initial acceleration reaches the target value. All operations were completed by the same personnel, and any discomfort experienced by the subjects during the operation should be promptly reported to the experimenters.

2.6 Experimental equipment

2.6.1 Transcranial pulse current stimulation (tPCS)

Developed by the National Key Technology R&D Program of China, the stimulation current is a bipolar current of 60–80 Hz, the pulse waveform is a square wave, the duty cycle is 29.7%, the stimulation intensity is in the range of 0–2 mA, and the stimulation time is determined by the experimental program. This product passed national security certification on 17 April 2021: report number CHTSM21040049.

2.6.2 Blood collection

We used 5 mL EDTA-anticoagulated vacutainer tubes to collect blood samples from the participants, which were produced by Lingen Precision Medical Products (Shanghai) Co., Ltd. (Shanghai, China).

2.6.3 Functional near-infrared spectroscopy

This study used an OctaMon + portable wireless near-infrared brain imaging system (Artinis, Netherlands) to collect Oxy Hb concentration data in the frontal lobe of the brain. OctaMon has a total of eight light source emission stages, generating 760 nm and 850 nm light waves, two light source detectors, and a sampling frequency of 50 Hz. According to the international 10–20 system brain electrode standard lead, the fNIRS channel position was registered with MNI spatial coordinates using a 3D locator and probability registration method. The fNIRS detector covered the frontal eye fields (FEF), dorsolateral prefrontal cortex (DLPFC), and orbitofrontal cortex (OFC), as shown in Figure 4.

TABLE 1 Training volume and training time.

Day	Total training distance/km	Main training Time/min	Auxiliary training Time/min	Borg
First day	20 km	138 min	42 min	8.53 ± 0.67
Second day	14 km	108 min	72 min	8.26 ± 0.44
Third day	22 km	144 min	36 min	8.80 ± 0.75
Fourth day	16 km	113 min	67 min	8.36 ± 0.60
Fifth day	18 km	124 min	56 min	9.00 ± 0.68
Sixth day	14 km	110 min	70 min	8.66 ± 0.65
Seventh day	18 km	130 min	50 min	8.93 ± 0.72

Note: Main training refers to formal running time and auxiliary training refers to technical movement preparation, simulation training, warm-up, and so on.

2.6.4 Behavioral testing instruments

We use instruments produced by the Science and Education Instrument Factory of East China Normal University. The model of the reaction time tester is EP202.203, and the model of the attention concentration tester is EP701C.

2.6.5 RPE questionnaire

Using the RPE 10-level scale, the higher the RPE score, the greater the level of fatigue (Zhu et al., 2020).

2.7 Testing indicators

All data collection was conducted using a sitting position test. The physiological indicators of oxygenated hemoglobin (Oxy-Hb) concentration, deoxyhemoglobin (HHb), total hemoglobin (HbTot) concentration, and hemoglobin difference (HbDiff) were measured by the OctaMon + portable wireless near-infrared brain imaging system; Biochemical indicators such as testosterone (T), cortisol (C), testosterone/cortisol (T/C), and creatine kinase (CK) were measured by a fully automated biochemical analyzer (RaytoRT-6100 China); The reaction time (RT) of behavioral indicators was measured by the EP202.203 reaction time tester produced by the Science and Education Instrument Factory of East China Normal University, and attention was measured by the EP701C attention concentration tester of East China Normal University.

2.8 Data collection

2.8.1 Collection of physiological indicators

First, nirsLAB v. 2013.1 software (Version 14, Revision 2, NIRx Medizintechnik GmbH, Berlin, Germany) was used to convert the collected original data into MATLAB format data. Then, the differential function DIFF in MATLAB was used to observe whether there was a horizontal signal in the signal. If there were more than 25% invalid horizontal signals in the signal, we excluded the signal of this channel. Then, Butterworth filtering was used to reduce the interference of high-frequency noise (0.3 Hz respiration and 1 Hz heart rate) and low-frequency noise (metabolic tremor of less than 0.01 Hz) in the signal and improve the signal-to-noise ratio

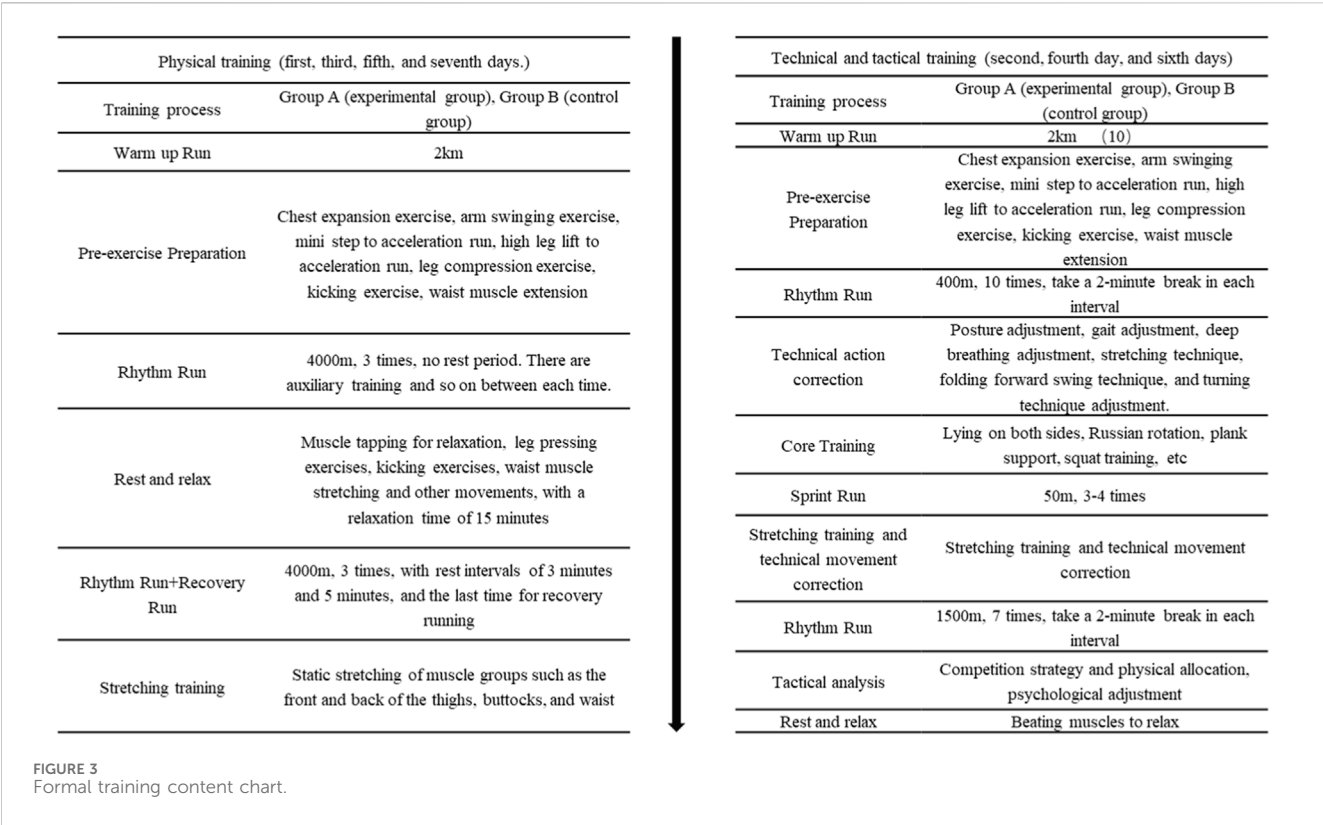
(BORG, 1988). Using the principal component analysis method proposed by Yücel (Yücel et al., 2014) to remove movement artifacts, the calculated monitoring time of the oxygenated hemoglobin concentration was 3 min. Finally, the oxygenated hemoglobin concentration data of all subjects were calculated, and the change in the prefrontal lobe oxygenated hemoglobin concentration (Oxy-Hb) was calculated according to the improved Beer–Brown law. Collection of Biochemical Indicators.

2.8.2 Collection of Biochemical Indicators

In this experiment, blood was collected twice, the day before and the day after the experiment. After the subjects reached the blood collection room (the temperature is 20°C), they sat quietly and rested for 10 min, and then the medical staff took 4 mL of left elbow venous blood and collected the blood sample in a vacuum blank tube to avoid shaking and vibration of the contents (blood). Within 30 min, serum was separated using a high-speed centrifuge (2000 R/min, 15 min. Shu Ke, China). The supernatant was extracted and stored in a medical refrigerator at −80°C (Boko, BDF-86V158, China). CK, T, and C were detected using ELISA kits (Wuhan Jianglai Biotechnology Co., Ltd. (Wuhan, China), according to the manufacturer's instructions, and the whole process was supervised by a principal investigator. All samples were sent to the laboratory for biochemical analysis within 48 h, and all analyses were repeated and performed by trained technicians. Serum samples were analyzed using an automatic biochemical analyzer (RaytoRT-6100 China). Serum CK, T, and C levels were recorded, and the serum T/C ratio was calculated.

2.8.3 Collection of behavioral indicators

The RT tester (EP202.203) produced by Shanghai East China Normal University Science and Education Instrument Co., Ltd. was applied to compare the choice RT of the subjects under light stimulation of four colors (red, green, yellow, and blue). By the way, a four-hole photoelectric contactless response key acted as the response component of the subjects, with 20 stimuli per test (5 times for each color). At the beginning of the test, the instrument automatically and randomly presented the four-color light stimulus. According to the presentation of the light stimulus, the fingers of the subjects left the middle position of the response keyboard and pressed the circular hole of the corresponding color. The instrument automatically recorded the time between

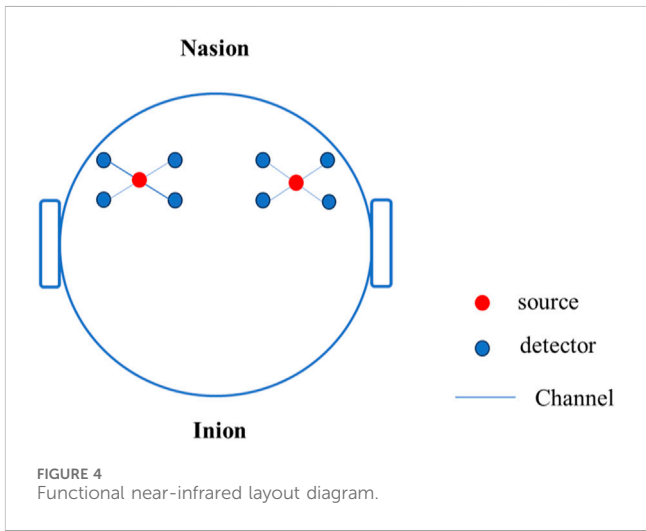


stimulus presentation and the fingers of subjects entering the circular hole of the corresponding response keyboard, and the mismatched color that subjects pressed would automatically be processed as an error by the instrument, and the corresponding time was not counted in the statistics. After completion of 20 tests, the buzzer in the instrument automatically sounded for 1 s for a hint. Finally, the total average RT was recorded (Deng et al., 2013).

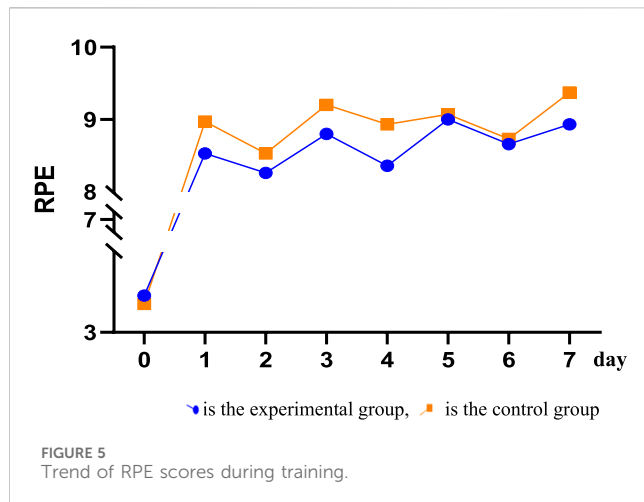
An attention concentration tester (EP701C) produced by Shanghai East China Normal University Science and Education Instrument Co., Ltd. was adopted for the examination of the attention concentration ability of individuals. To be specific, the instrument was placed on a table about 1.30 m high, and then the subjects held the induction handle with a handedness to trace the white sign of rotation in the hexagonal track. Before measurement, the instrument was adjusted in advance, the movement speed of the white mark was set as 30 r/min, and the test time as 20 s. The instrument automatically recorded the onattention and off-attention after completion of the test. The test rules were explained first, then the subjects were allowed to practice once and measure once (Li et al., 2017).

2.9 Statistical analysis

All data were analyzed using SPSS 25.0 and graphically plotted using Graph Pad Prism 8.0 software. Firstly, perform a normal distribution test on the data, and use Mann Whitney U test for data that does not conform to a normal distribution. Using stimulus conditions (Group A, Group B) as inter-group variables and time (pre-test, post-test) as intra-group variables, a two factor repeated



measures analysis of variance was conducted to investigate the effects of each variable on physiological, biochemical, and behavioral indicators. The effect size was represented by a bias of η^2 . Use independent sample t-test to analyze the RPE differences between the two groups. Pearson bilateral correlation test was used to analyze the correlation between various indicators, and linear regression was used to explore the contributions of central fatigue and peripheral fatigue to behavioral changes. The data for statistical testing is expressed as ($M \pm SD$), with a 95% confidence interval. $P < 0.05$ indicates significant differences, while $P < 0.01$ indicates very significant differences.



3 Results

3.1 Daily RPE score after training

After receiving tPCS intervention, all subjects underwent RPE score recording. The results showed that the daily RPE score exceeded eight points, and the RPE score after physical training was higher than 8.5 points, while the RPE score after technical and tactical training was less than 8.5 points. The independent sample T-test was used to analyze the RPE values of the two groups. The results showed that there were significant differences in RPE scores between the two groups on the first day, the third day, the fourth day, and the seventh day. The RPE scores of the control group were higher than those of the experimental group (Figure 5; Table 2).

3.2 Baseline data results before tPCS intervention

After completing exercise training, all participants measured their baseline values and conducted independent sample t-test analysis. It was found that there was no significant difference between Group A and Group B in physiological, biochemical, and subjective indicators (Table 3).

3.3 Changes in various indicators after tPCS intervention

3.3.1 Physiological indicators

After completing the training, participants underwent tPCS intervention. Two-way repeated measures ANOVA showed significant main effects for time on Oxy-Hb [$F(1,58) = 10.95$, $p = 0.00$, $\eta^2 = 0.16$], HHb [$F(1,58) = 4.89$, $p = 0.03$, $\eta^2 = 0.08$], HbTot [$F(1,58) = 14.54$, $p < 0.01$, $\eta^2 = 0.20$], and HbDiff [$F(1,58) = 8.89$, $p < 0.01$, $\eta^2 = 0.13$].

Post-hoc tests revealed that the experimental group showed a decrease in Oxy-Hb after intervention, with no significant differences compared to pre-intervention or the control group post-intervention. In contrast, the control group showed a

significant decrease in Oxy-Hb after intervention compared to pre-intervention ($p < 0.01$). The experimental group also exhibited increases in HHb, HbTot, and HbDiff post-intervention, with no significant differences compared to pre-intervention or the control group. The control group showed significant increases in HHb, HbTot, and HbDiff post-intervention compared to pre-intervention ($p < 0.01$) (see Table 5).

3.3.2 Biochemical indicators

After completing the training, participants underwent tPCS intervention. Two-way repeated measures ANOVA revealed significant interaction effects for cortisol, T/C ratio, and CK across stimulation conditions and time, specifically C [$F(1,58) = 14.48$, $p < 0.01$, $\eta^2 = 0.20$], T/C [$F(1,58) = 16.40$, $p < 0.01$, $\eta^2 = 0.22$], and CK [$F(1,58) = 5.17$, $p = 0.03$, $\eta^2 = 0.08$]. Significant main effects of time were observed for T [$F(1,58) = 9.33$, $p < 0.01$, $\eta^2 = 0.14$], C [$F(1,58) = 97.97$, $p < 0.01$, $\eta^2 = 0.63$], T/C [$F(1,58) = 15.68$, $p < 0.01$, $\eta^2 = 0.21$], and CK [$F(1,58) = 90.63$, $p < 0.01$, $\eta^2 = 0.61$]. The main effect for stimulation condition was also significant for C [$F(1,58) = 4.14$, $p = 0.05$, $\eta^2 = 0.07$].

Post-hoc tests indicated that the experimental group showed no significant differences in T and T/C after intervention compared to pre-intervention, but significant differences were noted compared to the control group ($p < 0.05$). The control group showed significant decreases in T and T/C after intervention ($p < 0.01$). Conversely, the experimental group exhibited significant increases in C and CK after intervention compared to pre-intervention and to the control group ($p < 0.01$), while the control group also showed significant increases in C and CK post-intervention ($p < 0.01$) (Tables 5, 6) ($p < 0.01$) (Tables 4, 5).

3.3.3 Behavioral indicators

After completing the training, participants underwent tPCS intervention. Two-way repeated measures ANOVA revealed a significant interaction effect of RT across stimulation conditions and time [$F(1,58) = 5.32$, $p = 0.03$, $\eta^2 = 0.08$]. There were significant main effects for time on RT [$F(1,58) = 25.18$, $p = 0.00$, $\eta^2 = 0.30$] and on target time [$F(1,58) = 43.36$, $p < 0.01$, $\eta^2 = 0.44$]. The main effect of the stimulation condition was significant for target time as well [$F(1,58) = 4.36$, $p = 0.04$, $\eta^2 = 0.07$].

Post-hoc tests indicated that the experimental group showed a significant increase in RT after the intervention compared to before and the control group. The control group also had a significant increase in RT post-intervention compared to pre-intervention. Conversely, the experimental group experienced a significant decrease in target time after the intervention compared to before and to the control group, while the control group also showed a significant decrease in target time post-intervention compared to pre-intervention ($P < 0.01$) (Tables 4, 5).

3.4 Correlation analysis of various indicators after tPCS intervention

Pearson correlation analysis revealed significant relationships between physiological, and biochemical indicators, and reaction time following tPCS intervention. In Group A, reaction time was significantly negatively correlated with T, T/C, and Oxy-Hb, and

TABLE 2 RPE scoring checklist.

Group	Baseline	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day
Experimental group (A)	4.07	8.53	8.26	8.80	8.36	9.00	8.66	8.93
Control group (B)	3.83	8.97	8.53	9.20	8.93	9.07	8.73	9.37
t	0.40	−2.70	−1.70	−2.10	−3.23	−0.36	−0.38	−2.47
p	0.69	0.01	0.09	0.04	0.00	0.72	0.70	0.02
RPE difference	−0.24	0.44	0.27	0.40	0.57	0.07	0.07	0.44

TABLE 3 Test results of various indicators before tPCS intervention.

Indicators	Group A	Group B	t	p
	M ± SD	M ± SD		
Oxy-Hb	−6.88 ± 8.189	−7.50 ± 7.23	0.31	0.76
HHb	4.06 ± 2.73	4.46 ± 2.24	−0.62	0.54
HbTot	12.77 ± 9.74	12.91 ± 8.34	−0.06	0.95
HbDiff	3.37 ± 5.19	3.62 ± 5.01	−0.19	0.85
T (nmol/L)	5.68 ± 0.71	5.57 ± 0.59	0.61	0.55
C (nmol/L)	255.11 ± 31.10	245.70 ± 18.05	1.43	0.16
T/C	0.07 ± 0.01	0.07 ± 0.00	−1.67	0.10
CK(ng/mL)	80.51 ± 10.38	79.11 ± 11.75	0.49	0.63
RT	0.37 ± 0.10	0.37 ± 0.13	−0.23	0.82
attention	14.14 ± 1.64	14.01 ± 1.46	−0.32	0.75

TABLE 4 Test results of various indicators after tPCS intervention.

Indicators	Group A	Group B	t	p
	M ± SD	M ± SD		
Oxy-Hb	−10.90 ± 8.95	−13.28 ± 10.08	0.97	0.34
HHb	5.07 ± 5.50	6.86 ± 5.75	−1.24	0.22
HbTot	18.30 ± 11.10	20.98 ± 13.43	−0.84	0.40
HbDiff	6.29 ± 0.66	6.82 ± 7.60	−0.29	0.78
T (nmol/L)	5.49 ± 0.71	5.15 ± 0.55	2.16	0.04
C (nmol/L)	287.02 ± 27.15	317.46 ± 34.94	−3.77	0.00
T/C	0.06 ± 0.01	0.05 ± 0.00	3.62	0.00
CK(ng/mL)	95.04 ± 10.81	102.77 ± 11.39	−2.69	0.01
RT	0.41 ± 0.06	0.48 ± 0.103	−0.07	0.00
attention	12.33 ± 1.96	11.12 ± 2.177	1.21	0.03

positively correlated with HHb, HbTot, and HbDiff. T was significantly positively correlated with T/C and Oxy-Hb, and negatively correlated with HHb, HbTot, and HbDiff. In Group B, reaction time was significantly negatively correlated with T, T/C, and Oxy-Hb, and positively correlated with HbDiff. T was

significantly positively correlated with T/C and Oxy-Hb, and negatively correlated with HHb (Table 6).

3.5 Regression analysis of central fatigue and peripheral fatigue on response time

A linear regression analysis was conducted with central and peripheral fatigue as independent variables and reaction time as the dependent variable. The results indicated a significant negative effect of central fatigue on reaction time in both groups A and B following tPCS intervention, with group A contributing 92.7% to the effect and group B contributing 86.1% (Table 7).

4 Discussion

This study investigates the intervention effects of tPCS on exercise-induced fatigue accumulation, and analyze which type of fatigue has a greater intervention effect from both central and peripheral perspectives. The results indicate that the fatigue levels of both groups increased after training, with significant differences in RPE scores compared to baseline. Following tPCS intervention, the experimental group exhibited lower RPE scores than the control group, but with no significant differences between the two groups. This suggests that while natural recovery can delay the rise in fatigue, tPCS intervention is more effective in delaying this increase, supporting Hypothesis 1. Analysis of oxygenated hemoglobin and routine biochemical markers revealed that tPCS impacts both central fatigue and peripheral fatigue, with a stronger effect on central fatigue, confirming Hypothesis 2. Regression results further indicate that the impact of tPCS on exercise behavior is primarily mediated through its effects on central fatigue.

