Participants

The sample size was calculated using the G*Power software (Version 3.1.9.7). To achieve 0.95% statistical power, a type I error of 0.05, and an effect size f = 0.55, which are parameters based on a similar study (Ouerghi N B22; Abassi W N25), a total of 30 participants was necessary. To account for potential dropouts, 34 healthy adolescent males were enrolled from middle schools in Dahmani (Kef Governorate, Tunisia). Participants were randomized to a high-intensity interval training group (HIITG, n=17) or a non-training control group (CG, n=17). Of the 34 pre-selected participants, six dropped out of the training program for personal reasons (two from the HIITG and four from the CG). Finally, 28 adolescent males (age, 14.9±0.74 yrs.; height, 1.66±0.07 m; body mass index (BMI), 18.2±2.67 kg/m^2) completed the study; 15 in HIITG and 13 in CG (Figure 1). The participants' usual physical activity consisted of weekly two-hour physical education lessons and one session of football training in the middle school's sports association each week (with an average duration of one hour and 30 min of football practice per week at their middle school). All participants have been members of the middle school football sports association club for an average of two years. This usual physical activity as well as habitual eating behaviors and tasks, were maintained over the intervention period in all participants.

Inclusion criteria were male sex, age from 14 to 16 years, classified as normal-weight according to the World Health Organization (WHO) child growth standards for BMI (normal-weight: BMI ≤ 85th percentile), usual physical activity consisting of weekly two-hour physical education lessons and one football training session/week in the middle school's sports association, and parental approval. Non-inclusion criteria were a medical condition that contraindicates intense physical exercise, participation in physical training at the time of the study or within the past 6 months (except physical education class and middle school sports association football training), and current or recent (within 3 months) dietary restriction or dietary supplementation. Exclusion criteria were consent retirement, non-compliance to the training program and incomplete data.

All procedures were approved by the local ethics committee of the High Institute of Sport and Physical Education of Kef (ISSEPK-058/2024). The study was conducted in accordance with the latest version of the Declaration of Helsinki. Written informed parental consent and participants' assent were obtained prior to the start of the study. All participants and their parents/legal representatives were fully informed about the experimental protocol and its potential risks and benefits.

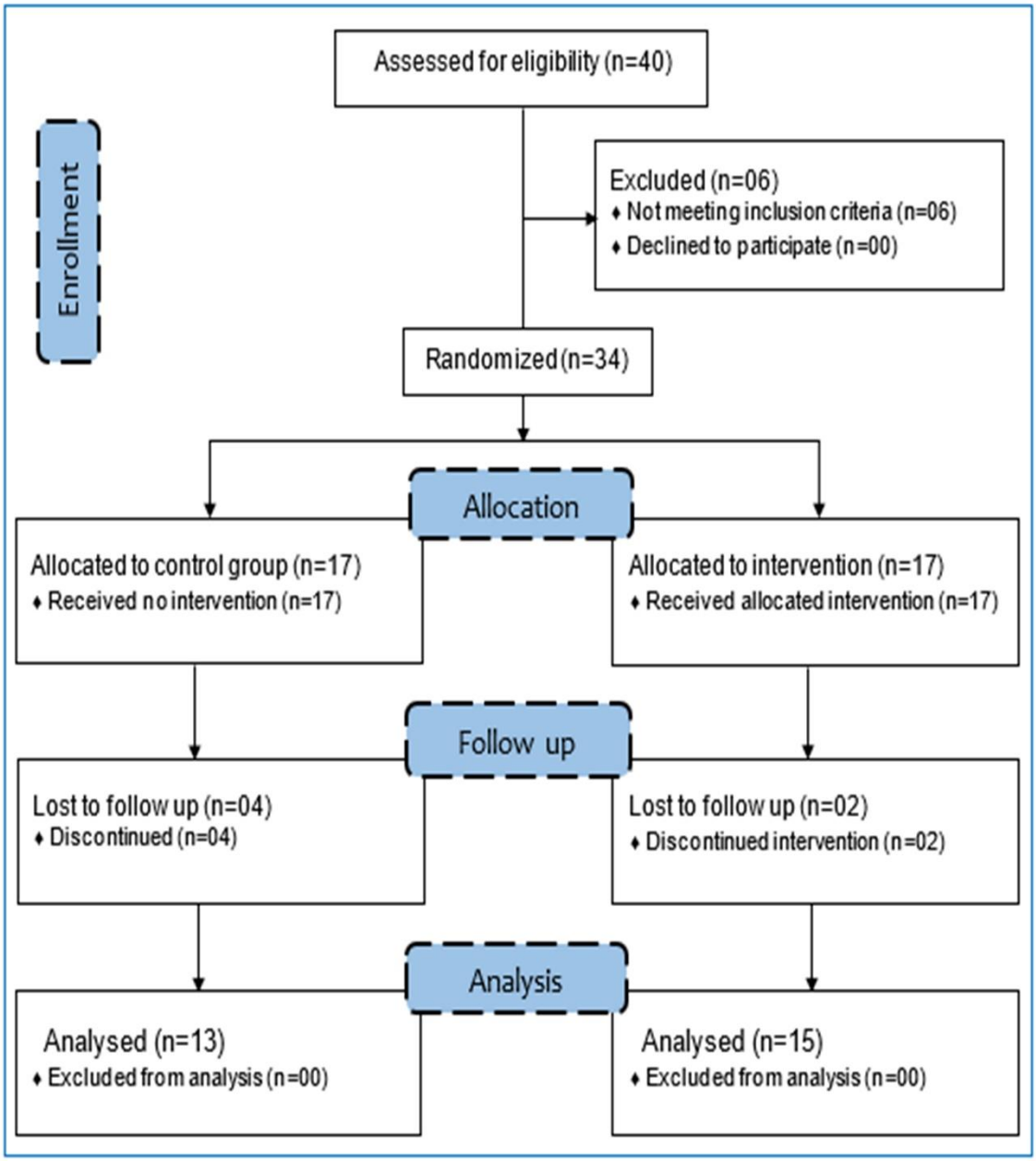


Figure 1. Flow chart of participant enrollment, randomized group allocation, and final analysis

Study design and procedures

The experimental protocol was carried out during the in scholar year period in the year 2024–2025. Anthropometric parameters', fitness components and a blood sample to measure circulating PTH levels were assessed in all participants before the beginning and after the 10-week HIIT program.