4.1 Central evidence of the effect of tPCS on fatigue

The results of fNIRS showed that after daily exercise, the physiological indicators Oxy Hb, HHb, HbTot, and HbDiff in the experimental group were lower than those in the control group. Among them, Oxy Hb, HHb, and HbDiff showed no significant changes compared to before the intervention. This indicates that using tPCS intervention after continuous exercise can maintain the blood oxygen status in the brain and delay the deepening of central fatigue.

TABLE 5 Test results of various indicators before and after tPCS intervention.

Indicators	Group	T	p	Chang rate	Change rate difference
Oxy-Hb	A	4.01	0.06	58.43%	18.64%
	B	5.78	0.00	77.07%	
HHb	A	-1.09	0.36	24.88%	28.93%
	B	-2.40	0.03	53.81%	
HbTot	A	-5.52	0.03	43.30%	19.21%
	B	-8.07	0.00	62.51%	
HbDiff	A	-2.92	0.05	86.65%	1.75%
	B	-3.21	0.03	88.40%	
T (nmol/L)	A	0.17	0.20	-3.35%	4.20%
	B	0.41	0.00	-7.54%	
C (nmol/L)	A	-31.91	0.00	12.51%	16.70%
	B	-71.76	0.00	29.21%	
T/C	A	0.00	0.95	-12.01%	14.94%
	B	0.00	0.00	-26.95%	
CK(ng/mL)	A	-14.53	0.00	18.05%	11.86%
	B	-23.53	0.00	29.91%	
RT	A	0.04	0.02	10.81%	18.92%
	B	-0.11	0.02	29.73%	
attention	A	1.81	0.00	-12.80%	7.83%
	B	2.89	0.00	-20.63%	

Note: The calculation method for the difference in rate of change $R = \left| \frac{B_1 - B_2}{B_1} - \frac{A_1 - A_2}{A_1} \right|$; A1 = numerical value after intervention in Group A; A2 = pre intervention value in Group A; B1 = value after intervention in Group B; B2 = pre intervention value for Group B.

In fact, the high or low oxygen content in the brain has been regarded as an important mechanism for the generation and prevention of central fatigue (Nybo and Rasmussen, 2007), and changes in cerebral blood oxygen reflect the activation status of the brain and the degree of central fatigue (Rasmussen et al., 2007). During brain activation, the excitability of neural activity increases, and local brain tissue blood flow, blood volume, and blood oxygen consumption all increase, but the proportion of increase varies. Oxygen consumption only slightly increases, and the increase in blood flow exceeds the increase in oxygen consumption, this difference leads to an increase in oxy-Hb concentration and a decrease in HHb concentration in the brain activation functional area (Hoshi, 2003). This is inconsistent with the results of this study, mainly because the fatigue of the subjects in this study has not been completely eliminated, and fatigue has accumulated after exercise. Therefore, the increase in oxygen consumption exceeds the increase in blood oxygen flow, manifested as a decrease in oxy-Hb concentration and an increase in HHb concentration in indicators; After tPCS intervention, although the concentration of oxy-Hb decreased and HHb increased, the difference in physiological changes in the experimental group was smaller than that in the control group, indicating that tPCS can delay the deepening of central fatigue.

Saavedra et al.'s study showed that tPCS stimulation in the frontal lobe can improve the excitability of the cerebral cortex, strengthen the connections between brain regions, and improve the blood oxygen transport capacity of the stimulated region (Saavedra et al., 2014), which to some extent explains the results of this study. In this study, both groups of subjects showed a decrease in blood oxygen concentration after exercise, but the group treated with tPCS intervention had a lower degree of decrease and no significant change compared to baseline. The reason may be that the subjects of this study received continuous 7 days of exercise training, and the physiological activation of the brain increased with the increase of exercise. Strong neuronal activity caused the demand for blood oxygen to exceed the transport of blood oxygen (Ding, 2012), leading to central fatigue. As time accumulated, the degree of fatigue increased, and the effect of tPCS on increasing the concentration of oxygenated hemoglobin was limited, Interventions that increase cerebral blood flow supply to maintain brain function can only alleviate central fatigue and cannot achieve the effect of eliminating central fatigue. The study by Inglese M et al. also supports this result. Inglese M found through his study of cerebral perfusion that as fatigue increases, cerebral blood flow decreases, and increasing blood flow supply can alleviate fatigue (Inglese et al., 2007).

TABLE 6 Correlation analysis of various indicators after tPCS intervention.

Group	Indicators	RT	Attention	T	C	T/C	CK	Oxy-Hb	HHb	HbTot	HbDiff
A	RT	1	0.02	−0.88**	0.03	−0.64**	−0.20	−0.96**	0.48**	0.46*	0.43*
	attention		1	−0.02	−0.13	0.07	0.015	−0.04	−0.17	−0.14	−0.08
	T			1	0.07	0.66**	0.09	0.88**	−0.54**	−0.48**	−0.42*
	C				1	−0.69**	0.12	−0.09	−0.08	−0.09	−0.01
	TC					1	−0.01	0.67**	−0.34	−0.28	−0.28
	CK						1	0.13	−0.07	−0.07	0.04
	Oxy-Hb							1	−0.55**	−0.48**	−0.39*
	HHb								1	0.83**	0.57**
	HbTot									1	0.88**
	HbDiff										1
B	RT	1	0.05	−0.58**	−0.32	−0.62**	0.29	−0.87**	0.05	−0.19	−0.39*
	attention		1	−0.09	0.22	−0.10	0.25	0.01	−0.06	−0.09	0.00
	T			1	0.24	0.96**	−0.32	0.86**	−0.38*	−0.33	0.10
	C				1	0.23	−0.07	0.35	0.02	−0.10	−0.08
	TC					1	−0.24	0.89**	−0.36	−0.22	0.17
	CK						1	−0.30	0.04	0.11	0.09
	Oxy-Hb							1	−0.25	−0.06	0.30
	HHb								1	0.52**	0.41*
	HbTot									1	0.054**
	HbDiff										1

Note: **P < 0.01,*P < 0.05.

4.2 Peripheral evidence of the effect of tPCS on fatigue

After 7 days of moderate-intensity training, the T and T/C values in the biochemical indicators of the two groups of subjects decreased, while the C and CK values increased, and peripheral fatigue significantly increased. The change rate of group indicators after tPCS intervention was lower than that of the control group, indicating that using tPCS intervention after moderate-intensity training can significantly delay the accumulation of peripheral fatigue. [Ma and Gao et al. \(2021\)](#) found in their study that during continuous high-volume training, the concentrations of CK and C increased over time, while T and T/C showed a significant decrease, which is consistent with the findings of this study. This study adopted a combination of moderate-intensity physical training and technical tactics and found that within a week, the T and T/C values of both groups of subjects were decreasing, while the C and CK values were increasing. This means that after 7 days of training, the physical functions of the subjects decreased and fatigue levels deepened. However, after intervention with tPCS, the changes in biochemical indicators in the experimental group were smaller than those in the control group, indicating that tPCS has the effect of delaying the deepening of fatigue. As [Ma and Gao et al. \(2021\)](#) continued their training, it was found that C and CK values showed a wave-like decrease, while T

and T/C showed an increasing trend. However, further research is needed to determine whether continuous tPCS intervention can further delay fatigue and even improve subject status. There are also scholars whose research results are different from this study. [Ma Haihao's](#) research shows that after a week of high-altitude training, the T and T/C values in athletes' blood significantly increase compared to a week ago, while the C value first increases and then decreases. The reason for the different results may be that the high-altitude environment increases the basal metabolic rate of the body, promotes protein breakdown, improves the body's functional status, and prevents the generation of peripheral fatigue ([Ma et al., 2023](#)).

In this study, the stimulation site of tPCS was located in the frontal lobe, and the pulse characteristics of tPCS current can stimulate deep brain nuclei. Therefore, tPCS may regulate the stimulation of the frontal lobe from top to bottom, ultimately causing changes in testosterone and cortisol ([Saavedra et al., 2014](#); [Datta et al., 2013](#); [Vasquez et al., 2017](#)). Scholars have found in their studies that using transcranial direct current stimulation to the dorsolateral prefrontal cortex (DLPFC) or C3 (brain localization based on the 10–20 EEG system) can reduce cortisol concentration ([Raimundo et al., 2012](#); [Moreno-Duarte et al., 2014](#)).

Both tPCS and tDCS belong to transcranial electrical stimulation, with certain similarities in the target area and effect

TABLE 7 Regression analysis of central and peripheral fatigue indicators on response time after tPCS intervention.

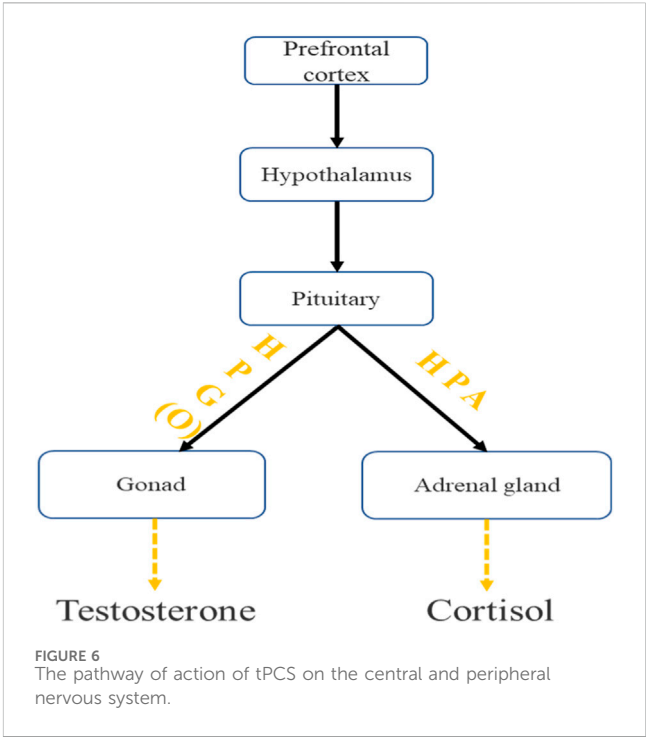
Group	Indicators	RT			
		SE	B	t	P
A	T	0.13	0.21	0.60	0.56
	C	5.68	−0.39	−1.19	0.25
	T/C	0.00	−0.46	−1.10	0.28
	CK	1.90	−0.08	−1.45	0.16
	Oxy-Hb	1.50	−0.90	−7.00	0.00
	HHb	0.50	−0.10	−0.79	0.44
	HbTot	1.78	−0.12	−0.57	0.57
	HbDiff	0.95	0.20	1.35	0.19
	R ²	0.93			
	F	47.19			
B	T	0.11	0.07	0.24	0.82
	C	3.30	0.04	0.54	0.59
	T/C	0.00	0.60	1.91	0.07
	CK	2.14	0.04	0.46	0.65
	Oxy-Hb	1.32	−1.49	−7.98	0.00
	HHb	0.41	−0.02	−0.22	0.83
	HbTot	1.52	−0.12	−1.15	0.27
	HbDiff	0.92	0.01	0.12	0.90
	R ²	0.86			
	F	23.43			

of stimulation, while the production of testosterone and cortisol is controlled by the hypothalamic-pituitary gonadal axis (HTPG) (Barbas et al., 2003), and there is a wide functional connection between the frontal lobe and the hypothalamus (Price et al., 2005). Therefore, We speculate that the pulse current of tPCS may affect the activity of HTPG through the frontal cortex (Shekhar et al., 2003; Shaffer et al., 2014), thereby promoting testosterone secretion, delaying the decrease in testosterone concentration, and ultimately maintaining individual motor behavior (Figure 6).

Moreover, the results of this study showed that the indexes T of peripheral fatigue in the two groups were highly correlated with the indexes Oxy-Hb and HbTot of central fatigue. This may suggest that there is an inherent connection between central fatigue and peripheral fatigue, but further physiological and biochemical experiments are needed to clarify their clear pathways of action.

4.3 The impact of fatigue on behavioral indicators

After exercise training, the behavioral indicators of both groups showed significant changes compared to before the tPCS intervention; Compared with the control group, the rate of



change in the experimental group was lower, in which the difference of the change rate at reaction time was 18.92%, and the difference of the change rate at attention was 7.83%. This suggests that tPCS intervention after continuous moderate-intensity exercise can delay the rise in response time and the fall in target time after fatigue to some extent. Through correlation analysis, it was found that response was significantly correlated with both central fatigue and peripheral fatigue, while attention was not significantly correlated with both central fatigue and peripheral fatigue.

The reaction time indicates the speed of information processing in the central nervous system of the brain. When the central nervous system accelerates information transmission, the reaction time is shortened. Many scholars have found that the increase in cerebral perfusion will lead to an increase in central neuron activation, especially the increase of blood oxygen content can be reflected in the transmission speed of central nervous system signals, and the response time will be shortened when the central nervous system is improved (Lucas et al., 2012a; Lambbrick et al., 2016; Lucas et al., 2012b) demonstrated this viewpoint through experiments that when cerebral blood flow in the frontal lobe increases, reaction time improves, which is consistent with the results of this study. This study found that after tPCS intervention, there was a significant negative correlation between the Oxy-Hb value and reaction time, the larger the Oxy-Hb value, the shorter the reaction time. Similar situations exist between attention and cerebral perfusion. Marshall et al. (2001) found in their study that after a decrease in cerebral blood flow, attention was impaired by 40%. However, in this study, no relationship was observed between target time and blood oxygen status. In fact, some scholars have proposed that both central fatigue and peripheral fatigue can affect reaction time and attention to a certain extent, but the degree of impact varies. Lin and Liu, (2014) found in long-term exercise-induced central fatigue that central

fatigue prolongs reaction time; When peripheral fatigue occurs, it can reduce muscle strength reserves, inhibit the body's athletic ability, and also affect reaction time (Skinner et al., 2005). Moreover, peripheral fatigue often occurs at the neuromuscular junction and muscle fibers (Ament and Verkerke, 2009; Maclaren et al., 1989), and the accumulation of fatigue metabolites can affect neuromuscular performance, leading to decreased motor performance (Chen et al., 2022). Neuromuscular fatigue can also affect motor control and proprioception, reducing the body's decision-making ability and reaction time (Borotikar et al., 2008). Due to the involvement of nerves and muscles in both reaction time and attention, both central fatigue and peripheral fatigue can affect the motor behavioral characteristics of this study. This is inconsistent with the results of this study, possibly because the central nervous system involvement required to complete reaction time and attention behavior is greater than the involvement of peripheral muscles. In this experiment, the accumulation of peripheral fatigue occurred more frequently in the lower limb major muscle group during exercise, with a lower correlation with the upper limb minor muscle group completing these two types of sports behaviors; Therefore, the correlation between peripheral fatigue and sport behavior in this study is not significant.

Peripheral fatigue contributes relatively more to the decrease in muscle activity after short-term high-intensity exercise, while central fatigue contributes relatively more in long-term moderate-intensity exercise; The elimination of peripheral fatigue depends more on time, while the elimination of central fatigue is closely related to external intervention (Carroll et al., 2017). Central fatigue occurs in the central nervous system, usually due to obstacles in the transmission of neural signals. The main mode of regulation is neural regulation, which is characterized by accuracy and speed (Guo et al., 2023); Peripheral fatigue includes neuromuscular junctions, peripheral nerves, muscle cell membranes, calcium release mechanisms, and sliding filaments, mainly regulated by humoral regulation, which is characterized by broad and slow regulation (Ament and Verkerke, 2009; Wu et al., 2022). Therefore, tPCS improved the cerebral blood oxygen status and delayed the degree of central fatigue by directly intervening in the frontal lobe. However, the intervention of tPCS cannot directly affect peripheral biochemical indicators and must be regulated through neurohumoral regulation. Therefore, the effect of tPCS on central fatigue is greater than that on peripheral fatigue (Liu T. et al., 2016).

Regression analysis shows that the coefficient of determination of central fatigue on reaction time is over 80%, the R^2 is 0.93 in the experimental group and 0.86 in the control group. This indicates that changes in reaction time are mainly influenced by central fatigue, and after tPCS intervention, the impact of central fatigue on reaction time further increases.

The research of Liu Jianxiu et al. (2016) supports the results of this experiment. He believes that reaction time can be used as a behavioral indicator to evaluate central fatigue. Reaction time mainly reflects the flexibility of brain neural activity. When fatigue occurs, phenomena such as decreased brain flexibility, limited individual activity level, and delayed response may occur.

The central nervous system is sensitive to factors such as arterial oxygen partial pressure, arterial oxygen content, and arterial oxygen saturation, which together affect brain function (Calbet et al., 2003). In this study, tPCS is stimulated in the frontal lobe of the brain, which

has a great effect on the central nervous system. Therefore, the impact of tPCS on response time is mainly achieved by changing the central fatigue state. Rasmussen et al. (2007) studied the effect of brain oxygenation on motor behavior activation through the contraction of finger static maximum isometric autonomous grip strength, they found that the decrease in finger grip level may not only be caused by peripheral neuromuscular factors but also by the imbalance of brain blood oxygen transport and demand caused by continuous exercise. When the brain's blood oxygen concentration decreases, it inhibits the activity of efferent neurons, ultimately, the individual's motor behavior performance was reduced, and the intervention of tPCS activated neurons, so the sport behavior can be improved. Kataoka et al. (2022) studied from the perspective of exercise programs and proposed that peripheral fatigue contributed more to the reduction of muscle contraction during short-duration, high-intensity exercise, while central fatigue contributed more to the reduction of muscle contraction during medium-intensity exercise with a longer duration. In this study, the subjects engaged in long-term moderate-intensity exercise, so we believe that under the intervention of tPCS, the changes in reaction time are mainly influenced by central fatigue. The study by Goodall et al. (2010) also showed that central fatigue caused by cerebral oxygenation can inhibit motor drive in the corticospinal cord, leading to changes in sports behavior. This provides a potential logic that central fatigue can affect sports behavior, which is consistent with the results of this study.

5 Limitations of research

- 1 This study lacks a clear mathematical relationship between central fatigue and peripheral fatigue, and cannot analyze the intrinsic relationship between central fatigue and peripheral fatigue in more detail. In fact, due to the widespread presence of the nervous system in the central and peripheral regions, central fatigue and peripheral fatigue have their own characteristics, but there is also a certain degree of intersection. However, distinguishing this independent yet unified relationship is a worthwhile and challenging task.
- 2 This study did not link sports or tasks with central and peripheral fatigue, so the application of tPCS in specific competitions is not clear enough and lacks detailed application guidelines.

6 Conclusion

- 1 tPCS intervention can delay the development of central fatigue and peripheral fatigue.
- 2 tPCS intervention has a greater effect on central fatigue than peripheral fatigue.
- 3 The effect of tPCS intervention on response time was mainly achieved by changing the central fatigue state.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Nantong University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

Q-CW: Writing–original draft, Writing–review and editing. SL: Data curation, Investigation, Methodology, Writing–review and editing. CW: Data curation, Investigation, Writing–review and editing. JL: Conceptualization, Methodology, Project administration, Writing–review and editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

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RECEIVED 29 October 2024

ACCEPTED 30 January 2025

PUBLISHED 25 February 2025

CITATION

Mengistu FA, Lake YA, Andualem ME,
Miherete YD and Zewdie SA (2025) Impact of
aerobic, resistance, and combined training on
cardiometabolic health-related indicators in
inactive middle-aged men with excess body
weight and obesity.
Front. Physiol. 16:1519180.
doi: 10.3389/fphys.2025.1519180

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Impact of aerobic, resistance, and combined training on cardiometabolic health-related indicators in inactive middle-aged men with excess body weight and obesity

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Methods: Twenty physically inactive men (49.15 ± 2.581 years) and BMI with 27.66 ± 0.91 , participated in an 8-month training programme involving concurrent exercise (CT), resistance training (RT), and aerobic training (AT) program to determine the effects on fasting blood glucose (FBG), insulin resistance (IR), blood pressure (BP) and waist-to-hip ratio (WHR) in overweight and obese adult persons. This study was used a randomized repeated measures parallel experimental design.

Results: Pre-to-post mean values of FBG, IR, SBP, DBP and WHR significantly decreased. Exercise modality had a significant effect on FBG ($F(2, 26) = 10.656$, $p = 0.001$, $\eta^2 = 0.571$), with RT and CT showing greater reductions than AT. IR decreased more in RT than in AT ($MD = 0.410 \pm 0.101$, $p = 0.03$). SBP also varied significantly between modalities ($F(2, 26) = 13.103$, $p = 0.02$, $\eta^2 = 0.528$), with CT and RT showing larger reductions than AT. WHR differed significantly ($F(2, 16) = 18.175$, $p = 0.001$, $\eta^2 = 0.694$), with AT and CT showing more reductions than RT. Diastolic blood pressure (DBP) showed no significant effect from exercise modality.

Conclusion: These findings highlight the importance of tailored exercise interventions, with short rest RT and CT emerging as the most effective method for inactive overweight and obese individuals.

KEYWORDS

fasting blood glucose, insulin resistance, resistance training, aerobic training, concurrent training

1 Introduction

A significant public health concern is the increasing prevalence of adult obesity and related conditions. Obesity is a condition in which an abnormal or excessive accumulation of fat poses a health risk. The highest prevalence of obesity was 44.3% among middle-aged adults (40–59 years old) (Boutari and Mantzoros, 2022). The combined estimates of the prevalence of obesity and overweight in Middle Eastern countries are 21.17% and 33.14%,

respectively (Okati-Aliabad et al., 2022). Like in many other countries, overweight and obesity are becoming increasingly prevalent public health issues in Ethiopia (Kassie et al., 2020). It is important to prioritize strategies that can help reduce the healthcare costs associated with obesity. Researchers strongly suggest that interventions improving both blood sugar control and cholesterol levels would be highly effective in preventing cardiovascular diseases (De Backer et al., 2003).

As highlighted in the 2025 ACSM Worldwide Fitness Trends, the Exercise is Medicine (EIM) initiative emphasizes incorporating physical activity into routine healthcare practices. Since its debut on the trends list in 2017, EIM has consistently ranked among the top 20 trends, reflecting its enduring relevance and impact (Newsome et al., 2024). Despite progress in understanding how different forms of exercise can mitigate obesity and related health risks, there remains significant debate and uncertainty about the optimal activity levels required for maximum benefits. Aerobic exercise, for example, predominantly utilizes fat as an energy source, enhancing the body's ability to break down stored fat through lipolysis (Brown, 2002; Noland, 2015). This process not only reduces fat stores but also has a cascade of beneficial effects on metabolic health. Enhanced lipolysis reduces ectopic fat accumulation in organs like the liver and muscle, which is a key driver of insulin resistance. As a result, insulin sensitivity improves, leading to better regulation of blood glucose levels (Bruno et al., 2018). Additionally, the reduction in fat mass also alleviates strain on the cardiovascular system, aiding in the normalization of blood pressure. This occurs through mechanisms such as improved endothelial function, reduced arterial stiffness, and enhanced nitric oxide production, which collectively support better vascular health and circulation.

High-intensity strength training burns mostly carbohydrates for immediate energy (Brown, 2002). It also triggers the release of hormones such as growth hormone and testosterone (Kraemer et al., 2020). By influencing the body's chemistry, hormones promote muscle growth and make it easier to access the body's ability to burn glucose derived from fat stores (gluconeogenesis) (Coll-Risco et al., 2016; Loucks and Caiozzo, 2012). To assess the impact of exercise programs on health accurately, participants need to follow a strict dietary monitoring protocol (Matthews et al., 2012) to ensure that any observed changes are primarily due to the exercise intervention itself and not influenced by significant shifts in their dietary habits (Beck et al., 2015).

Researchers have previously studied the separate effects of aerobic exercise and strength training on health (Doewes et al., 2023; Kim et al., 2019; Mann et al., 2014). While some research shows that these exercises combined can help older adults with obesity manage their blood sugar and cholesterol (Azarbayjani et al., 2014; Batrakoulis et al., 2022), blood pressure and insulin resistance (Cornelissen et al., 2011; Kolahdouzi et al., 2019), a knowledge gap exists in comparing aerobic, resistance, and concurrent training, particularly in studies that control for participants' dietary practices. We lack a clear understanding of how these training modalities differ under controlled dietary conditions.

Hence, the objective of this study was to assess and compare the efficacy of various exercise modes (aerobic, resistance training, and combined exercise) and their ability to increase metabolic

biomarkers, pressure and anthropometric indices over time among adults with overweight and obesity.

2 Methods and materials

This study is reported following the CONSORT guidelines.

2.1 Research setting and design

This study used a randomized parallel group experimental design to evaluate between-group differences to track between-subject difference after 12-week training.

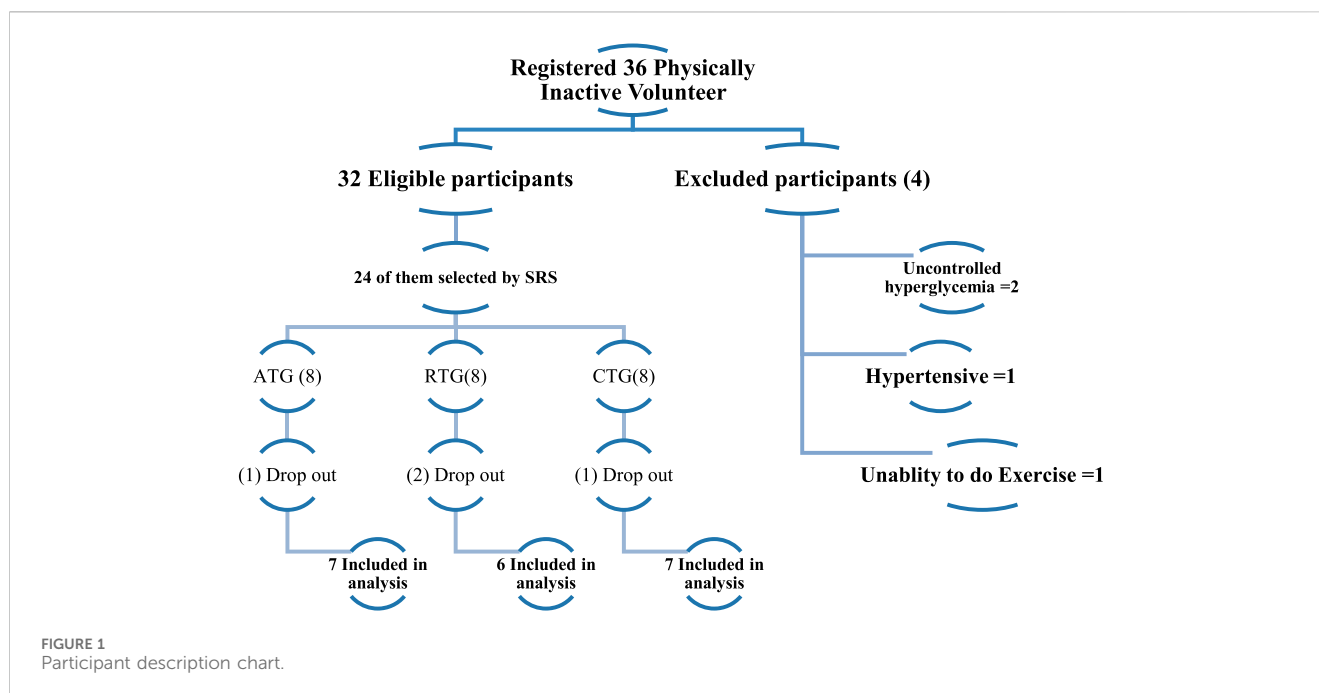
Males aged 45–60 years who were physically sedentary and had a BMI greater than 24.9 kg/m² were included in the study. The volunteers were chosen from among the inactive citizens of Debre Markos town, Ethiopia, and were notified via notice boards and local radio.

The inclusion criteria were as follows: (a) had a BMI >24.9 kg/m² (b) were aged between 45 and 60 years, (c) volunteered to participate, (d) were physically inactive (did not achieve 30–60 min per day or 150 min per week of moderate intensity exercise or 20–60 min per day (75 min per week) of vigorous intensity (Cohen, 1991) and cleared a medical history from the physical activity readiness questionnaire), and (d) were able to perform the necessary exercises. The exclusion criteria were as follows: (a) any cardiovascular, respiratory, or musculoskeletal disorders precluding physical exercise; (b) uncontrolled hyperglycaemia (≥ 126 mg/d) or hypertension (a resting blood pressure $\geq 140/100$ mmHg); and (c) active infection, (d) acute myocardial infarction, stroke, trauma, surgery or severe liver dysfunction.

Using the G*Power tool, we were able to identify the optimal sample size for our experiment. In accordance with Sousa et al. (2014), we calculated a sample size of 21 individuals, accounting for zero correlations between AT TGC, total-c, LDL-c, and HDL-c, a significance threshold (α) of 0.05, lower effect size 0.25, with 15 number of measurements and a power of 0.95 (Sousa et al., 2014). Considering a 10% non response rate, a total sample size of 24 was needed. By successfully detecting the specified effect size with this sample size, we were able to reach the desired statistical power and significance level for our investigation.

Thirty-six men who were overweight or obese were recruited and registered for the current study. Of the 36 volunteers we had originally registered as physically inactive, 32 remained when the inclusion criteria were applied. Using a straightforward random selection process, the researchers produced a final study group of 24 volunteers. Those who participated were then randomly assigned to one of three exercise groups, each consisting of eight participants: aerobic, resistance, or combination training. Balanced randomization (1:1:1) and homogeneous samples were used in the investigation (Figure 1). Throughout the investigation, the data collectors were blinded to one another.

To ensure ethical conduct, participants were fully informed about all procedures, risks, and guidelines before providing their informed consent. According to Cohen, 1991, this met the standards established by the American College of Sports Medicine (Cohen, 1991). Additionally, all procedures involving human subjects were



examined and given feedback by Debre Markos University Sport Science Academy Ethics Review Committee (ERC) (SPSC05/22). Finally, the ethical guidelines outlined in the 2000 amendment of the Declaration of Helsinki were followed when the study was conducted. This trial was retrospectively registered on the Open Science Framework (OSF) platform. The registration can be accessed at <https://doi.org/10.17605/OSF.IO/34MKQ>.

The first set of data was gathered before the intervention began, and the second set was gathered at the completion of the 8-week intervention. The Debre Markos Referral Hospital is where the data were gathered. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and the waist-to-hip ratio were secondary objectives, whereas fasting blood glucose (FBG) and insulin resistance (IR) were the primary outcomes. The follow-up (intervention) periods spanned April 20/2022–2022.

2.2 Measurement of study variables

2.2.1 Fasting glucose and insulin resistance

To minimize acute exercise effects and ensure accurate results, blood samples were collected 48 h post training. The participants fasted for 12 h and abstained from alcohol, coffee, and high-fat meals prior to testing. Blood samples were drawn by a trained medical laboratory technician at Debre Markos Referral Hospital via antecubital vein puncture, both before and after 12 weeks of exercise training, with a total volume of 5 mL collected.

The samples were centrifuged to separate the plasma from the cellular components and were stored at -80°C to maintain integrity for analysis. Glucose levels were measured via the hexokinase method (COBAS, Roche), with an intra coefficient of variation between 1.58% ($\mu = 64.7 \text{ mg/dL}$) and 1.38% ($\mu = 369 \text{ mg/dL}$) (Landberg et al., 2021). Fasting plasma insulin was quantified via

a Human Insulin ELISA Kit (Shanghai Kehua Bio-Engineering Co., Ltd., China) (Shakil-Ur-Rehman et al., 2017).

The homeostasis model of insulin resistance (HOMA-IR index), which is the product of glucose and insulin concentrations divided by a factor, was used to quantify insulin resistance (Vogesser et al., 2007). The HOMA-IR index was calculated as follows:

$$\text{HOMA-IR} = \frac{\text{fasting serum glucose (mmol.L}^{-1}\text{)} * \text{fasting serum insulin (}\mu\text{U.mL}^{-1}\text{)}}{22.5}$$

2.2.2 Blood pressure

Blood pressure was measured via an automated Sphygmocor XCEL device (AtCor Medical, CardieX, Sydney, Australia) (De la Torre Hernández et al., 2021). The appropriately sized cuff was placed on the participant's left upper arm while they lay still on the catheterization laboratory table. After standard Oscillometric brachial blood pressure was recorded, the cuff was reinflated to sub diastolic pressure to capture volumetric waveforms for 5 s. The participants were instructed to keep their arms relaxed and refrain from movement during the measurement.

2.2.3 Waist-hip ratio (WHR)

Waist and hip circumferences were measured via a Sammons Preston Tape (Narang Medical Limited, New Delhi) (Khan et al., 2009), with measurements taken to the nearest 0.1 cm. When waist circumference was measured, the participants were instructed to breathe normally and wear lightweight clothing to ensure accurate measurement. The measurement was taken at the narrowest point of the waist, usually located just above the navel. For hip circumference, measurements were taken at the site of the greatest circumference around the buttocks (Hu et al., 2010). The waist-to-hip ratio was calculated as the waist circumference divided by the hip circumference (Bredella et al., 2009). All measurements

were conducted by a trained data collector who has received comprehensive instruction in these measurement procedures. This training ensures that the data collector adheres to standardized protocols, promoting accuracy and consistency in the measurement process.

2.2.4 Average daily energy intake

We utilized a 24-hour interactive questionnaire with several passes that was developed and validated for use in developing countries (Gibson and Ferguson, 1999). The three 24-hour sessions were held on Monday, Wednesday and Saturday to capture variation in intake throughout different days of the week. We applied the Ethiopian food composition table to estimate nutrient and energy levels from dietary data. The names of foods and drinks, their descriptions, cooking methods, and amounts from both 24-hour periods were coded and submitted to the NutriSurvey200 (Regassa et al., 2021). After the frequency of consumption per day was determined, we used the product sum approach to determine daily food intake. Daily food intake = \sum (food item's stated consumption frequency, translated to times per day) * (portion size ingested of that food). The daily average energy intake was also determined as follows: $ADEi = \sum \text{daily food intake} / \text{number of data collection days}$.

2.3 Exercise intervention protocol

Over the course of 8 weeks, the exercise routines consisted of three weekly sessions, each lasting 60 min. The participants warmed for five to 10 minutes before beginning 30–40 min of main training for each exercise session. They finished with a cool-down that lasted five to 10 min. The six exercises in the RT program were designed to target the body's major muscle groups. Standing plantar flexion, squatting, machine leg pressing, neutral rowing, dumbbell curl, triceps pulley, bicep curl, and vertical bench pressing were the workouts that were performed. Three sets of eight to twelve repetitions at an intensity between fifty and seventy-five percent of their one-repetition maximum (1RM) were part of the training regimen. The remaining time between training sessions and between sets was approximately 48–72 h and 1–1.5 min, respectively (Jambassi Filho et al., 2020).

To determine protocol loads, the 1-RM test will be applied by gradually increasing resistance until the volunteer succeeded in performing no more than one repetition. Start with a warm-up that includes a small weight that is roughly 40%–60% of the perceived maximum load in order to calculate each individual's 1-RM. To make sure the muscles are prepared for the subsequent section of the test, give everyone a minute to relax after finishing the warm-up. provide the individual 12–15 repetitions after increasing the weight to a moderate load (60%–80% of the perceived maximum). This set should be difficult, but not impossible. Rest for one to 2 minutes after finishing this set. After establishing a reasonable weight, encourage the subject to do up to 10 repetitions with a 10% increase in load. Allow the individual an additional one to 2 min of break before continuing if they are able to perform ten or more repetitions with the new weight. Keep an eye on the participant throughout this rest period

to make sure they aren't exerting themselves excessively. Increase the weight by 10% again and have them try repetition with the larger load if they are able to finish the set of 10 or more reps. After that, the resistance will be gradually raised until the individuals could only complete each exercise nine repetition or less. Reaching the target number of repetitions in between 3 and 6 tries is the aim of increasing the resistance. Three minutes of rest are permit in between each particular exercise, and 2 min will be permitted between each try. Brzycki 1-RM prediction equation (Brzycki, 1993) will then use to estimate the 1-RM based on the resistance and repetitions recorded on the last try. The mathematical expression for the equation is $1RM = W / [102.78 - 2.78(R)] / 100$, where R is the maximum number of repetitions and W is the weight was using (Abdul-Hameed et al., 2012).

A treadmill was used for aerobic activity, and the intensity level ranged from 50% to 75% of the maximal heart rate (HRmax). Following a training regimen consisting of three exercises per session, the CTs completed the same number of workouts as the ATs and RTs did.

We will use the heart rate reserve (HRR) approach, which is based on the Karvonen formula, to determine the target heart rate (THR) in order to manage the intensity of the exercise (Yabe et al., 2021). This approach is appropriate for a broad spectrum of adult fitness levels (ACSM's, 2013). The following is the formula:

$$THR = HR_{rest} + [(HR_{max} - HR_{rest}) \times Intensity]$$

Participants wore Polar heart rate monitors (Polar Electro, Kempele, Finland) to track their heart rate (Hernández-Vicente et al., 2021), ensuring that aerobic exercise was conducted within the desired percentage of their maximum heart rate (HRmax). These approaches ensured that participants performed the exercises at the prescribed intensity levels safely and effectively.

In each session, participants performed endurance exercises before moving on to strength exercises. Details of the general training intervention approach are outlined in Table 1.

This specific exercise order was selected to explore the impact of aerobic training preceding strength training (Leppers et al., 2001). All interventions were conducted under the supervision of qualified professionals to ensure adherence to the protocol, monitor participant safety, and provide guidance during the exercise sessions. And to minimize potential confounding factors, participants were explicitly advised not to engage in any additional resistance-type or aerobic training throughout the duration of the study.

2.4 Statistical analysis

SPSS version 26 was used to analyse the data (SPSS Inc., Chicago, IL). By using a Bonferroni adjustment for multiple comparisons in their ANCOVA, with average daily energy intake as the covariate, the researchers were able to secure dependable results for FBG, IR, SBP, DBP, and WHR. The various exercise types performed by the groups were compared, with statistical significance set at a p-value of 0.05 or lower. All statistical analyses were conducted using two-tailed tests.

TABLE 1 Training protocol details.

Weeks	Intensity				Duration			
	AT (HR _{max})	RT (1RM)	CT		AT (minutes)	RT (3 sets)	CT	
			AT	RT			AT	RT
Week 1 and 2	50%–55%	50%–55%	50%–55%	50%–55%	25	10–12	13	10–12
Week 3 and 4	55%–60%	55%–60%	55%–60%	55%–60%	30	10–12	15	10–12
Week 5 and 6	60%–65%	60%–65%	60%–65%	60%–65%	35	10–12	17	10–12
Week 7 and 8	65%–75%	70%–75%	65%–75%	65%–75%	40	10–12	20	10–12

TABLE 2 Baseline and follow-up data while adjusting dietary practice.

	ATG (Age = 49.00 ± 2.08, ADEi = 2885.00 ± 109.180)		RTG (Age = 49.83 ± 3.06) ADEi = 2636.00 ± 98.82)		CTG (Age = 48.71 ± 2.87) ADEi = 2753.70 ± 178.12)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
FBG	98.49	94.44 ^a	98.70	86.07 ^a	96.83	88.61 ^a
IR	2.822	2.17 ^a	2.793	1.76 ^a	2.820	1.963 ^a
SBP	132.14	125.13 ^a	130.50	123.72 ^a	131.42	120.67 ^a
DBP	87.14	87.147 ^a	85.83	86.064 ^a	84.00	83.798 ^a
WHR	1.17	0.934 ^a	1.21	1.074 ^a	1.15	0.868 ^a

Note. FBG: fasting blood glucose; IR: insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure and WHR: waist to hip ratio. The values are presented as the means.

^aCovariates appearing in the model were evaluated at average daily energy intake (ADEi) = 2671.5620_±.

3 Results

The baseline descriptive data characteristics and the adjusted absolute changes in FBG, IR, SBP, DBP, and WHR levels over the course of the study are summarized in Table 2. Twenty people completed the study and were analysed: seven in the AT, six in the RT, and seven in the CT. Four participants were dropped from the experiment due to exercise-induced injuries, parting a total of 20 participants who finished the study and were included in the analysis, 7 in the AT, 6 in the RT and 7 in the CT. The normality of the data was assessed using the Shapiro-Wilk test. The results indicated that all variables were normally distributed at baseline and follow-up ($p > 0.05$).

The results revealed no significant differences in any of the variables among the three groups in the pretest, suggesting successful randomization of the study participants. The average ages of the participants in the respective groups were AT = 49.00 ± 2.08, RT = 49.83 ± 3.06 and CT = 48.71 ± 2.87. The average body mass indices of the participants in the AT, RT and CT groups were 27.84 ± 1.10, 27.45 ± 0.74 and 27.70 ± 0.85, respectively (Table 2).

As Table 3 shows, exercise modality had a significant main effect on FBG ($F(2, 26) = 10.656$, $P = 0.001$, $\eta^2 = 0.571$), as shown in Table 3. Post hoc analyses using the Bonferroni *post hoc* criterion revealed that RT (-8.376 ± 2.032 , $p = 0.002$) and CT ($MD = -5.837 \pm 1.936$, $P = 0.025$) were significantly greater than AT was. The IR from the HOMA-IR revealed a more substantial decrease in RT than AT did ($MD = 0.410 \pm 0.101$, $p = 0.03$); nonetheless, there was no noticeable shift from the concurrent training group.

Systolic blood pressure parameters $F(2, 26) = 6.33$, $p = 0.009$, and $\eta^2 = 0.442$ were substantially lower in the CT groups than in the AT and RT groups ($MD = 4.457$, $SE = 0.782$, $P < 0.001$) and ($MD = 3.042$, $SE = 0.101$, $P < 0.042$), respectively. The waist-to-hip ratio varied significantly between the training modalities ($F(2, 16) = 18.175$, $P = 0.001$, $\eta^2 = 0.694$). Both AT and concurrent training significantly decreased compared with RT ($MD = 0.140$, $SE = 0.029$, $P = 0.01$) and ($MD = 0.206$, $SE = 0.035$, $P < 0.001$), respectively. Even though the diastolic blood pressure data were significantly different between the pretest and posttest values, the independent variables had no discernible effect on these variables.

4 Discussion

The current study revealed significant reductions in FBG across all groups, with the most substantial decreases observed in the short rest resistance training (RT) and concurrent training (CT) groups. Studies have shown that both concurrent (Al-Mhanna et al., 2024; Al-Mhanna et al., 2023; Castaneda et al., 2002; Sigal et al., 2007) and resistance training (Castaneda et al., 2002; Fink et al., 2018) improve glycemic control in adults with overweight and obesity. However, Cai and Zou (2016) conducted a meta-analysis of randomized controlled trials and reported that regular aerobic exercise can help manage FBG levels (Cai and Zou, 2016). Our research, however, casts doubt on this idea by showing that short rest RT can dramatically reduce FBG, suggesting that resistance training could be essential for glycemic control. This result was supported by

TABLE 3 Test between subject effect changes in outcomes within treatment groups.

Variables	Between-subjects effects			Pairwise comparison					
	F	Sig. ^b	η^2	Treatment groups	Mean difference	Std. Error	Sig. ^b	95% CID	
								Lower bound	Upper bound
FBG	10.651	0.001	0.571	AT-RT	8.376	2.032	0.002	2.945	13.808
				AT-CT	5.837	1.936	0.025	0.661	11.012
				RT-CT	-2.540	2.444	NS	-9.073	3.993
IR	8.957	0.002	0.528	AT-RT	0.410	0.101	0.003	0.139	0.680
				AT-CT	0.211	0.096	NS	-0.047	0.469
				RT-CT	-0.199	0.122	NS	-0.524	0.127
SBP	6.33	0.009	0.442	AT-RT	1.415	0.915	NS	-1.032	3.862
				AT-CT	4.457	0.872	0.000	2.125	6.789
				RT-CT	3.042	1.101	0.042	0.099	5.986
DBP	4.849	0.023	0.377	AT-RT	1.084	2.005	NS	-4.276	6.443
				AT-CT	3.349	1.910	NS	-1.758	8.456
				RT-CT	2.265	2.412	NS	-4.181	8.712
WHR	16.175	0.001	0.594	AT-RT	-0.140	0.029	0.001	-0.218	-0.062
				AT-CT	0.066	0.028	NS	-0.008	0.140
				RT-CT	0.206	0.035	0.000	0.112	0.300

Note: FBG: fastening blood glucose; IR: insulin resistance; SBP: systolic blood pressure; DBP: diastolic blood pressure and WHR: waist to hip ratio; NS: not significant. Covariates appearing in the model are evaluated at the following values: average daily energy intake = 2671.5620.