Anthropometric parameters [height, body mass, BMI, percentage of body fat (%BF) and waist circumference (WC)] were evaluated in barefoot lightly clothed subjects as previously described (Ouerghi N BS17a). All participants were in puberty and this was determined by

pubic hair development according to the Tanner classification (Tanner ADC66) at the beginning and the end of the 10-week training period.

Physical fitness measures

After familiarization procedures, all physical tests were assessed on three non-consecutive days separated by 48 h. On the first day, five-jump test (FJT), squat jump (SJ) and countermovement jump (CMJ) were assessed. On the second day, linear sprint (5 m, 10 m, 20 m and 30 m sprint) were performed. On the third day, the participants performed a graded exercise test until exhaustion, the 20 m shuttle run test, to evaluate aerobic capacity. Before all the physical tests, the participants completed a standardized warm-up (10-min of jogging, jumping exercises, short distance accelerations and dynamic stretching). The tests were performed at the same times and under the same experimental conditions. All participants were strongly encouraged to achieve maximum performance during the tests, and the best performance of three trials was recorded for all anaerobic performance tests. The FJT, SJ, CMJ and linear sprint tests were performed using techniques, experimental procedures and equipment's as previously described (Ouerghi N BS17a). The intraclass correlation coefficient (ICC) and 95% confidence interval (95% CI) confirm the reproducibility of the following tests: FJT, SJ, CMJ, FJT, 5m, 10 m, 20 m and 30 m, with mean ICC values ranging from 0.831 to 0.978, and mean 95% CIs ranging from 0.741 to 0.990.

Aerobic capacity

Maximal aerobic speed (MAS) and maximal oxygen consumption (VO_{2max}) were determined using an incremental running test: 20-m shuttle run test, conducted as previously described by Léger LA (EJAPOP82). It was designed to assess the aerobic capacity of schoolchildren and healthy adolescents and adults. Participants ran back and forth between two lines at a measured distance of 20-m. The test started with an initial speed of 8 km/h, which increased by 0.5 km/h every minute in accordance with the recorded "beep" sound (Léger LA EJAPOP82). The subject was encouraged throughout the test. The test ended when the subject failed to reach the line (within 2-m) on two consecutive occasions. The reproducibility of tests was determined using pilot data from 28 participants collected during familiarization trials (MAS: ICC=0.837, 95% CI=[0.647-0.924]; VO_{2max}: ICC=0.850, 95% CI= [0.676-0.931]).

High-intensity interval training intervention

The training program consisted of high-intensity interval exercise sessions performed three times per week on nonconsecutive days (i.e., Tuesday, Thursday and Saturday) for a period of 10 consecutive weeks. The training protocol was carried out as previously described by Ouerghi N BS17a, OF17b) and Buchan DS (BMCPH13) in young males. The HIIT period commenced

2 days after the pretesting procedure. Each training session was started by a standardized 15-min warm-up consisting of light aerobic exercise followed by high-intensity interval runs. The protocol consisted of three series of 4-8 repetitions of 30-s of work at 100-110% of MAS and 30-s of active recovery at 50% of MAS. Participants were required to increase the number of repetitions from 4 to 8, and intensity from 100 to 110 % of MAS (Detailed in Table 1). Each training sessions ended with a 10-min cool-down followed by 5 min of static stretching. Participants in the CG continued their regular physical education classes but did not participate in any additional training program.

Blood sampling and laboratory analysis

Fasting blood samples were collected from the antecubital vein in the morning (around 8 a.m.) the day before the start and two days after the completion of the training program. All participants fasted for at least 12 hours before sampling, and blood was drawn via venipuncture. The samples were then centrifuged, and the plasma was frozen at -80°C until analysis. Plasma PTH levels were measured in duplicate by radioimmunoassay using a commercial reagent kit (Immunotech, France).

Statistical Analysis

Data were expressed as means and standard deviations (SD). Normality of data of each group was verified by Shapiro-Wilk test and the homogeneity of variance was assessed using the test of Levene. The reliability of all physical tests was assessed by the intra-class correlation coefficient (ICC) and 95% confidence interval (95%CI). Anthropometric parameters, physical fitness tests and PTH concentrations were assessed using a two-way analysis of variance with repeated measures (ANOVA) (2 groups x 2 times). If group-by-time interactions turned out to be significant, a post-hoc analysis was used to identify significant pairwise comparisons. Partial eta squared (η_p^2) values were derived from ANOVA outputs and used to determine effect sizes. Independent t-tests were performed to examine the differences between the two groups (HIITG *vs*. CG). The effect size (d) was calculated using Cohen's d; these calculations were based on Cohen's classification of a small ($0.2 < d < 0.5$), moderate ($05 < d < 0.8$) and large ($d \geq 0.8$) effect size (Cohen J 1988). Statistical analyses were performed using the SPSS software version 22.0 (SPSS, Inc., Chicago, IL, USA). The level of statistical significance was set at $p \leq 0.05$.