Baldi and Snowling (2003) and Marquis-Gravel et al. (2015). In contrast, research on sprint interval training (SIT) by Ghaedi et al. (2020) did not support this outcome.

The effectiveness of exercise in improving insulin sensitivity was highlighted by the significant decreases in IR observed in all groups, especially in short rest RT, which supported various findings (Mavros et al., 2013; Van Der Heijden et al., 2010; Yaspelkis, 2006) and also improves in SIT training (Ghaedi et al., 2020). Conversely, de Matos et al. (2018) reported that obese individuals engaging in high-intensity interval training showed no improvement in insulin resistance. In contrast to our findings, however, studies have shown that regular aerobic exercise (Bruno et al., 2018; Motahari-Tabari et al., 2014) and concurrent training (Bharath et al., 2018; Medeiros et al., 2015) can improve insulin resistance in obese individuals. This discrepancy, which may result from differences in study design, exercise order in concurrent training or intervention duration, emphasizes the potential advantages of including resistance training in exercise programs for the treatment of IR.

The observed decreases in SBP were most pronounced in the CT and RT groups, supporting findings from Lemes and his teammates (2016), which suggest that resistance training is effective in lowering systolic blood pressure in metabolic syndrome patients (Lemes et al., 2016). Another study demonstrated that concurrent training decreased systolic blood pressure in hypertensive individuals (Aguiar et al., 2017; Caminiti et al., 2022). Conversely HIIT

training did not demonstrate significant improvement in resting blood pressure (Dunstan et al., 2002). Dimeo et al. (2012), on the other hand, noted that aerobic training significantly decreased systolic and diastolic daytime ambulatory blood pressure (Dimeo et al., 2012). Our findings support a dual-modality exercise strategy by confirming that resistance training and combination training offer greater advantages for reducing SBP.

Although all the groups showed significant reductions in DBP (Wewege et al., 2018), no substantial differences emerged between the training modalities (Moeini et al., 2015) except for the control group (Ambelu and Teferi, 2023). This finding is in line with that of Cornelissen and Smart (2013), who noted that diastolic pressure may be influenced by a variety of factors, including baseline health and diet. Conversely, a study reported that 9 months of aerobic exercise and concurrent training exercise can differentially affect diastolic blood pressure (Sousa et al., 2013), indicating that the length of training may influence how these interventions affect DBP.

Significant improvements in WHR were observed, particularly in the CT and aerobic training groups. This result supports previous findings that aerobic exercise (Chiu et al., 2017; Marandi et al., 2013) and concurrent exercise positively influence WHR and metabolic health (Ho et al., 2012; Monteiro et al., 2015). However, according to Ramos-Campo et al. (2021), resistance exercise can effectively reduce body composition (Ramos-Campo et al., 2021). Our results challenge this by demonstrating substantial reductions in WHR across both aerobic and combined training modalities,

emphasizing the importance of various exercise approaches in managing body composition. Overall, the findings of this study reinforce the significant role of tailored exercise interventions in improving key metabolic and cardiovascular health markers in overweight and obese adults. The contrasting results with the literature highlight the complexities of exercise effects and underscore the need for further research to elucidate the optimal exercise strategies for different populations.

One limitation of this study is the absence of a non-intervention control group, which restricts the ability to fully attribute observed changes solely to the intervention. While the study was designed with random assignment and control of key confounding factors, the lack of a traditional control group limits the ability to isolate the effects of the intervention from other potential influences. Future research should consider incorporating a non-exercising control group to strengthen the validity of causal inferences and provide a more comprehensive understanding of the intervention's impact. However, the observed differences in the effectiveness of each modality suggest that individualized exercise prescriptions could optimize outcomes, especially for older adults. Future studies should explore the long-term effects of these interventions and evaluate whether combining different modalities yields synergistic benefits. Additionally, more diverse participant groups and the inclusion of non-exercising control groups will be essential in validating these results and refining clinical guidelines.

5 Conclusion

The results of the current study confirm the undeniable benefits of regular exercise for overweight and obese adults. Specifically, concurrent training (CT), which combines short rest resistance training and continuous aerobic training, proves to be the most effective approach for improving metabolic and cardiovascular health indices, as well as enhancing the waist-to-hip ratio in overweight and obese individuals. These findings highlight the importance of incorporating CT as a primary exercise strategy for improving overall health and managing weight-related conditions.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

The trial was approved by the Ethics Review Board of Debre Markos University, Sport Science Academy (Reference number:

SPSC 05/22). All the participants were informed about the intervention and possible adverse events before the commencement of the trial and signed an informed consent form.

Author contributions

FM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. YL: Funding acquisition, Investigation, Methodology, Project administration, Software, Writing–review and editing. MA: Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing–review and editing. YM: Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing–review and editing. SZ: Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The research for this project was supported by the Annual Research Funding from Debre Markos University. Their financial contribution has been essential in advancing our work. However, the funding did not cover the publication fees.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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OPEN ACCESS

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RECEIVED 23 October 2024

ACCEPTED 17 March 2025

PUBLISHED 28 March 2025

CITATION

Feige S, Peter S, Weickmann J, Michaelis A, Gebauer RA, Weidenbach M, Dähnert I, Münch D, Poschart M, Wüstenfeld J and Paech C (2025) Physiologic response to distance diving in healthy children and young adults.
Front. Sports Act. Living 7:1515674.
doi: 10.3389/fspor.2025.1515674

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Physiologic response to distance diving in healthy children and young adults

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Introduction: Diving and fin swimming are well established sports, including competitive sports, but little is known about the short-term adaptation of physiological parameters in Children and Adults during submersed dynamic apnea near the individual maximum. The current study provides data on the physiological adaptation of Children and young Adults to dynamic apnea diving in real-life conditions.

Methods: This study provides data from 11 healthy elite fin swimmers (<30 years), including transcutaneous oxygen saturation and heart rate performing various diving protocols.

Conclusion: The results suggest that apnea duration primarily affects oxygen saturation, while dive speed influences cardiovascular workload. Oxygen levels often decline post-dive, indicating a delayed oxygen debt requiring recovery. Future research should explore broader demographics, including recreational divers and medically restricted populations.

KEYWORDS

swimming, diving, children, pediatrics, adults, fin swimming, dynamic apnea, physiology

1 Introduction

Diving and fin swimming are well established-sports, including competitive sports, but little is known about the short-term adaptation of physiological parameters in Children and Adults during submersed dynamic apnea. Diving skills are vital in contact with open water and essential in the prevention of drowning accidents. Concerning the cardiovascular effects of immersion and submersion on the human body, patients with severe cardiopulmonary restrictions and congenital heart disease are partly restricted from taking part in swimming or diving activities to prevent potential harm based on physiologic assumptions (1, 2). Immersed in water, the human body has physiological mechanisms of adaption at its disposal to bridge the missing oxygen supply during apnea, which is most crucial to prevent blackout (3–5). The mammal diving response supports breath holding and leads to a reduced heart rate by activation of the vagal system (6). Peripheral vasoconstriction helps maintaining adequate blood flow to the brain and causes a centralization of blood to the oxygen sensible inner organs (7). Both mechanisms are already observable when breath holding with cold water face

immersion (4, 8). Moreover, a reduction in spleen volume, caused by reflexive contraction, is observable not only in aquatic but in terrestrial mammals (9, 10). Due to hydrostatic pressure, full body water immersion increases stroke volume, reduces venous capacity, leads to restrictive ventilation and central hypervolemia causing renal diuresis (7, 11). Nevertheless, the human body seems to cope quite well with these external stressors, allowing to swim and dive to a remarkable extent (12). Previous studies of our working group justify the contemplation, that also cardiopulmonary restricted patients could possibly adapt quite well to this stressor (8, 13, 14). To assess expectable changes in transcutaneous O₂ saturation (tO₂%), heart rate (HR) and perfusion index (PI) during and following shallow dives in Children and young Adults and how these parameters behave when confronting the body with different diving times and workloads during the dive, this study provides data from elite fin swimmers in life-like conditions. As a consequence, this study may contribute to a more profound advice for athletes and patients who wish to safely participate in leisure or competitive swimming and apnea diving activities. We also wish to provide a data base for further considerations and research on this subject.

2 Methods

2.1 Participants' characteristics

Eleven healthy study subjects were recruited from local swimming and sports clubs. Inclusion criteria were young age (<30 years), experience with swimming and diving, i.e., participation in the regional fin swimming club, and the absence of cardiovascular disease in the patient history. Exclusion criteria were signs of reduced general condition and acute illness, signs of limitations of cardiopulmonary function, mental handicap and genetic diseases. Participants with common pre-existing respiratory conditions, such as mild allergies (two with house dust mite allergy and one with allergies to dog, cat, and grass pollen) or a history of bronchial hyperreactivity, were included, provided they were asymptomatic at the time of testing and showed no limitations in their physical activity. No signs of obstructive or restrictive ventilatory disorders were detected in any participants, and acute respiratory infections were excluded prior to testing. None of the measured blood parameters fell outside the normal reference values. The subjects or their legal guardians gave their informed consent. The study received ethical approval by the ethics committee University of Leipzig and is listed under the reference 034/24-ek.

2.2 Medical assessment and testing conditions

All study subjects underwent a thorough medical work up 14–20 days before the testing. Anthropometric data were measured. Cardiovascular and pulmonary data were collected by performing echocardiography (EPIQ CVx, Philips), electrocardiography (resting ECG, custo cardio 400, customized),

and body plethysmography (Q-Box, CosMed). Blood samples were taken to assess hemoglobin levels and erythrocyte count, as these parameters are essential for oxygen transport. Data on potential medical history and medication was obtained from personal interviews. The testing took place in a local public swimming hall. Water temperature was 28°C and ambient temperature was 30°C. The pool was 2 m in depth. All dives were performed apnea and with monofins. The tests were supervised by a medical doctor and a study nurse.

2.3 Diving protocol

Prior to the start of the testing, the subjects were instructed to the following protocol. The protocol was designed in view of usual distances in diving competitions and in close consultation to the coaches of the fin swimming club, to warrant the study subjects safety on the one hand, and to set distances challenging enough for the study subjects to accomplish a dive near the personal maximum, on the other hand.

2.3.1 Diving protocol for adults

Physiological data during and following a dive of different distance and different workloads was collected via pulse oximeter for an overall time of 70 min. The participants were asked to perform a 50, 75 and 100 m dive in a convenient, self-selected pace and two dives over 75 m of which one was economical pace and the other was at the study subjects individual maximum speed. Before and after, as well as between dives, monitoring in a sitting position for 10 min was applied. Mobile ultrasound measurement of spleen size in two diameters was performed prior to the first and after the last dive. An illustration of the test procedure for adults can be found in [Supplementary Figure 1](#).

2.3.2 Diving protocol for children (age 8–16)

Physiological data during and following a dive of different distances was collected via pulse oximeter for an overall time of 45 min. The participants were asked to perform a 25, 50 and 75 m dive in a convenient, self-selected pace. Before and after, as well as between dives, monitoring in a sitting position for 10 min was applied. Mobile ultrasound measurement of spleen size in two diameters was performed prior to the first and after the last dive. An illustration of the test procedure for children can be found in [Supplementary Figure 2](#).

2.4 Assessment of physiological parameters during testing

Transcutaneous oxygen saturation, heart rate, and perfusion index (PI), as a surrogate of peripheral vascular tone (15, 16), were recorded for the whole testing period. PI is derived from the photoelectric plethysmography signal of the pulse oximeter and calculated as the ratio between the pulsatile component and the non-pulsatile component of the light reaching the detector. As the change in peripheral perfusion leads to a change in the

pulsatile (arterial) component, whereas the non-pulsatile part, which is determined by the tissue, does not change, the ratio between both components changes (15). Thus, PI reflects peripheral vasomotor tone and is a surrogate for vasoconstriction or vasodilatation, promoting a volume shift. The perfusion index subsumes vascular tone and systemic blood flow, which is influenced by stroke volume (15).

Measurements were done using a Masimo Rad-97™ patient monitor and Masimo RD SET sensors (Masimo Corporation, Irvine, USA) protected from the water by hydrophobic coating with vaseline, which were placed on an index finger like demonstrated in the picture (Supplementary Image 1). The first mobile ultrasound measurement of spleen size was performed during the rest phase before the first dive, and the second measurement was performed after the last dive of the protocol, using a GE VScan with Dual Portable Ultrasound. Measurement was taken in transvers maximal length and maximal dorsoventral width, calculating spleen surface in cm².

2.5 Statistical analysis

For the statistical analysis, IBM SPSS Statistics for Windows (V29) was used. Anthropometric measurements of the participants were analyzed and a Mann–Whitney *U* test was conducted to compare gender-specific differences within the study population. Differences in recovery time, oxygen and heart rate responses were assessed, with specific attention to variations between Adults and Children, as well as gender differences, using the Mann–Whitney *U* test. For the statistical analysis of the 75-meter dives, a Wilcoxon signed-rank test was employed to compare the physiological variables between the two different dive speeds. Spearman's correlation was applied to estimate the influence of dive time, speed and anthropometric data on oxygen and heart rate progression during the dives. The correlation coefficient (ρ) and *p*-value are included in the manuscript.

3 Results

The total study population of 11 fin swimmers was divided into a group of Children (<17 years) and a group of Adults (≥17 years). The characteristics of the study population are shown in Table 1. Children and Adults did not differ significantly in anthropometric as well as in cardiovascular and pulmonary data. There were significant differences between male and female patients. *P*-values for sex differences are shown in the last row of Table 1. Detected via mobile echocardiography, the mean reduction in spleen surface was 4.03%, indicating a notable adaptation over time.

3.1 Physiological adaptation parameters during the dives

The dives over different distances were analyzed over time for heart rate and transcutaneous oxygen saturation separately with a

5 min follow-up period after emersion. Graphical representation for each distance and group is provided in Figures 1–6.

The time under immersion varied between the study subjects as the diving speed was individually determined. This was not taken into account in the diagrams (Figures 1–6) in favor of showing the phase directly after surfacing. Heart rate reduction following the immersion was not obligatory but most clearly observable during the 50 m dive with an average drop of 18 bpm (range 6–39 bpm; *N* = 6). While heart rate changes were evident in all other participants over 50 m, two Children and two Adults showed no change in heart rate. One adult individual even experienced an increase in heart rate by 10 bpm during the 50 m dive. Looking at the maximum heart rate of the 25–75 m dives, all participants reached their maximum heart rate only after surfacing when they had left the water, which is indicative of the diving bradycardia response. An exception was the longest dive distance in this protocol of 100 m. All participants experienced an average increase in heart rate of 24 bpm (range 14–37 bpm) during the underwater time. Four study subjects reached their maximum heart rate when emerging while 2 study subjects reached their peak after leaving the water. The lowest oxygen saturation values were measured after surfacing, although in average there was a slight drop in oxygen saturation levels during the longer dives. The curve of minimal oxygen saturation exhibited noticeable fluctuations, which can largely be attributed to the limited number of participants in this study. Given the small sample size, individual variability had a pronounced impact on the overall trend, as each participant's values were only recorded during a single event of desaturation. This overlap suggests that the desaturation phase occurred later in some participants than others. These factors should be considered when interpreting the results, and future studies with a larger cohort may provide a more consistent curve. One exception must be made for an adult participant, in which the lowest values were reached continuously during the dive (50 m: 95% SpO₂; 75 m: 87% SpO₂; 100 m 89% SpO₂).

Children and Adults were expected to follow similar courses of changes in oxygen saturation and heart rate in response to the immersion. In this study, the group of Children differed significantly from the group of Adults in minimal oxygen saturation levels post diving (*p* = 0.019). There was a greater drop in oxygen saturation levels in the adult group after emersion, most noticeable over 75 m.

3.2 Factors influencing minimum oxygen saturation and heart rate

No sex differences in the investigated parameters could be found. An analysis of all dives indicates a significant connection between dive time and cardiac work load. Dive time correlates moderately with mean heart rate (Spearman's ρ = .491, *p* = .005) as well as with maximum heart rate (Spearman's ρ = .528, *p* = .002). The time under water does also correlate with the intraindividual range from highest to lowest heart rate measured

TABLE 1 Characteristics of the study population.

Subjects characteristics	Group of children				Group of adults				Total study population		
	Female (N = 3)		Male (N = 2)		Female (N = 3)		Male (N = 3)		Female (N = 6)	Male (N = 5)	p-value
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Median	
Age (years)	15	15–15	15.5	15–16	17	17–23	18	17–29	15–23	15–29	0.396
Anthropometry											
Size (cm)	162.3	161.9–167.2	180.9	178.4–183.4	169	166.7–170	176.9	170.2–192.7	161.9–170.0	170.2–192.7	0.006*
Weight (kg)	63.9	56.8–65.6	74.6	67.2–82.0	59.3	59.3–68.0	84.5	64.4–98.9	56.8–68.0	64.4–98.9	0.028*
BMI (kg/m ²)	24.3	20.3–25.0	22.75	21.1–24.4	21.3	20.5–23.8	26.6	22.2–27.0	20.3–25.0	21.1–27.0	0.201
Body fat (%)	21.1	17.9–25.3	14.45	14.3–14.6	17.2	13.9–20.7	12.5	10.2–13.0	13.9–25.3	10.2–14.6	0.018*
Lean body mass (%)	49	46.6–50.4	63.8	57.6–70.0	51.1	49.1–53.9	75.9	56.4–86.0	46.6–53.9	56.4–86.0	0.006*
Haemogram											
Erythrocytes (Mio./μl)	4.62	3.89–4.86	4.97	4.97–4.97	4.85	4.60–5.00	4.84	4.76–5.42	3.89–5.00	4.76–5.42	0.200
Hemoglobin (mmol/L)	8.7	7.8–8.9	9.2	8.9–9.5	9	8.4–9.6	9.2	9.1–10.1	7.8–9.6	8.9–10.1	0.082
ECG											
QRS complex (ms)	91	86–96	109	101–117	103	102–109	106	99–118	86–109	99–118	0.144
Echocardiography											
Ejection fraction (%)	66.47	57.70–66.65	62.65	62.38–62.92	62.49	62.26–67.47	61.1	58.76–72.55	57.70–67.47	58.76–72.55	0.715
Absolute heart volume ^a (ml)	613.7	540.47–706.48	888.06	866.71–909.40	637.83	587.74–696.33	948.4	743.62–1,098.34	540.473–706.475	743.624–1,098.340	0.006*
Relative heart volume ^a (ml/kgKG)	9.60	9.52–10.77	11.99	11.09–12.90	10.24	9.91–10.76	11.2	11.11–11.55	9.52–10.77	11.09–12.90	0.006*
PFTs											
FEV1 (L)	3.08	2.56–3.17	4.59	4.19–4.99	3.93	3.36–3.97	4.82	3.86–5.49	2.56–3.97	3.86–5.49	0.018*
RV (L)	1.07	0.73–1.13	1.2	0.61–1.78	1.05	0.87–1.05	1.02	0.36–1.24	0.73–1.13	0.36–1.78	0.855
TLC (L)	4.96	4.81–5.29	6.96	5.49–8.42	5.51	5.04–5.95	7.57	5.41–7.85	4.81–5.95	5.41–8.42	0.045*
VC (L)	4.16	3.74–4.23	5.76	4.88–6.64	4.46	4.17–4.90	6.33	5.05–6.83	3.74–4.90	4.88–6.83	0.011*

^aFormula was based on the work of Dickhuth.

*Significant at a level of $\alpha = 0.05$.

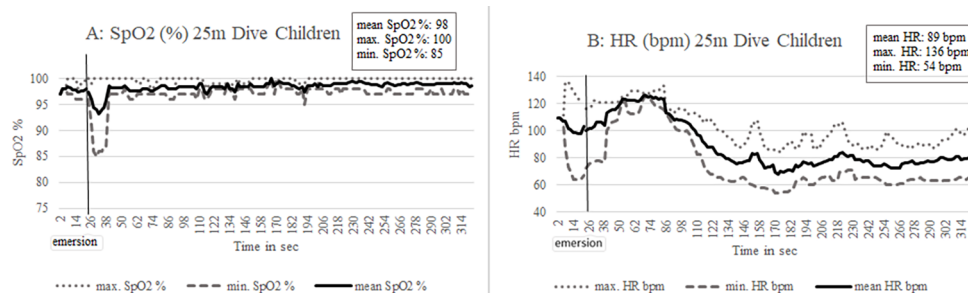


FIGURE 1

Twenty-five meters dive children: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

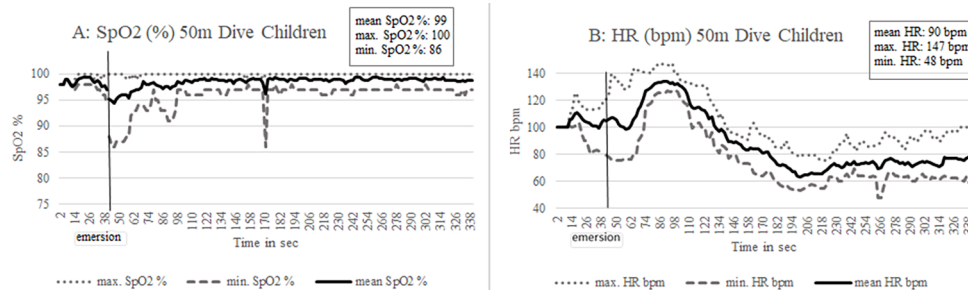


FIGURE 2

Fifty meters dive children: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

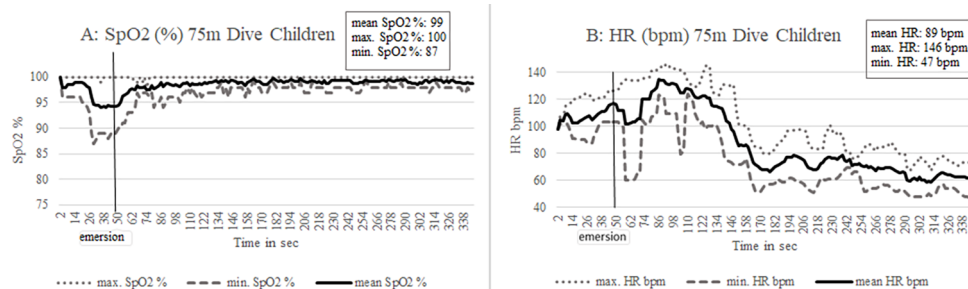


FIGURE 3

Seventy-five meters dive children: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

while diving (Spearman's $\rho = .473$, $p = .007$). Consequently, a longer dive time is associated with a higher heart rate, indicating a rising activation of autonomic nervous system. No correlation was found between dive time and minimal transcutaneous oxygen saturation under water (Spearman's $\rho = -.073$, $p = .696$). The PI showed significant drop with immersion, but no significant trend in the PI can be identified during the dives based on the available data.

3.3 Time for recovery

There was a resting period of 10 min after each dive. Table 2 shows the time in seconds until average values of transcutaneous oxygen saturation and heart rate prior to dive are restored after emersion. No statistically significant difference in recovery times for 50 and 75 m dive was found between Children and Adults ($\alpha = 0.05$). Average recovery times for oxygen saturation were

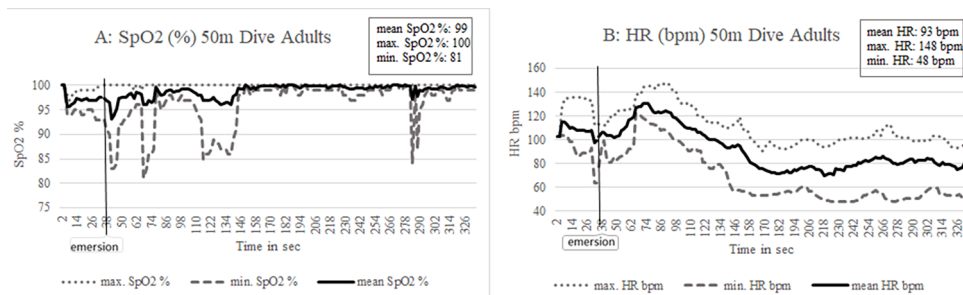


FIGURE 4

Fifty meters dive adults: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

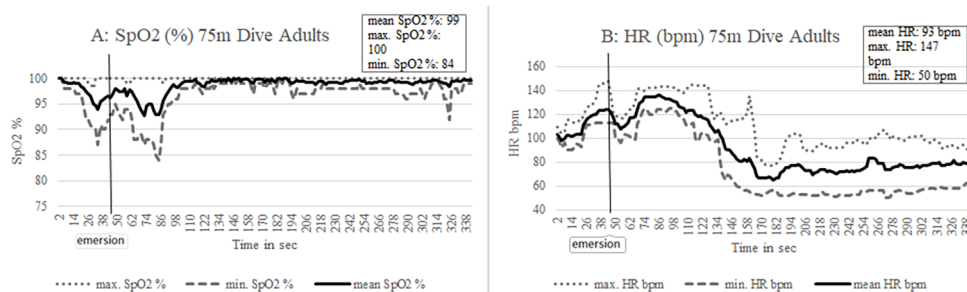


FIGURE 5

Seventy-five meters dive adults: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

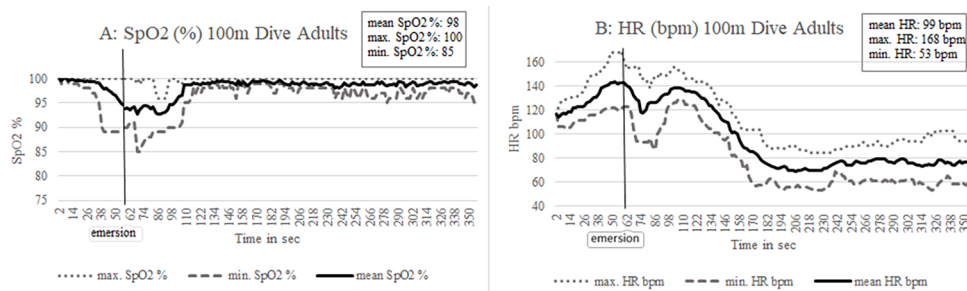


FIGURE 6

Hundred meters dive adults: average, maximum and minimum values of transcutaneous oxygen saturation (A) and heart rate (B) are displayed over dive time (total average, maximum and minimum values are shown in the box).

below 1 min with a maximum of 72 s (75 m dive, Adults) to regain the base level. Normalization of the heart rate takes at a minimum 1 min, with average values around 100 s and maximum times of 160 s (50 m dive, Children). Dive time did not correlate significantly with minimum oxygen saturation or maximum heart rate post diving in Spearman's correlation [min SpO₂ post diving (%): $\rho = .011$, $p = .956$; max heartrate post diving (1/min): $\rho = .244$, $p = .202$]. Minimum oxygen saturation level reached after emersion

strongly affects the time to recover to baseline levels (Spearman's $\rho = -.744$, $p = .000$). The time needed to recover baseline oxygen saturation is also correlated moderately with maximum heart rate during the dive (Spearman's $\rho = .429$, $p = .023$), whereas the time to recover baseline heart rate shows no significant correlation with any oxygen saturation parameters during or after the dive.

The ratio between body fat percentage and lean body mass influences the minimal oxygen saturation post diving as well as

TABLE 2 Recovery times for oxygen saturation and heart rate in children and adults.

	Diving distance	Group of children (N = 5)			Group of adults (N = 6)			p-value
		Mean	Min.	Max.	Mean	Min.	Max.	
Time to normalize tO ₂ % (s)	25 m	4.67 (N = 3)	0	14				
	50 m	22.00	0	64	26.00 (N = 5)	0	44	0.597
	75 m	18.40	0	40	44.00 (N = 4)	20	72	0.086
	100 m				44.00	16	68	
Time to normalize heart rate (s)	25 m	125 (N = 2)	94	156				
	50 m	115.60	90	160	103.4 (N = 5)	86	125	0.602
	75 m	94.00	60	116	104.75 (N = 4)	94	122	0.462
	100 m				104.33	86	120	

the time to normalize SpO₂%. There was a negative correlation between lean body mass and minimal oxygen saturation post diving (Spearman’s $\rho = -.405$, $p = .027$). Besides, lean body mass correlated with time to normalize SpO₂% (Spearman’s $\rho = .492$, $p = .008$). Body fat percentage behaved in exactly the opposite way, correlating negatively with time to normalize SpO₂% (Spearman’s $\rho = -.504$, $p = .006$) and positively with minimal oxygen saturation post diving (Spearman’s $\rho = .387$, $p = .035$).

3.4 Influence of dive speed

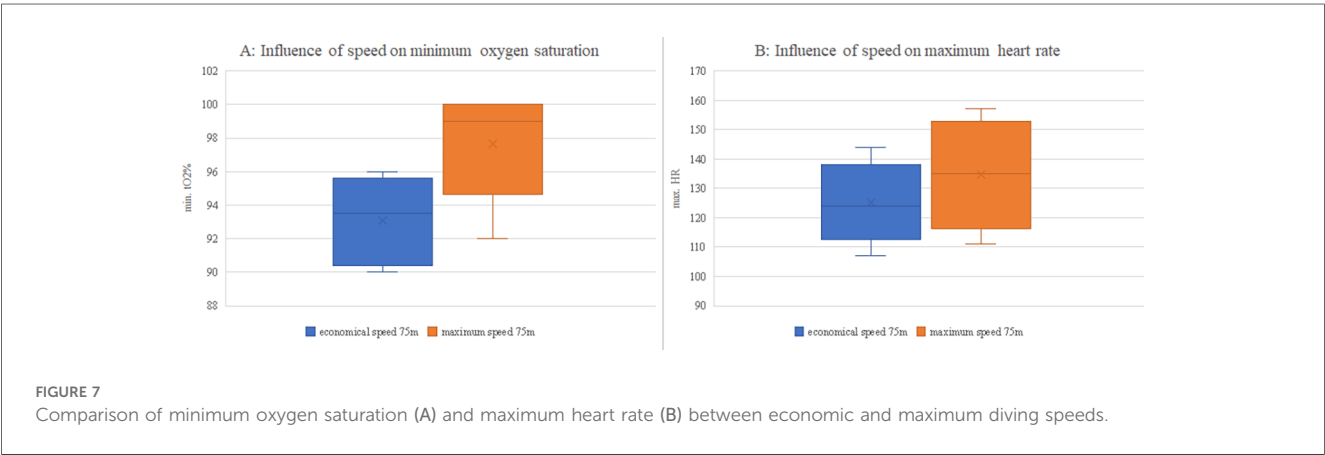
Adult participants were asked to perform two 75-meter dives: one at an economical pace and the other at their individual maximum speed, to determine the influence of speed on oxygen saturation and heart rate. They adhered to the instruction and completed the second 75 m much faster than the first with an average pace of 1,96 m/s for the economical and 2,63 m/s for the maximum speed lap ($p = 0.028$). This had an impact on oxygen saturation and heart rate within the apnea phase as seen in Figure 7. Despite the expected lower muscular work load, a significantly larger drop in mean oxygen saturation ($p = 0.028$) and minimum oxygen saturation ($p = 0.042$) was seen while diving the individual more comfortable economical pace which automatically led to more apnea time. The cardiac response reflected the difference in muscular work load over the two dives. A dive with maximum speed, significantly increases mean heart rate ($p = 0.028$), minimum heart rate ($p = 0.028$) and maximum

heart rate ($p = 0.027$) compared to a dive at an economical pace over the same distance. In total, the speed of the 75-meter dive was significantly correlated with the minimum heart rate underwater (Spearman’s $\rho = 0.669$, $p = .017$), underlining the relationship between diving speed and cardiac workload. Interestingly, the recovery parameters seem to not differ significantly depending on the dive speed.

4 Discussion

This is the first study that examined the short-term physiological adaptations during dynamic apnea dives under life-like conditions. It provides a dataset of 11 elite fin swimming athletes, who were analyzed for heart rate, oxygen saturation, and perfusion index (PI) using transcutaneous pulse oximetry to better understand physiologic adaption parameters during dynamic apnea. The findings contribute important insights into the physiological demands of shallow apnea diving.

A central mechanism during apnea diving is the diving response (bradycardia and central volume shift by peripheral vasoconstriction), a phenomenon provoked by vagal influence (6). Our study corroborated this response in most subjects, with heart rate reductions observed after entering the water particularly over the 50 m dive. This aligns with other studies documenting diving-induced bradycardia in both Children and Adults (4, 8). The exception of increased heart rate over the 100 m dive with maximal heart rates reached already when



emerging, most likely reflects sympathetic activation overpowering the parasympathetic trigger in autonomic regulation due to the heightened physical and metabolic stress and possibly psychological stress of longer dives (3). This assumption is underpinned by the moderate correlation found between dive duration and the intraindividual range from highest to lowest measured heart rate while diving. Interestingly, this phenomenon seems to be related to the work load and stress levels as no such correlation could be found between dive time and minimal oxygen saturation during the dive. The lowest oxygen saturation levels were reached after resurfacing, rather than during the dive itself. This supports the idea that the diving response—including bradycardia and peripheral vasoconstriction—helps conserve oxygen even under dynamic apnea (7). Once the dive ends and normal circulation resumes, the oxygen debt from peripheral tissues has to be paid off. This finding has practical implications for apnea training and safety protocols. Trainers and medical professionals should be aware that oxygen levels may not reach their lowest point during the dive itself, but rather just after it, making post-dive recovery observation, for at least 1 min, essential. Individuals with higher lean body mass showed lower post-diving $\text{SpO}_2\%$ and delayed recovery in oxygen saturation levels. During a prolonged breath-hold, oxygen stores are gradually depleted and the body shifts toward anaerobic metabolism to sustain muscle function (17). Individuals with more lean body mass may accumulate higher levels of anaerobic byproducts like lactate and also have larger myoglobin for oxygen storage, which increases the oxygen debt that needs to be replenished after resurfacing.

Nevertheless, interindividual differences should be taken into account as in our small cohort there was one participant whose lowest oxygen values were reached continuously during the dive. No pre-disposing condition was found, that could explain these findings in this participant.

The protocol included both fast and slow dives over a distance of 75 m, allowing for a direct comparison of the impact of speed on heart rate and oxygen saturation. Faster dives resulted in greater cardiovascular stress, as seen in higher heart rates during maximum-speed dives. In contrast, a slower, subjectively more economical dive resulted in a more pronounced drop in oxygen saturation, as the apnea duration was extended. This suggests that oxygen reserves are primarily affected by the length of the apnea period rather than the intensity of physical effort when diving the same distance. On the other hand, faster dives are associated with a higher cardiac workload, as reflected by the significant increase in heart rate. Taking into account, that recovery parameters remain largely the same across both dive types, it highlights how different dive strategies can affect physiological responses. Based on specific goals it can be beneficial to either minimizing oxygen consumption or managing cardiovascular strain. While this study focused on the influence of speed over a fixed distance, it is worth investigating how speed affects physiological responses when dive duration is controlled instead of distance. By allowing participants to dive at varying speeds over the same time period, the interplay between speed, oxygen saturation, and heart rate might be more clearly

delineated, offering a more comprehensive understanding of the effects of different diving strategies.

One main interest of this study was to find out if Children and Adults respond differently exposed to immersed dynamic apnea. Summarized, the investigated parameters of Children in comparison to Adults over 50 and 75 m behave very similar. Basic heart rate responses were equal. One exception must be made for the transcutaneous oxygen saturation levels after emersion, which were significantly lower in the adult group. Nevertheless, these findings have to be interpreted with caution, with the small number of study subjects in mind and with consideration for the fact that the two groups have similar cardiovascular and pulmonary data. Further research is needed to investigate differences in the diving response for dynamic apnea of Children and Adults with attention to the oxygen saturation levels after emersion. Interestingly, no sex differences in diving response were observable. There are expectable differences in anthropometric data as well as in cardiovascular and pulmonary data but these variations seem to have no general influence on the requested performance. Looking at world records stated by AIDA (International Association for the Development of Apnea) for static apnea, to date, 11:35 min are held by male and 09:07 min by female athletes (12). When it comes to peak performances, male subjects could have a slight physiological advantage as seen in many competitive sports. A comprehensive analysis of the study subjects characteristics shows a study population of moderate to highly trained subjects. Many participants of this study showed an incomplete right bundle branch block, indicating an increased vagal tone (18, 19). Former findings of studies on diving responses suggest a longer apnea time and more pronounced bradycardia reached by trained divers compared to untrained individuals (4). This allows the assumption, that the performance by trained fin swimmers seen in this study is above average which also means, that diving distance and apnea time would probably not be reached by merely recreational divers. Consequently, this dataset can be seen as an upper baseline, showing normal to above-average divers and their diving response and further studies are needed to investigate standard population and in the longer term even medically restricted subpopulations to complete the whole picture.

The diving response in mammals includes a reflexive contraction of the spleen whose significance still needs to be examined more closely (9, 10). Schagatay described a mean reduction of 18% in spleen volume after three apneas (9). Due to the test setting, we measured spleen surface in two diameters with mobile echocardiography. A slight reduction of 4% in surface area could be seen in our study population, underlining the interplay between immersion and spleen contraction already observed in former studies.

The analysis of the PI reveals no consistent trend. The authors consider multiple factors that may have contributed to these results, including contact with cold water by hand during the preparation phase and the limited measurement area. Other studies have documented a decrease in PI when the diving response is triggered by cold water face immersion due to the peripheral vasoconstriction (4, 8).

5 Conclusion

This study provides one of the first real life data sets reporting on physiological adaptations during dynamic apnea in the young, emphasizing significant distinctions between Children and Adults. Notably, while both Children and Adults exhibited similar heart rate responses, Adults experienced significantly lower post-dive oxygen saturation levels, emphasizing the need for further research into age-related differences in diving physiology. The results indicate that the duration of apnea primarily affects oxygen saturation, while dive speed influences cardiovascular workload. Interestingly, oxygen levels often decline only after the dive, suggesting a post-dive oxygen debt that must be addressed during recovery. This highlights the importance of post-dive recovery monitoring, as oxygen levels may continue to decrease after surfacing. This study lays a foundational dataset for understanding how the body adapts to the demands of dynamic apnea in Children and young Adults and future research should expand upon these findings by examining a broader demographic, including recreational divers and medically restricted populations.

6 Limitations

This prospective monocentric study was non-blinded, owed to the test setting and active surveillance of all participants while performing their protocol. The small number of 11 participants is the main limitation of this study. Data shows long diving periods with real-life locomotory muscle activity, but study subjects did not reach complete exhaustion. Thereby, we cannot predict critical cut-off values with certainty. A further limitation is the use of a clinical device not qualified for use under water. Although the pulse oximeter was well-prepared, we can not completely exclude aberrant values because of off-label use.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethik-Kommission an der Medizinischen Fakultät der Universität Leipzig. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SF: Data curation, Formal analysis, Visualization, Investigation, Writing – original draft, Writing – review & editing. SP: Data

curation, Investigation, Writing – original draft, Writing – review & editing. JW: Writing – review & editing. AM: Writing – review & editing. RG: Writing – review & editing. MW: Writing – review & editing. ID: Writing – review & editing. DM: Writing – review & editing. MP: Writing – review & editing. JW: Writing – review & editing. CP: Supervision, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The current study was funded by the Deutsche Herzstiftung e.V.

Acknowledgments

The authors would like to thank Stefanie Makosch (Senior Clinical Specialist) for providing essential technical support during the experimental phase. We would like to acknowledge the support of the Institute for Applied Training Science (Leipzig) for providing the necessary resources to carry out this research. We are also deeply grateful to the athletes who generously volunteered their time and effort to participate in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fspor.2025.1515674/full#supplementary-material>

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RECEIVED 18 November 2024

ACCEPTED 28 February 2025

PUBLISHED 08 April 2025

CITATION

Domaradzki J, Popowczak M,
Kochan-Jacheć K, Szkudlarek P,
Murawska-Ciałowicz E and Koźlenia D (2025)
Effects of two forms of school-based
high-intensity interval training on body fat,
blood pressure, and cardiorespiratory fitness
in adolescents: randomized control trial with
eight-week follow-up—the PEER-HEART
study.
Front. Physiol. 16:1530195.
doi: 10.3389/fphys.2025.1530195

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Effects of two forms of school-based high-intensity interval training on body fat, blood pressure, and cardiorespiratory fitness in adolescents: randomized control trial with eight-week follow-up—the PEER-HEART study

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Introduction: This study examined the effects of 8-week interventions based on two variants of typical exercises, namely, high-intensity interval training (HIIT) and high-intensity plyometric training (HIPT), on body fat (BF%), blood pressure, and cardiorespiratory fitness (CRF). In addition, the sustainability of the effects after another 8 weeks was assessed.

Methods: The project was designed as a randomized controlled trial with eight groups of participants (two variants, two sexes, and two groups (experimental and control)) and was conducted in a school physical education (PE) program. The outcomes analyzed were the BF%, systolic (SBP), diastolic blood pressure (DBP), and CRF expressed in terms of maximum oxygen uptake (VO_{2max}). A total of 307 healthy adolescents participated in this study and were randomly assigned into the two groups. During the 8 weeks, the participants completed two exercise sessions each week with progressively increasing volumes. For the first 2 weeks, the sessions involved four rounds of 20 s of intense effort followed by 10 s of rest; this increased to six rounds during weeks 3–4 and eight rounds during weeks 5–8. The HIPT program was based on plyometric exercises, whereas the HIIT was based on bodyweight resistance exercises.

Results: Multidimensional analysis of variance (ANOVA) indicated a statistically significant second-order interaction (time \times variant \times group: $\Lambda = 0.943$, $F = 2.20$, $p < 0.027$, $\eta^2_{pG} = 0.057$, $d = 0.25$), confirming the changes in the BF%, SBP, DBP, and VO_{2max} dependent on the type of intervention and group assignment. The ANOVA results revealed significant main and interaction effects for BF%, SBP, and DBP, with time and the HIIT variant as the main contributors (BF%: $F = 3.911$, $p = 0.023$, $\eta^2_{pG} = 0.001$, $d = 0.04$ vs. $F = 9.900$, $p < 0.001$, $\eta^2_{pG} = 0.001$, $d = 0.03$;

SBP: $F = 31.801$, $p < 0.001$, $\eta^2_{pG} = 0.012$, $d = 0.16$ vs. $F = 8.939$, $p = 0.003$, $\eta^2_{pG} = 0.026$, $d = 0.16$; DBP: $F = 3.470$, $p = 0.033$, $\eta^2_{pG} = 0.002$, $d = 0.06$ vs. $F = 4.982$, $p = 0.026$, $\eta^2_{pG} = 0.014$, $d = 0.12$). The second-order interaction for VO_{2max} (time \times sex \times group: $F = 6.960$, $p = 0.001$, $\eta^2_{pG} = 0.003$, $d = 0.05$) indicated that the improvements over time were not related to the training variant. Although these effects were small (low eta values), post hoc tests (all comparisons in post-intervention, $p > 0.05$) showed that both the HIIT and HIPT groups exhibited beneficial changes compared to controls; however, no statistically significant differences were observed between the experimental and control groups. Furthermore, the observed improvements were maintained through the 8-week follow-up period, as demonstrated by no significant changes between the post-intervention and follow-up measurements ($p > 0.05$). Discriminant analysis showed that BF% and SBP were the key variables for the two exercise variants in men, with HIPT yielding greater reductions in SBP and HIIT resulting in more pronounced decreases in BF%.

Discussion: In conclusion, both HIIT and HIPT interventions effectively improved health-related parameters, providing valuable enrichment to the PE lessons in schools. These benefits were also sustained for at least 8 weeks post-intervention.

KEYWORDS

physical education, adolescent, body composition, cardiovascular fitness, health, school-based setting, plyometric exercises

1 Introduction

Over the past three decades, obesity among youth has become a global health concern because of its increasing prevalence. According to data from the World Health Organization, over 390 million children and adolescents aged 5–19 years were overweight in 2022, including 160 million affected by obesity (World Health Organization, 2023). The projections indicate that the obesity rates could reach 20% in boys and 18% in girls by 2035 (Lobstein et al., 2023). This situation is particularly concerning in Poland, where approximately 29% of the adults were obese in 2019 (Pawlewicz, 2024); forecasts predict further growth of this number, with obesity affecting more than 35% of men and 25% of women by 2035 (Lobstein et al., 2023). Furthermore, the WHO European Region has yet to implement measures to tackle this epidemic (World Health Organization Regional Office for Europe, 2021). The health consequences of obesity in youth are severe and tend to worsen in adulthood. Obese adolescents are prone to higher risks of cardiometabolic diseases, including hypertension, type 2 diabetes, and atherosclerosis, which could lead to increased morbidity and mortality compared to their normal-weight peers (Després et al., 2008; Kumar and Kelly, 2017; Di Angelantonio et al., 2016; Baker et al., 2007; Weihrach-Blüher et al., 2019). In addition, obesity negatively impacts mental health, contributing to depression, reduced quality of life, discrimination, and poor academic performance (Pont et al., 2017; Ma et al., 2021; Ladd et al., 2017). A study across 32 countries found that normal-weight children have a 13% higher chance of better academic performance than those with obesity (Devaux and Vuik, 2019).

Regular physical activity is a key intervention for preventing obesity and improving metabolic health in youth. High-intensity interval training (HIIT) has been shown to be effective in

improving body composition, increasing cardiorespiratory fitness (CRF), and reducing cardiovascular risk (Carson et al., 2014; Khalafi et al., 2022; Robinson et al., 2015). Owing to its short duration and efficiency, HIIT is particularly appealing in school settings, where it offers significant health benefits with less time commitment from youth who may otherwise be discouraged by longer activities (Weston et al., 2021; Chuensiri et al., 2018). Physical education (PE) classes typically lasting 45 min can provide an ideal environment for integrating HIIT, especially when using the Tabata protocol (Peake et al., 2015). Recent studies have shown that prolonged exercise sessions during PE in schools are unattractive to youth, resulting in decreased motivation and lower exercise intensity (Weston et al., 2021; Duncombe et al., 2024). Thus, identifying student-friendly exercise formats that can sustain high intensity is essential. Comparative experiments have demonstrated varying effectiveness among exercise variants (Murawska-Ciałowicz et al., 2021). Our previous research that used a modified Tabata program was shown to reduce body fat, lower blood pressure, and improve CRF; however, the intensity and motivation declined over time (Domaradzki et al., 2020, 2022). In the present study, we introduce high-intensity plyometric training (HIPT) as a potentially more engaging and effective variant for students; its effectiveness has been supported by studies in athletes (Vácsi et al., 2013; Söyler et al., 2024). The protocol emphasizes plyometric exercises aimed at maximizing rapid repetitions and total work done (Davies et al., 2015). Such explosive movements engage the fast-twitch muscle fibers, potentially enhancing muscle power and anaerobic performance. Plyometric training is characterized by rapid eccentric-concentric transitions that can improve motor function, muscle strength, and endurance in youth (Staniszewski et al., 2024). Combined with HIIT, it may further enhance muscle strength, power, and local

muscular endurance (Hammami et al., 2021; Moran et al., 2017). For instance, Racil et al. (2016) found that adding plyometric exercises to a HIIT program improved body composition and jumping ability in obese adolescent girls. For comparison, we introduce two variants in the present study: the standard HIIT protocol (Domaradzki et al., 2020, 2022) and a modified HIPT protocol incorporating plyometric exercises. Both programs were administered with the aim of maximizing the number of repetitions during the work phase, thereby increasing the demands on the cardiovascular-respiratory systems and energy metabolism. In our present study, the HIIT program entailed bodyweight resistance exercises to impose a greater load on the musculoskeletal system, whereas the HIPT program utilized high-speed plyometric exercises to facilitate a higher number of repetitions, potentially increasing the total work performed. Further research on HIIT variants, including those with plyometric elements, are crucial for developing effective school health programs, especially considering that the sustainability of these effects remain unexplored in school settings.

Therefore, the aim of the present study was twofold: (1) to determine whether body fat percentage (BF%), systolic and diastolic blood pressure (SBP/DBP), and CRF change after 9 weeks of high-intensity training using the two intervention variants, namely, HIIT and HIPT; (2) to assess the sustainability of the results induced by these interventions 8 weeks after completion. Many studies lack assessments of the durability effects and focus only on the intervention period. It is hypothesized that integrating both types of interventions into PE classes could serve as time-efficient and effective methods for enhancing physical fitness while mitigating obesity-related health issues among youth. Specifically, both programs are expected to significantly reduce body fat percentage and improve CRF and blood pressure compared to the baseline values. Moreover, we anticipate that the HIPT variant will produce greater improvements in these parameters by allowing a higher number of repetitions through high-speed plyometric movements. Finally, these beneficial effects are expected to be sustained for at least 8 weeks after completion of the intervention.

2 Materials and methods

2.1 Ethics committee

The Ethics Committee of the Wrocław University of Health and Sport Sciences granted approval for this study (ECUPE no. 33/2018; approval date: 31 October 2018). The research adhered to all ethical principles for medical research involving human subjects, as outlined in the Declaration of Helsinki by the World Medical Association.

2.2 Clinical trial registration

The study was conducted in Wrocław, Poland, in 2024 as project supported financially by the state budget under the Polish Ministry of Science and Higher Education program titled “Science for Society II” (project no. NdS-II/SP/0521/2023/01). The study has been registered as a clinical trial at clinicaltrials.gov with ID: NCT06431230 and the acronym PEER-HEART for

“Physical Education dosE Response Health markErs Adolescents inteRval Training.”

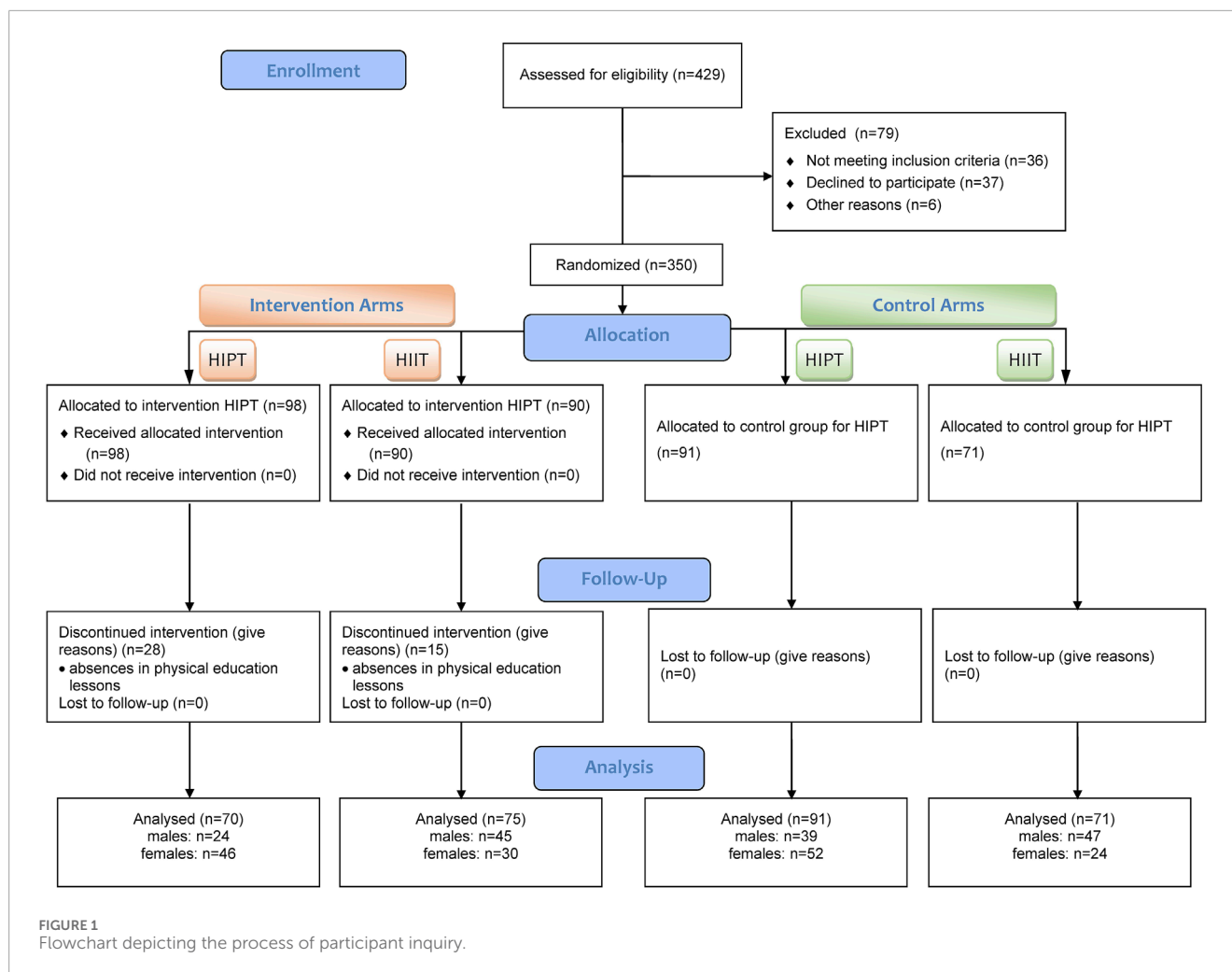
2.3 Participants

G*Power v.3.1 (Faul et al., 2007) software was used for the basic calculations by assuming the multidimensional analysis of variance (MANOVA) method for eight groups (two schools, boys and girls separately under the experimental and control groups) and three repeated measurements (baseline, post-intervention, and follow-up). For an effect size of 0.20, an alpha level of 0.05, and a power of 0.95, the minimum sample size required was 310 participants. The study sample consisted of individuals from two schools, each participating in two versions of high-intensity training, namely, the plyometry-based HIPT method and the previously used modified Tabata protocol (HIIT) as the standard, along with two control groups from both schools. Initially, 429 participants were assigned to the project across thirteen classes in each school. The group assignments were determined through a simple, non-returnable randomization using the online tool available at www.randomization.com (accessed on 08.01.2024). Each class was labeled with a number from 1 to 13 in both schools, and the students from each class were at the same educational level and followed the same PE curriculum. The HIIT inclusion criteria were as follows: the participants must be first-year high school students who are regularly enrolled in and participate in PE classes; they must be free from any medical conditions or contraindications, such as cardiovascular, respiratory, or musculoskeletal disorders, that could prevent them from safely engaging in high-intensity exercises; they should not be involved in any additional structured high-intensity training programs outside the standard school curriculum; lastly, informed consent must be provided, and parental or guardian consent must be obtained for minors. Prior to the group allocations, some of the subjects were excluded owing to refusal to participate, medical contraindications, or participation in additional sports activities within the past 6 months ($n = 79$). A few more students were excluded during the intervention due to absence exceeding 20% during the PE lessons ($n = 43$). No adverse effects were observed from the HIIT interventions. The final analysis included ($n = 307$) adolescent students. The flow structure of participant selection for the interventions is detailed in [Figure 1](#). Participation was voluntary, and the students could withdraw at any time. Both students and their parents/guardians were informed about the study objectives and procedures. Written informed consent was obtained from the school principals, parents, and study subjects before participation.

2.4 Anthropometric measurements

2.4.1 Procedures

Morphological measurements were obtained at three time points: before the 8-week intervention, immediately after the intervention, and at the end of an 8-week follow-up period. These assessments were conducted on a single day between 8:00 a.m. and 1:00 p.m. in sports halls under standardized conditions for each group. The participants wore T-shirts, shorts, and shoes for the tests, although the anthropometric measurements were obtained without shoes. First, the body height and composition were measured. On a



different day, the blood pressure was measured, following which the beep test was performed. The students, parents, and teachers were informed of the specific rules for all tests before commencement of the project. All procedures were conducted thrice during the three time points mentioned above.

2.4.2 Body morphology

Body height measurements were obtained to the nearest 0.1 cm using anthropometers (GPM Anthropological Instruments, DKSH Ltd., Switzerland). The body height was additionally measured using an anthropometer in the standing position barefoot according to the International Society for the Advancement of Kinanthropometry (ISAAK) (Marfell-Jones et al., 2006). The bodyweight and BF% were measured with a Tanita Inner Scan V (BC-601 model, Tanita Co., Tokyo, Japan). The reliabilities of these methods were verified in a prior research by Ramírez-Vélez et al. (2016). Prior to the measurements, the students received instructions on the examination procedures. They were asked to void their bladders, avoid excessive water intake, and maintain their usual breakfast routines. For the measurements, the participants were required to be barefoot and shirtless, ensuring that their heels were placed on the rear electrodes of the scale, with legs straight at the knee and hip joints, arms slightly abducted and flexed at the shoulder joints, elbows straight, and fingers touching the manual electrodes. These

measurements were conducted in the evening at least 3 h after the last meal of the day. The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

2.5 Blood pressure measurements

All blood pressure measurements were performed using an Omron BP710 Automatic Blood Pressure Monitor (Omron Healthcare, Inc., Hoffman Estates, IL, United States) (Nasir et al., 2021). The appropriate cuff sizes were selected based on the participants' upper arm circumferences. The participants were asked to sit quietly for 10 min before the measurements, and the blood pressure readings were acquired three times at 10-min intervals. The average of these three readings was used for the analysis. The blood pressure readings were measured before and after the intervention period as well as during follow-up.

2.6 Multistage fitness test

To assess the maximal heart rate, we performed the multistage fitness test (MSFT), and the participants were measured using the Polar Verity Sense sensors (Polar Electro, Kempele, Finland)

(Navalta et al., 2023). The MSFT involves continuous running between two lines spaced 20 m apart and synchronized to a series of recorded beeps. The participant starts running at a speed of 8.5 km/h, stops at each marked line, turns 180°, and runs back. As the test progresses, the pace increases by 0.5 km/h every minute, which is indicated by a sound signal. The test continues until the participant can no longer maintain the required speed. The maximum oxygen uptake (VO_{2max}) was calculated based on the following formula (Ramsbottom et al., 1988):

$$VO_2 \text{ max} = 3.46 * (L + SN / (L * 0.4325 + 7.0048)) + 12.2,$$

where L is the level and SN is the number of shuttles.

2.7 Interventions

The HIIT program based on the Tabata method was implemented during two PE classes each week over an 8-week period, where the training volume increased progressively. The intensity of the workouts was monitored using the Polar Verity Sense devices (Polar Electro, Kempele, Finland), and the target intensity was set to a specific percentage of the maximum heart rate. In the other PE classes, the students followed the school's standard curriculum for first-year students, which focused on improving their skills in various sports. The Polar Verity Sense sensors were used for monitoring the heart rate to control the effort intensity target at 75%HR_{max} as established by the beep test (Duncombe et al., 2024).

During the sessions incorporating the intervention protocol, the students began with a 10-min standardized warm-up. Then, they engaged in HIIT exercises comprising four rounds of 20 s of intense effort, followed by 10 s of rest for the first 2 weeks; this pattern shifted to six rounds during the third and fourth weeks and increased to eight rounds during the final 4 weeks (Table 1). The HIPT program included exercises such as ankle hops, burpees, high knees, shoulder taps to hand claps, butt kicks, two-leg mountain climbers, squat jumps, and alternating leg mountain climbers. The HIIT program featured squats, no-jump burpees, lunges, shoulder taps, lateral squats, push-ups, standing abs twists, and sit-ups. All sessions of the intervention program were supervised. Students received constant feedback over the duration of each work and rest rounds from the supervisor, and they could also see the timer. All exercise rounds were performed in an “all-out” manner. The students were constantly motivated to perform as many repetitions as possible. Subsequently, a standard PE program was implemented to develop comprehensive motor skills, including both team games (e.g., soccer, volleyball, and basketball) and individual disciplines (e.g., athletics and gymnastics). The lesson structure for the control groups was identical, except for the absence of the HIIT and HIPT interventions. The two intervention structures are presented in Figure 2.

The mean resting heart rate (HR) values for the four groups of men (HIIT E, HIIT C, HIPT E, and HIPT C) were assessed at three time points: before intervention (Pre), immediately after intervention (Post), and at follow-up (FU). In the HIIT E group, the measured HRs were 72.3 ± 7.5 bpm (70.1–74.0) at Pre, 72.1 ± 6.3 bpm (70.2–74.0) at Post, and 76.0 ± 8.9 bpm (73.3–78.7) at FU; similarly, the values for the HIIT C group were 71.5 ± 6.3 bpm (69.7–73.4) at Pre, 72.6 ± 6.9 bpm (70.5–74.6) at Post, and 72.2 ±

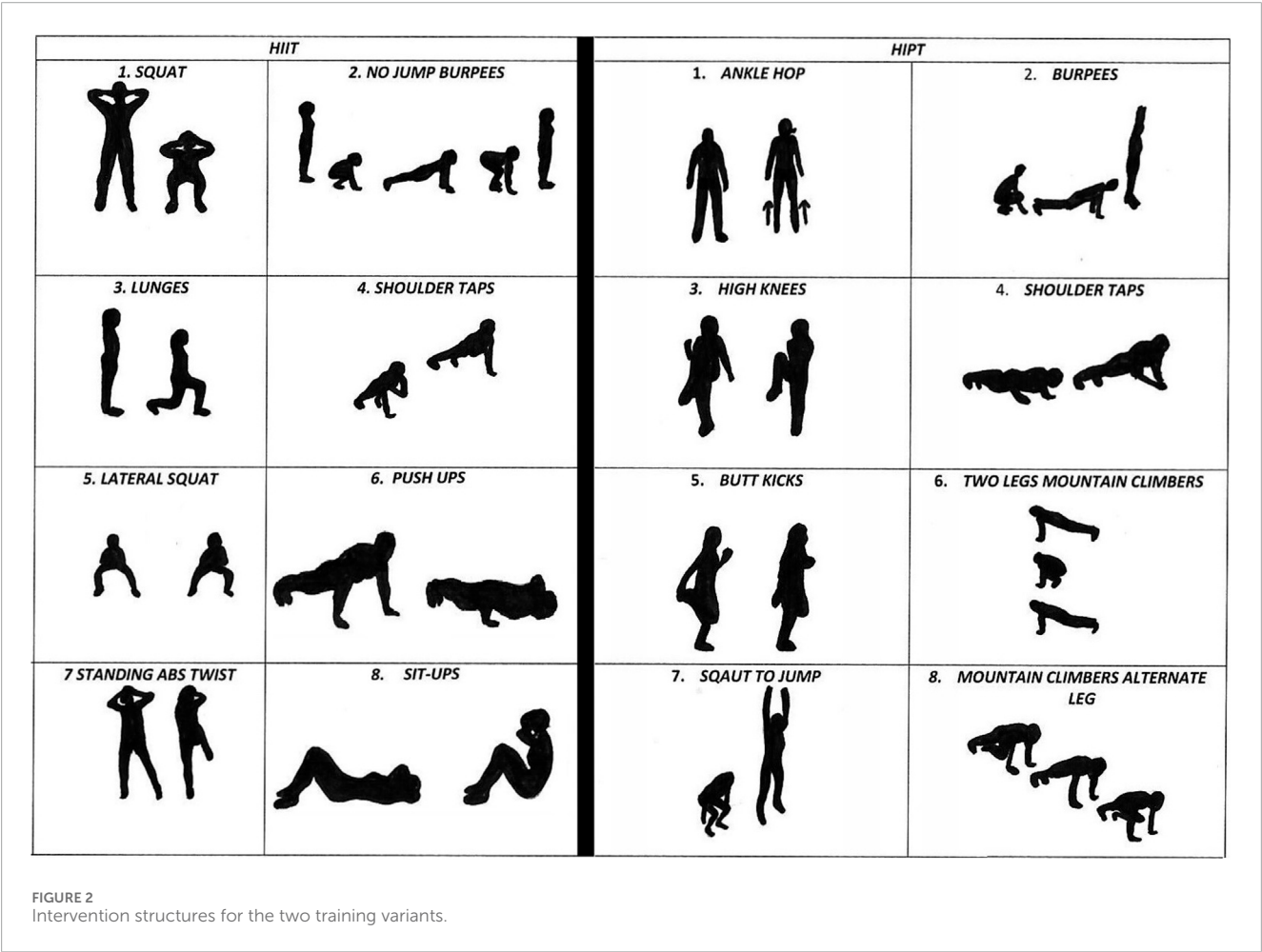
8.5 bpm (69.5–74.5) at FU. In the HIPT E group, the measured HRs were 75.0 ± 5.7 bpm (72.6–77.5) at Pre, 75.6 ± 5.6 bpm (73.3–78.0) at Post, and 71.5 ± 7.1 bpm (68.5–74.6) at FU; similarly, the values for the HIPT C group were 73.4 ± 6.8 bpm (71.2–75.6) at Pre, 71.3 ± 6.4 bpm (69.2–73.4) at Post, and 71.0 ± 8.5 bpm (68.5–73.7) at FU. The mean resting HR values for the four groups of women were as follows: in the HIIT E group, the measured HRs were 72.1 ± 5.7 bpm (69.9–74.3) at Pre, 74.9 ± 6.3 bpm (72.6–77.1) at Post, and 72.0 ± 8.3 bpm (68.9–75.2) at FU; in the HIIT C group, the measured HRs were 72.8 ± 8.5 bpm (69.2–76.4) at Pre, 70.8 ± 7.4 bpm (67.7–73.9) at Post, and 76.2 ± 7.4 bpm (73.1–79.3) at FU; in the HIPT E group, the measured HRs were 75.0 ± 6.0 bpm (73.2–76.8) at Pre, 74.7 ± 6.1 bpm (72.9–76.9) at Post, and 74.3 ± 8.6 bpm (71.7–76.9) at FU; in the HIPT C group, the measured HRs were 74.1 ± 5.8 bpm (72.4–75.7) at Pre, 74.3 ± 6.7 bpm (72.4–76.1) at Post, and 74.2 ± 8.7 bpm (71.8–76.7) at FU. All data values are presented as mean ± standard deviation, with the 95% confidence intervals shown within parentheses.

2.8 Statistics

Before conducting the statistical analysis, all assumptions were verified and the usefulness of the chosen methods was confirmed. Given the large sample size, the Kolmogorov–Smirnov test was used to assess the normality of the continuous variable distributions. A slight deviation from normal distribution was observed for body fat values; in this case, the inference was confirmed using non-parametric tests, including the Kruskal–Wallis analysis of variance (ANOVA) for comparisons across the groups or the Wilcoxon signed-rank test for repeated measures within each group. When the interpretation of results from both methods was consistent, it was assumed that the parametric ANOVA was robust against violations of variance homogeneity assumptions. MANOVA and mixed-effect analysis of variance (ME ANOVA) were employed to test the effects of the interventions on the studied variables. Four-way models were used with sex (males (M) and females (F) as levels), group (experimental (E) and control (C) as levels), intervention variants (levels) as the between-group factors, and time (three points: pre-intervention, post-intervention, and follow-up denoted by PRE, POST, and FU, respectively, as levels) as the within-group factors. Before analysis, the homoscedasticity and sphericity were tested using Levene's and Mauchly's tests, respectively, along with the Greenhouse–Geisser correction when the sphericity assumption was violated. The effect size was assessed using the generalized partial eta square (η^2_{pG}) and converted to Cohen's d value (d) (Lakens, 2013). In cases where we observed significant effects, a post hoc test with Bonferroni correction was used. All procedures were conducted twice, once for the basic anthropometric measurements to study the general differences between the groups and then for the outcomes of interest, namely, BF%, SBP, DBP, and CRF (in terms of VO_{2max}). The effects between the two variants were compared using the discriminant analysis (DA) method; this approach allows identification of the set of variables that can best distinguish between the two categories, which unlike the ANOVA enables multivariate analyses. Standard DA was conducted using the delta (Δ) of the body fat percentages, blood pressure values, and maximum oxygen uptake as pre–post differences, with Wilks'

TABLE 1 Weekly HIIT variant intensities vs. percentage of the maximal heart rate [%].

Week	HIPT (n = 70)		HIIT (n = 75)	
	Mean ± SD (95% CI) [%]	Min–max [%]	Mean ± SD (95% CI) [%]	Min–max [%]
1	80.7 ± 6.8 (79.1–82.3)	65–97	85.7 ± 6.1 (84.3–87.1)	66–97
2	79.7 ± 6.8 (78.1–81.3)	61.9–94.8	82.4 ± 5.8 (81–83.7)	66.1–96.2
3	78.4 ± 8.2 (76.4–80.3)	52.6–90.1	81.3 ± 6.2 (79.9–82.7)	65.3–92.5
4	78.7 ± 7.7 (76.9–80.6)	53.1–92	81.2 ± 6.3 (79.8–82.7)	62.7–93.8
5	76.4 ± 8.6 (74.3–78.4)	48.7–90.4	79.2 ± 8 (77.4–81.1)	52.9–93
6	76 ± 9.5 (73.7–78.3)	49.1–89.9	78.5 ± 8 (76.6–80.3)	41.3–91.7
7	77.4 ± 6.6 (75.8–79)	62.4–91.8	79.5 ± 6.3 (78.1–81)	57.9–90.9
8	75.9 ± 8.6 (73.8–77.9)	56.5–90.6	80.9 ± 8.5 (79–82.9)	50.7–96.5



Lambda value set as the criterion for assessment of the model built using all four variables. In addition, partial Wilks’ Lambda value was calculated as the individual load of each variable on the model. Moreover, the tolerance values were calculated as (1-R²) for each variable in relation to the remaining variables. Tolerance is defined as the proportion of a variable’s variance that is not accounted for by other independent variables in the equation. The alpha level was fixed at α = 0.05, and calculations with *p* < 0.05 were considered to be statistically significant. All analyses were conducted using Statistica 13.0 software (StatSoft Poland 2018, Cracow, Poland) and R software with RStudio (PBC, Boston, MA, United States; <http://www.rstudio.com/>) (accessed on 15 September 2024).

3 Results

3.1 General sample characteristics: basic anthropometric measurements

Descriptive statistics of the baseline, post-intervention, and follow-up anthropometric measurements are presented in [Table 2](#). MANOVA was conducted for the basic anthropometric measurements and showed statistically significant multidimensional main effects for all four factors: variant ($\Lambda = 0.941$, $F = 6.13$, $p < 0.001$, $\eta^2_{pG} = 0.058$, $d = 0.35$), sex ($\Lambda = 0.716$, $F = 39.20$, $p < 0.001$, $\eta^2_{pG} = 0.284$, $d = 0.89$), group ($\Lambda = 0.936$, $F = 6.74$, $p < 0.001$, $\eta^2_{pG} = 0.064$, $d = 0.37$), and time ($\Lambda = 0.002$, $F = 22,430.06$, $p < 0.001$, $\eta^2_{pG} = 0.998$, $d = 22.3$). There were no statistically significant interactions between the factors, except for time: time \times variant ($\Lambda = 0.902$, $F = 5.30$, $p < 0.001$, $\eta^2_{pG} = 0.098$, $d = 0.33$), time \times sex ($\Lambda = 0.523$, $F = 44.74$, $p < 0.001$, $\eta^2_{pG} = 0.477$, $d = 0.95$), and time \times group ($\Lambda = 0.909$, $F = 4.89$, $p < 0.001$, $\eta^2_{pG} = 0.091$, $d = 0.32$). This confirmed that the interventions affected the basic anthropometric features; however, the results were dependent on the type of HIIT and were related to sex. ANOVA showed changes over time related to the natural growth of the adolescents in terms of height ($F = 601.832$, $p < 0.001$, $\eta^2_{pG} = 0.002$, $d = 0.05$) and sexual dimorphism in height ($F = 265.809$, $p < 0.001$, $\eta^2_{pG} = 0.470$, $d = 1.33$). There were significant sexual differences in bodyweight ($F = 54.570$, $p < 0.001$, $\eta^2_{pG} = 0.019$, $d = 0.20$), but the effects were related to the variant of HIIT used ($F = 5.725$, $p < 0.002$, $\eta^2_{pG} = 0.019$, $d = 0.20$). Similarly, the effects on BMI mirrored differences in bodyweights (sex: $F = 54.571$, $p < 0.001$, $\eta^2_{pG} = 0.018$, $d = 0.19$; variant: $F = 5.720$, $p < 0.002$, $\eta^2_{pG} = 0.018$, $d = 0.19$). Detailed comparisons with the post hoc tests revealed no significant differences in any of the variables (all $p > 0.1$) at baseline.

3.2 Detailed effects on the outcomes of interest

The descriptive statistics of the outcomes of interest at baseline, post-intervention, and follow-up are presented in [Table 3](#) and illustrated in [Figures 3–6](#). MANOVA showed statistically significant multidimensional main effects on all four factors: variant ($\Lambda = 0.968$, $F = 2.41$, $p = 0.049$, $\eta^2_{pG} = 0.032$, $d = 0.26$), sex ($\Lambda = 0.546$, $F = 61.43$, $p < 0.001$, $\eta^2_{pG} = 0.454$, $d = 1.3$), group ($\Lambda = 0.942$, $F = 4.58$, $p < 0.001$, $\eta^2_{pG} = 0.058$, $d = 0.35$), and time ($\Lambda = 0.006$, $F = 5,683.87$, $p < 0.001$, $\eta^2_{pG} = 0.994$, $d = 12.8$). The effect of the time factor, which was actually an intervention effect, was immense; however, it was related to the variant and group but not to sex. This was confirmed through the second-order interaction term (time \times variant \times group: $\Lambda = 0.943$, $F = 2.20$, $p < 0.027$, $\eta^2_{pG} = 0.057$, $d = 0.26$).

3.3 Body fat percentage

ANOVA of the body fat percentage showed sexual dimorphism toward more fat tissues in female than male participants ($F = 268.128$, $p < 0.001$, $\eta^2_{pG} = 0.462$, $d = 1.31$) and effects of the intervention independent of sex ($F = 3.911$, $p = 0.023$, $\eta^2_{pG} = 0.001$, $d = 0.03$) ([Figure 3](#)). However, changes over time were modified by

the variant of HIIT used ($F = 9.900$, $p < 0.001$, $\eta^2_{pG} = 0.001$, $d = 0.04$). Detailed comparisons with post hoc tests revealed no significant differences in body fat percentage (all $p > 0.05$) at baseline. Over the study duration, men in both variant groups showed decreased body fat percentages; however, only the HIIT variant was statistically significant ($p < 0.001$). In the men of both variant groups, the follow-up measurements were similar to the post-intervention measurements, indicating sustained effects that were confirmed by similar fat levels and no statistically significant differences (HIIT variant: $p = 1.000$; HIPT: $p = 0.075$). The female groups also showed decreased body fat percentages, but the effects were reversed between the variants. Female participants practicing HIPT showed a significant difference ($p < 0.001$), while those in the HIIT variant did not ($p = 1.000$). The results were sustained, and the follow-up measurements did not differ significantly from the previous values (both variants: $p = 1.000$). The effects of the interventions on the experimental groups (both men and women) were not large enough to show significant differences from the control groups (all comparisons, $p = 1.000$). The men had significantly lower body fat percentages than the women in each comparison (pre, post, and follow-up) (all $p < 0.001$).

3.4 Systolic blood pressure

ANOVA of the SBP showed sex-based differences and higher values in men than women ($F = 35.404$, $p < 0.001$, $\eta^2_{pG} = 0.095$, $d = 0.46$). Further, the variant of the HIIT showed differences between the groups ($F = 8.939$, $p = 0.003$, $\eta^2_{pG} = 0.026$, $d = 0.23$). Intervention expressed over time was another factor affecting the results ($F = 31.801$, $p < 0.001$, $\eta^2_{pG} = 0.012$, $d = 0.11$) ([Figure 4](#)). However, the third-order interaction term (time \times sex \times variant \times group: $F = 2.20$, $p = 0.027$, $\eta^2_{pG} = 0.057$, $d = 0.25$) confirmed that changes over time were related to all of the factors. Detailed comparisons with post hoc tests revealed no significant differences in the SBP (all $p > 0.05$) at baseline. Over the study duration, men in both variant groups showed significantly decreased SBP values ($p < 0.001$ and $p = 0.016$). In the men of both variant groups, the effects were sustained ($p = 1.000$). The female groups also showed decreased SBP values ($p < 0.001$ for both variants) with sustained effects ($p = 1.000$). The effects of the interventions on the experimental groups (both men and women) were not large enough to show significant differences from the control groups (all comparisons, $p = 1.000$). There were no significant differences in SBP values between men and women (experimental and control groups of both variants) (all $p > 0.100$).

3.5 Diastolic blood pressure

ANOVA of the DBP showed significant effects of the interventions (time) ($F = 3.470$, $p = 0.033$, $\eta^2_{pG} = 0.002$, $d = 0.05$). However, these were related to the variant of the HIIT used ($F = 4.982$, $p = 0.026$, $\eta^2_{pG} = 0.014$, $d = 0.12$) ([Figure 5](#)). The second-order interaction term (time \times group: $F = 4.120$, $p = 0.018$, $\eta^2_{pG} = 0.002$, $d = 0.05$) showed that the temporal changes were linked with the experimental groups. Detailed comparisons with post hoc tests revealed no significant differences in the DBP (all $p > 0.05$) at baseline. Over the study duration, despite the decreasing

TABLE 2 Descriptive statistics of the basic anthropometric measurements of the pre-intervention (Pre), post-intervention (Post), and follow-up (FU) values based on variant, sex, and group.

Variable	Time	HIIT		HIPT	
		Men			
		E (n = 45)	C (n = 47)	E (n = 24)	C (n = 39)
		Mean ± SD (95% CI)	Mean ± SD (95% CI)	Mean ± SD (95% CI)	Mean ± SD (95% CI)
Age [years]	Pre	15 ± 0.6 (14.8–15.3)	15.1 ± 0.6 (14.9–15.2)	14.9 ± 0.5 (14.8–15.1)	15 ± 0.4 (14.9–15.1)
Body height [cm]	Pre	175.4 ± 6.6 (172.6–178.2)	178.1 ± 6.8 (175.9–180.4)	176 ± 6.1 (174.2–177.9)	176.7 ± 6.9 (174.7–178.8)
	Post	175.7 ± 6.6 (172.9–178.4)	178.5 ± 6.8 (176.3–180.7)	176.3 ± 6.1 (174.5–178.2)	177.1 ± 7 (175–179.1)
	FU	176 ± 6.6 (173.2–178.8)	178.8 ± 6.8 (176.6–181)	176.8 ± 6.1 (174.9–178.6)	177.5 ± 7.1 (175.4–179.5)
Bodyweight [kg]	Pre	65.5 ± 9 (61.7–69.3)	68.8 ± 16. 6 (63.4–74.2)	65.2 ± 11.6 (61.7–68.7)	62.6 ± 8.7 (60–65.1)
	Post	64.9 ± 7.7 (61.6–68.1)	69.7 ± 16.8 (64.2–75.1)	64.5 ± 10.6 (61.4–67.7)	63.2 ± 9.5 (60.4–66)
	FU	64.8 ± 8.6 (61.1–68.4)	69.7 ± 16.6 (64.3–75.1)	65.5 ± 11.1 (62.1–68.8)	63.4 ± 8.5 (60.9–65.9)
Body mass index [kg/m²]	Pre	21.3 ± 2.8 (20.1–22.5)	21.5 ± 3.9 (20.2–22.7)	21 ± 3.3 (20–22)	20 ± 2.3 (19.3–20.7)
	Post	21 ± 2.5 (20–22.1)	21.7 ± 3.9 (20.4–22.9)	20.7 ± 3 (19.8–21.6)	20.1 ± 2.6 (19.4–20.9)
	FU	20.9 ± 2.7 (19.8–22)	21.6 ± 3.9 (20.3–22.9)	20.9 ± 3.2 (20–21.9)	20.1 ± 2.2 (19.5–20.7)
Women					
Variable	Time	E (n = 30)	C (n = 24)	E (n = 46)	C (n = 52)
Age [years]	Pre	15.1 ± 0.5 (14.9–15.2)	15 ± 0.6 (14.9–15.2)	14.8 ± 0.4 (14.6–14.9)	15 ± 0.4 (14.8–15.2)
Body height [cm]	Pre	165.6 ± 6 (163.9–167.4)	165.2 ± 6.2 (163.5–166.9)	162.9 ± 5.4 (160.8–164.9)	164 ± 5.7 (161.6–166.4)
	Post	165.8 ± 5.9 (164–167.6)	165.5 ± 6.2 (163.8–167.2)	163.2 ± 5.4 (161.2–165.2)	164.3 ± 5.7 (161.9–166.8)
	FU	166.1 ± 6 (164.3–167.9)	165.7 ± 6.2 (164–167.4)	163.6 ± 5.3 (161.6–165.5)	164.7 ± 5.8 (162.2–167.1)
Bodyweight [kg]	Pre	58.2 ± 10.9 (55–61.5)	57.3 ± 8.3 (54.9–59.6)	54.2 ± 6.5 (51.8–56.7)	55.8 ± 7.5 (52.7–59)
	Post	58.4 ± 9.9 (55.4–61.3)	58.1 ± 8.7 (55.7–60.5)	54 ± 5.9 (51.9–56.2)	56 ± 7.5 (52.8–59.1)
	FU	58.1 ± 10.4 (55–61.1)	57.5 ± 8.7 (55–59.9)	54.7 ± 6.6 (52.3–57.2)	56.2 ± 7.5 (53–59.4)
Body mass index [kg/m²]	Pre	21.2 ± 3.6 (20.1–22.3)	21 ± 3 (20.2–21.8)	20.5 ± 2.4 (19.6–21.4)	20.8 ± 2.8 (19.6–22)
	Post	21.2 ± 3.2 (20.2–22.2)	21.2 ± 3 (20.4–22.1)	20.3 ± 2.1 (19.5–21.1)	20.7 ± 2.6 (19.6–21.8)
	FU	21 ± 3.4 (20–22)	20.9 ± 3 (20.1–21.8)	20.5 ± 2.4 (19.6–21.4)	20.7 ± 2.6 (19.6–21.8)

trend of the DBP values, there were no significant differences in the pre–post and follow-up results (both men and women; all $p = 1.000$). Consequently, there were no differences with the control groups (all $p = 1.000$). Moreover, there were no significant differences between the male and female groups (all $p = 1.000$).

3.6 Cardiorespiratory fitness (VO_{2max})

ANOVA of the CRF based on VO_{2max} showed a significant effect of the intervention (time) ($F = 29.390$, $p < 0.001$, $\eta^2_{pG} = 0.014$,

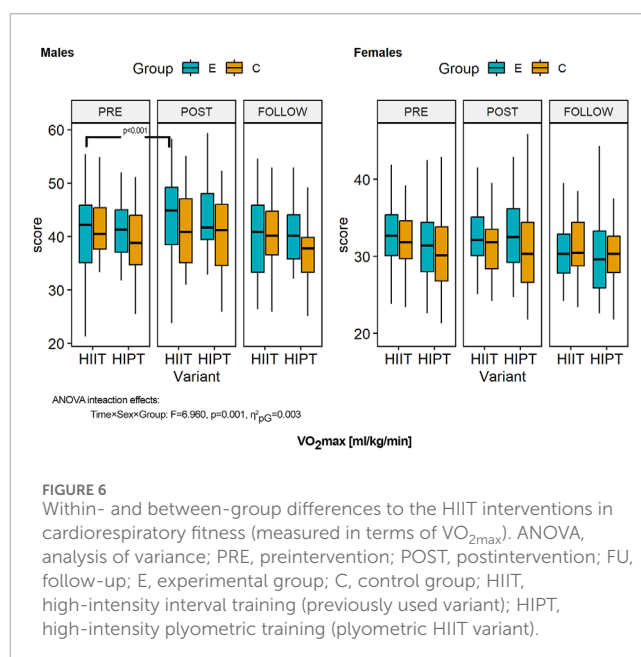
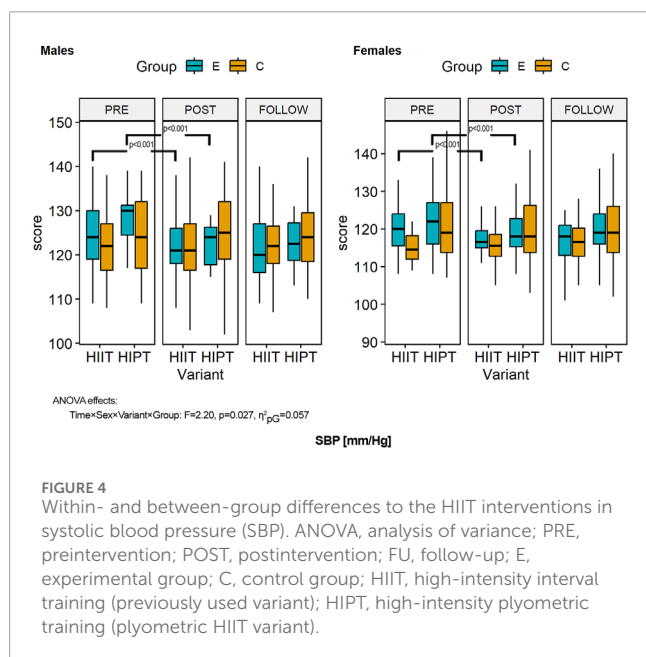
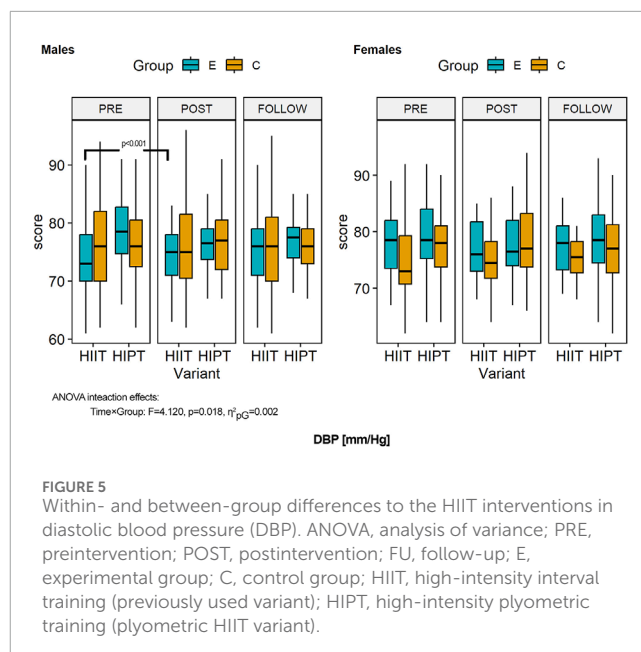
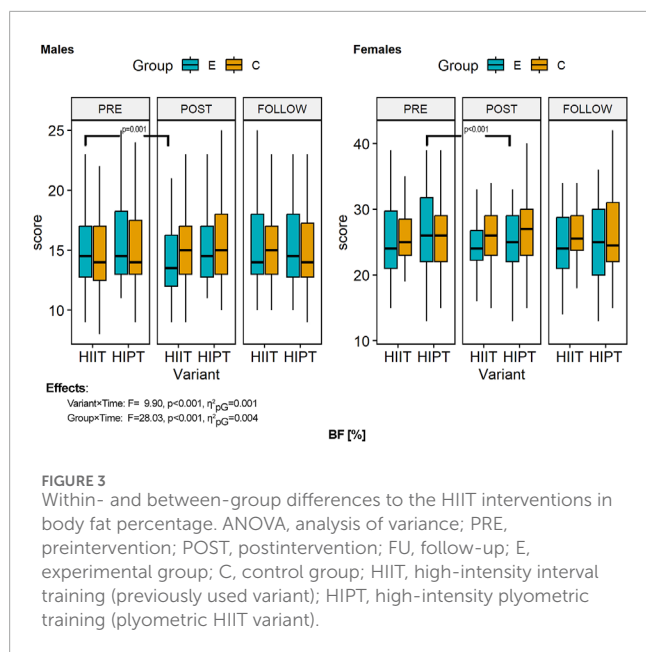
$d = 0.12$); however, this was related to sex ($F = 159.578$, $p < 0.001$, $\eta^2_{pG} = 0.313$, $d = 0.67$) (Figure 6). The second-order interaction term (time \times sex \times group: $F = 6.960$, $p = 0.001$, $\eta^2_{pG} = 0.003$, $d = 0.06$) showed that the temporal changes were linked with sex and the experimental groups but not to the variant of HIIT used. Detailed comparisons with post hoc tests revealed no significant differences in CRF between the experimental and control groups (all $p > 0.05$) at baseline. Over the study duration, an increasing trend was observed for the CRF; however, significant improvements were observed in the HIIT group and especially men ($p < 0.001$), while all other differences in the pre–post comparisons were not

TABLE 3 Descriptive statistics of the body fat, systolic and diastolic blood pressures (SBP/DBP), and VO_{2max} values at pre-intervention (Pre), post-intervention (Post), and follow-up (FU) based on variant, sex, and group.

Variable	Time	HIIT		HIPT	
		Men			
		E (n = 45)	C (n = 47)	E (n = 24)	C (n = 39)
		Mean ± SD (95% CI)	Mean ± SD (95% CI)	Mean ± SD (95% CI)	Mean ± SD (95% CI)
Fat [%]	Pre	15.8 ± 3.4 (14.4–17.2)	16.9 ± 5.6 (15–18.7)	16.4 ± 5.8 (14.7–18.2)	14.8 ± 3.5 (13.8–15.8)
	Post	15.1 ± 3 (13.8–16.4)	17.3 ± 5.8 (15.4–19.1)	15.1 ± 4.5 (13.8–16.5)	15.1 ± 3.3 (14.1–16)
	FU	15.1 ± 3.3 (13.7–16.5)	16.4 ± 5.8 (14.5–18.3)	16.1 ± 5.1 (14.6–17.6)	15.3 ± 2.9 (14.4–16.1)
SBP [mm/Hg]	Pre	127.8 ± 5.7 (125.4–130.2)	124.5 ± 8.6 (121.7–127.3)	124.6 ± 8.9 (121.9–127.3)	122.5 ± 7.4 (120.3–124.6)
	Post	122.5 ± 4.7 (120.5–124.4)	124.9 ± 9.4 (121.9–127.9)	121.8 ± 6.6 (119.9–123.8)	122.4 ± 8.5 (119.9–124.9)
	FU	122.7 ± 5.6 (120.3–125.1)	123.8 ± 7.9 (121.2–126.3)	121.4 ± 7.7 (119.1–123.7)	122.1 ± 6.6 (120.2–124.1)
DBP [mm/Hg]	Pre	78.8 ± 7.1 (75.7–81.8)	76.6 ± 7.3 (74.2–79)	74.6 ± 8.1 (72.2–77)	76.1 ± 7.9 (73.8–78.4)
	Post	76.1 ± 5.1 (74–78.3)	76.6 ± 5.7 (74.8–78.4)	74.4 ± 5.9 (72.6–76.2)	75.8 ± 8.1 (73.4–78.2)
	FU	76.6 ± 4.4 (74.7–78.4)	76.2 ± 5.3 (74.5–77.9)	75.5 ± 6.7 (73.5–77.5)	75.7 ± 8.1 (73.3–78.1)
VO _{2max} [mL/kg/min]	Pre	41.1 ± 5.8 (38.7–43.5)	39 ± 6.9 (36.8–41.3)	40.1 ± 8 (37.7–42.4)	42.3 ± 6.1 (40.5–44.1)
	Post	43.2 ± 6.4 (40.5–45.9)	40.5 ± 7.8 (37.9–43)	43.9 ± 8.5 (41.3–46.4)	41.6 ± 6.8 (39.6–43.6)
	FU	40.7 ± 6 (38.1–43.2)	37.5 ± 5.1 (35.8–39.17)	40.3 ± 7.7 (38–42.6)	40.6 ± 6.1 (38.8–42.4)
Females					
Variable	time	E (n = 30)	C (n = 24)	E (n = 46)	C (n = 52)
Fat [%]	Pre	26.3 ± 6.6 (24.3–28.2)	26.1 ± 5.3 (24.6–27.5)	24.8 ± 5.9 (22.6–27)	25.4 ± 6.1 (22.8–28)
	Post	24.7 ± 5.2 (23.2–26.3)	26.8 ± 5.6 (25.3–28.4)	24.2 ± 4.9 (22.4–26)	26.3 ± 4.9 (24.3–28.4)
	FU	24.8 ± 6 (23.1–26.6)	25.8 ± 5.9 (24.1–27.4)	24.6 ± 4.9 (22.8–26.5)	26 ± 4.9 (24–28.1)
SBP [mm/Hg]	Pre	121.8 ± 8.4 (119.3–124.3)	120.2 ± 8.8 (117.7–122.7)	120 ± 6.2 (117.7–122.3)	115.5 ± 5.1 (113.4–117.7)
	Post	118.8 ± 6.1 (117–120.6)	119.9 ± 9 (117.4–122.4)	116 ± 6 (113.7–118.3)	115.9 ± 6.4 (113.2–118.6)
	FU	119.2 ± 6.4 (117.3–121.1)	119.7 ± 9.2 (117.1–122.3)	116.2 ± 7.7 (113.3–119)	116.9 ± 6.9 (114–119.8)
DBP [mm/Hg]	Pre	79.6 ± 7.3 (77.4–81.8)	78 ± 7.2 (75.9–80)	77.8 ± 6.2 (75.5–80.1)	75.5 ± 7.6 (72.3–78.7)
	Post	77.6 ± 5.7 (75.9–79.3)	78.3 ± 6.9 (76.4–80.3)	76.4 ± 4.9 (74.6–78.3)	75.6 ± 7.2 (72.6–78.7)
	FU	79.1 ± 6.3 (77.2–80.9)	77.5 ± 7.1 (75.6–79.5)	77.3 ± 4.8 (75.5–79.1)	76.4 ± 7.4 (73.3–79.5)
VO _{2max} [mL/kg/min]	Pre	32.5 ± 5.4 (30.9–34.1)	31.2 ± 6 (29.5–32.8)	32.7 ± 4.3 (31.1–34.3)	33 ± 5.9 (30.5–35.5)
	Post	33.2 ± 5.1 (31.6–34.7)	31.5 ± 6.2 (29.7–33.2)	33.1 ± 4.6 (31.4–34.8)	32.5 ± 6.2 (29.9–35.1)
	FU	30.8 ± 5.5 (29.1–32.4)	31.5 ± 6.4 (29.7–33.3)	30.2 ± 4.8 (28.4–32.1)	32.6 ± 6.6 (29.8–35.4)

significant ($p > 0.05$). Follow-up results showed sustained effects and no significant differences (except for women in the HIPT group: $p = 0.032$). There were no significant differences compared to the

control groups (all $p = 1.000$). However, all sex-based differences were statistically significant (all $p < 0.001$) in the post-intervention and follow-up measurements.



The results of the DA are limited to the set of variables that have the most deviations between the two groups of participants in terms of the two variants of HIIT. **Table 4** presents the calculated statistics for the men and women separately. Only the model built for men was statistically significant (Wilks' $\Lambda = 0.82$, $F = 3.43$, $p = 0.013$), while there were no such differences among the women (Wilks' $\Lambda = 0.96$, $F = 0.82$, $p = 0.512$). This means that none of the variables could distinguish between the variants in the case of female participants. This was further confirmed through detailed statistical calculations for each of the variables (**Table 5**). In the case of male participants, two variables were statistically significant, namely, the Δ body fat ($p = 0.031$) and Δ SBP ($p = 0.033$) (**Table 5**). Both variants of the HIIT differed significantly in terms of the effects

on these outcomes. However, these effects were opposition: HIPT supported immense changes in the SBP, whereas HIIT supported changes in BF%.

4 Discussion

In this study, we investigated the effectiveness of 8-week interventions of two HIIT protocols on body fat percentage, blood pressure values, and CRF. Moreover, the sustainability of the effects was assessed at 8-week follow-up. The effects of both interventions were observed on each variable in both sexes; however, these effects were rather small compared to the control groups. Variation in

TABLE 4 Descriptive statistics of the delta values for variants and sex.

Variable	HIPT	
	Men (n = 24)	Women (n = 46)
	Mean \pm SD (95% CI)	Mean \pm SD (95% CI)
Δ Fat [%]	-0.67 ± 1.55 (-1.32 to -0.01)	-1.54 ± 2.66 (-2.33 to -0.75)
Δ SBP [mm/Hg]	-5.37 ± 4.32 (-7.2 to -3.55)	-3.07 ± 4.7 (-4.46 to -1.67)
Δ DBP [mm/Hg]	-2.62 ± 6.25 (-5.27 – 0.02)	-2 ± 5.09 (-3.51 to -0.49)
Δ VO _{2max} [mL/kg/min]	2.14 ± 3.91 (0.49 – 3.79)	0.67 ± 2.84 (-0.17 – 1.52)
Variable	HIIT	
	Men (n = 45)	Women (n = 30)
	Mean \pm SD (95% CI)	Mean \pm SD (95% CI)
Δ Fat [%]	-1.31 ± 1.66 (-1.81 to -0.81)	-0.63 ± 2.37 (-1.52 – 0.25)
Δ SBP [mm/Hg]	-2.73 ± 5.28 (-4.32 to -1.15)	-4 ± 6.89 (-6.57 to -1.43)
Δ DBP [mm/Hg]	-0.22 ± 5 (-1.72 – 1.28)	-1.37 ± 5.63 (-3.47 – 0.74)
Δ VO _{2max} [mL/kg/min]	3.81 ± 4.67 (2.41 – 5.21)	0.41 ± 3.25 (-0.8 – 1.63)

TABLE 5 Wilks' Lambda (Λ), *F*-value, and *p*-value for models built separately for men and women. Partial Wilks' Lambda (Λ_p), *F*-value, *p*-value, and tolerance are also shown for each variable in each model.

Variable	Wilks' Λ_p	<i>F</i>	<i>p</i>	Tolerance
Men: Wilks' $\Lambda = 0.82$, <i>F</i> = 3.43, <i>p</i> = 0.013				
Δ Fat [%]	0.93	4.87	0.031	0.87
Δ SBP [mm/Hg]	0.93	4.76	0.033	0.82
Δ DBP [mm/Hg]	0.98	1.49	0.226	0.87
Δ VO _{2max} [mL/kg/min]	0.97	1.80	0.185	0.97
Women: Wilks' $\Lambda = 0.96$, <i>F</i> = 0.82, <i>p</i> = 0.512				
Δ Fat [%]	0.97	2.08	0.154	0.99
Δ SBP [mm/Hg]	0.99	0.73	0.396	0.91
Δ DBP [mm/Hg]	0.99	0.54	0.466	0.91
Δ VO _{2max} [mL/kg/min]	0.99	0.04	0.839	0.99

the effects between the two variants was observed in men but not in women. In men, HIPT had a more significant impact on the SBP, whereas HIIT influenced BF%. Thus, both programs induced positive adaptations; however, these effects appear to be sex-specific and dependent on the type of intervention. The interventions significantly reduced body fat independent of sex, although the changes were more influenced by the HIIT program.

Men in the HIIT group showed significant reductions in body fat percentages, which were sustained at follow-up, whereas women in the HIPT group achieved comparable reductions. Despite the initial assumption of differences in the variant of exercise performed, the observed differences between the programs were not significant and were rather dependent on sex within the effects. The interventions also produced significant reductions in SBP in both sexes across both variants, and these reductions were maintained at follow-up in both experimental groups. However, no significant differences were noted between men and women. Despite a general trend toward lower DBP in both sexes, no significant differences were found in the pre-post or follow-up comparisons, and there were no differences compared to the control groups. This suggests minimal effects of the interventions on the DBP. Significant improvements in VO_{2max} were observed, particularly among men in the HIIT group (*p* < 0.001), indicating a positive impact of HIIT on CRE. This effect persisted at follow-up in the men, while women in the HIPT group showed minor improvements. However, the observed changes were not statistically different from those of the control groups, indicating that the HIIT was effective even though the changes were not large enough to significantly differentiate between the experimental and control groups.

Previous studies have demonstrated that even short but intensive intervention protocols, such as the Tabata protocol, could have considerable positive effects on physical fitness, cardiorespiratory system functions, and body composition in youth and adolescents within the limited time available during PE classes (Martin-Smith et al., 2020; Ekstrom et al., 2017; Domaradzki et al., 2020; Ricci et al., 2022). In our previous study, we reported significant decreases in the mean values of bodyweight and body fat percentage in response to HIIT implemented during PE

lessons (Domaradzki et al., 2022). However, these effects were observed predominantly among men, suggesting sex as a factor differentiating the effects of HIIT; this was also confirmed in the present study and is likely attributable to differences in the hormonal profiles and muscle fiber compositions between the sexes during adolescence (Xu et al., 2022). Bogataj et al. (2021) reported positive impacts of HIIT on body composition among obese girls, indicating that women can also benefit from HIIT interventions. Racil et al. (2016) showed that programs combining HIIT with plyometric exercises could improve the lean body mass and jumping performance in obese adolescent girls, highlighting the potential of HIIT interventions for women. However, in the present study, we found notable sex-based differences in body fat reduction based on the variant of protocol used. Men experienced significant reductions in body fat percentage with the HIIT, and these results were sustained during the follow-up period. In contrast, women showed comparable reductions when engaging in HIPT. This differential response may be attributed to sex-specific metabolic pathways associated with hormones and differences in body compositions between men and women (González-Gálvez et al., 2024). Dominic and Kishore (2021) demonstrated the effectiveness of interval effort on body fat reduction, which was confirmed in a meta-analysis by Khodadadi et al. (2023). However, our findings indicate that the type of HIIT protocol used could interact with sex to influence the body composition outcomes. The differential responses may be attributable to physiological and hormonal differences between the sexes during adolescence, affecting the mechanisms by which men and women metabolize fat during high-intensity exercises. Additional factors, such as variations in insulin sensitivity, adipokine profiles, and muscle fiber distribution, may also contribute to the observed differences in body fat reduction between the sexes (Lundsgaard and Kiens, 2014; Gado et al., 2024). Our study showed that the improvements in body fat percentage were associated with better CRF—a relationship that is more pronounced in men. This finding aligns with the results reported by Lan et al. (2022) and Guo et al. (2023), who noted associations between fat loss and increased CRF. The utilization of fatty acids during aerobic metabolism may explain the link between reduced body fat and enhanced CRF (Hargreaves and Spriet, 2020). These findings highlight the importance of tailoring the intervention programs to optimize fat reduction and fitness improvements based on sex. Nonetheless, the lack of straightforward differences between the protocols indicates that multiple physiological factors interact to evoke positive adaptations beyond the type of interval training employed (Yu, 2025; Takahashi et al., 2025).

Excess body fat is closely linked to elevated blood pressure, which contributes to hypertension in overweight and obese individuals (Shariq and McKenzie, 2020). Although cardiovascular diseases mainly manifest in the mid-life years, they have their origins in adolescence, making early interventions a public health priority (Chung et al., 2015). Obesity causes functional and structural changes in microcirculation that could impair the microvascular functions underlying elevated blood pressures (El Meouchy et al., 2022). HIIT has been shown to improve cardiovascular health by reducing endothelial damage that precedes atherosclerosis (Tjønnå et al., 2009). Tjønnå et al. (2009) observed similar improvements in blood pressure independent of sex. Significant

reductions in the SBP were observed for both sexes across both intervention variants, and these reductions were maintained during the follow-up period. Delgado-Floody et al. (2019) reported SBP decreases (Δ –8.70 mmHg) in adolescents following HIIT but did not note any relationships between the improvements in CRF and blood pressure. Martínez-Vizcaíno et al. (2014) observed reduced body fat and cardiometabolic risk through improvements in the blood lipid profiles in girls, supporting the effectiveness of HIIT in enhancing the cardiovascular parameters among women. Despite the general trend toward lower DBP, no significant differences were found in our study's pre-post or follow-up comparisons for either sex, suggesting minimal effects of the interventions on the DBP. This may indicate that the SBP is more responsive to HIIT interventions during adolescence and that sex-specific physiological factors could influence these outcomes. The differential responses between the sexes could be attributed to hormonal variations, vascular adaptations, and differences in autonomic regulation of blood pressure.

CRF is a key indicator of health and a predictor of the risk of cardiovascular diseases (Raghuveer et al., 2020; Belanger et al., 2022). HIIT workouts require less time commitment and have been shown to improve CRF (Carson et al., 2014; Khalafi et al., 2022; Robinson et al., 2015). Our previous study showed improvements in aerobic capacity following HIIT implemented during PE lessons (Domaradzki et al., 2020), with sex-based differences influencing the outcomes. In the present study, significant improvements in VO_{2max} were observed among the men participating in the HIIT group ($p < 0.001$). This positive impact on CRF was sustained during the follow-up period, especially in men. In contrast, women engaging in HIPT showed minor improvements that were not significantly different from those of the control groups. These findings suggest that men may possess a greater capacity for cardiovascular adaptation to high-intensity stimuli, possibly owing to their higher baseline cardiac outputs and muscle masses, thereby facilitating more pronounced improvements in oxygen uptake (Mauro et al., 2024; Svane et al., 2024). This suggests that men may respond more favorably to the HIIT variant in terms of CRF enhancement, while women may require different training stimuli to achieve similar benefits. Burgomaster et al. (2008) revealed no changes in VO_{2max} after HIIT in men, while women exhibited improvements (Talanian et al., 2007). Astorino et al. (2012) also observed differences between the sexes in terms of CRF and blood pressure responses. Our results contrast with some of these findings, showing more significant improvements in boys than girls for both CRF and body fat percentage. Boys may experience greater increases in stroke volume and cardiac output during high-intensity exercises, leading to more substantial improvements in VO_{2max} . Hormonal differences, such as higher testosterone levels in men, may enhance their muscle masses and oxygen utilization, contributing to the differential adaptations observed (Pataky et al., 2023). Additionally, fatigue resistance and muscle fiber composition could differ between the sexes, affecting the performance and training outcomes (Pataky et al., 2023). Women may have higher proportions of type I muscle fibers, which are more resistant to fatigue but may not respond as dramatically to high-intensity training as type II fibers that are more prevalent in men (Haizlip et al., 2015). These observations highlight the importance of considering sex-specific responses

when designing intervention programs for adolescents. Future research efforts should further investigate the interplay between hormonal, metabolic, and cardiovascular factors to better tailor the HIIT and HIPT protocols for optimizing health outcomes in both sexes. Tailoring the protocols to optimize the benefits for each sex may enhance the effectiveness of the intervention aimed at improving CRF to reduce cardiovascular disease risk. For example, incorporating plyometric exercises may be more beneficial for women, as suggested by the improvements observed in body composition and functional performance in studies like those reported by [Racil et al. \(2016\)](#).

Our research shows that very intensive forms of training with anaerobic metabolism lasting only up to 4 min, when implemented during PE lessons over a period of 8 weeks, can affect body composition and cardiorespiratory system functions. However, the trends in the changes were different depending on the variant of intervention used and sex, indicating the need for further research. These suggest the necessity of studying the dose–response phenomena with intervention time as the load of effort ([Domaradzki et al., 2023](#)). At present, it is not clear which variant of the intervention is more efficient and could depend on the measurements and sex. Therefore, future research should investigate hybrid versions of both of the variants considered herein. Future research should also investigate whether adolescents with different weight statuses (e.g., underweight, normal weight, and overweight/obese) respond differently to the interventions. Examining these group-specific effects could provide a more nuanced understanding of how the baseline weights may moderate the impacts of the interventions.

Given the abovementioned findings, we are aware that this study has some limitations that should be addressed in the future. One of the main drawbacks is the lack of control over participants' caloric intakes and daily physical activities, which are factors that could have influenced the observed outcomes. Additionally, the absence of an assessment of the maturation status of each participant constitutes a significant confounding variable, as variations in the developmental stage could affect the observed results. These limitations should be taken into account when interpreting the findings and also highlight the need for more controlled methodologies in future research.

5 Conclusion

Our results prove the positive effects of HIIT exercises lasting only up to 4 min on body fat percentage, blood pressure, and CRF, when implemented during PE lessons over a period of 8 weeks. However, the trends of the changes were different depending on the variant of intervention used and sex of the participant. Body fat reduction was observed in men performing HIIT, while HIPT was observed to be more effective in women. Unlike DBP, SBP showed reductions in both variants of the intervention in both sexes. The effects on CRF were clearer in men performing HIIT than HIPT; however, this was not noted in women irrespective of the variant of intervention performed. The overall effects on the

study participants were generally poor, which may be attributed to the short duration of exercises (up to 4 min). Nonetheless, both programs are valuable for PE teachers and practitioners in reducing body fat and blood pressure while enhancing CRF. These interventions can be considered low-dose but feasible owing to their brief duration (4 min) and impact on youth health, making them easy to incorporate into PE lessons. Dedicating such a short portion of the class time can yield positive effects without compromising on the abilities of the teachers to meet their curriculum requirements.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Wrocław University of Health and Sport Sciences (ECUPE No. 33/2018; approval date: 31 October 2018). The studies were conducted in accordance with all local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

JD: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, and writing – review and editing. MP: conceptualization, data curation, investigation, methodology, project administration, supervision, validation, writing – original draft, and writing – review and editing. KK-J: data curation, investigation, methodology, supervision, writing – original draft, and writing – review and editing. PS: data curation, investigation, software, writing – original draft, and writing – review and editing. EM-C: supervision, writing – original draft, and writing – review and editing. DK: conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, supervision, validation, writing – original draft, and writing – review and editing.

Funding

The authors declare that financial support was received for the research and/or publication of this article. This study was funded by a grant from the state budget under the Polish Ministry of Science and Higher Education program titled “Science for Society II” (project no. NdS-II/SP/0521/2023/01).

Acknowledgments

The authors would like to thank the employees of the Krzysztof Kamil Baczyński Secondary School No. VII in Wrocław and Agnieszka Osiecka Secondary School No. XVII in Wrocław, Poland, for their participation in this research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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