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Effects of vitamin D supplementation during autumn and winter on blood biomarkers and physical performance in runners and non runners

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This study investigated the effects of an 8-week daily 2000 IU vitamin D_3 supplementation, administered via orodispersible films, on serum vitamin D, leukocyte count, and performance parameters in healthy recreational runners and non-runners, followed by a 12-week post-supplementation follow-up. Forty-five participants were randomized into four groups: supplemented runners (RUN-SUPPL, $n = 13$, age 33.4 ± 7.5 y), non-supplemented runners (RUN-CON, $n = 10$, age 34.8 ± 8.6 y), supplemented non-runners (NON-RUN-SUPPL, $n = 11$, age 29.3 ± 7.5 y), and non-supplemented non-runners (NON-RUN-CON, $n = 11$, age 28.3 ± 6.0 y). Assessments were conducted at baseline (T0, October, pre-supplementation), post-intervention (T1, December, after 8 weeks), and follow-up (T2, March, 12-weeks post-supplementation). Supplementation significantly increased serum 25(OH)D_3 in runners (30.45 ± 7.0 to 35.35 ± 8.9 ng/mL, $p < 0.001$) and non-runners (25.0 ± 8.7 to 30.2 ± 9.7 ng/mL, $p < 0.001$), while non-supplemented non-runners showed a marked decline (25.93 ± 6.6 to 17.8 ± 7.3 ng/mL, $p < 0.001$). After follow-up, serum vitamin D decreased significantly in all groups (e.g., RUN-SUPPL 23.52 ± 5.6 ng/mL, $p < 0.001$). A significant time-by-supplementation interaction was observed for total leukocytes ($p = 0.001$) and neutrophils ($p = 0.03$), indicating more stable immune profiles in supplemented participants. No significant changes were found in VO_2max or countermovement jump ($p > 0.05$), while maximal isometric force showed a trend toward improvement in supplemented subjects ($p = 0.056$). Despite these physiological benefits, no ergogenic effects on aerobic or explosive performance were observed.

Keywords 25-hydroxyvitamin d, VO_2max, Maximal isometric force, Leukocytes, Neutrophils, Monocytes

Approximately 80% of vitamin D is produced in the human body by UVB radiation[1], converting 7-dehydrocholesterol to pre-vitamin D. Ergocalciferol or cholecalciferol can also be obtained from food[2]. These levels may vary based on season, sun exposure duration, and ethnicity, among other factors. It is estimated that around one billion people globally are vitamin D deficient[3]. Serum 25(OH)D_3 concentrations below 20 ng/mL, between 20 and 29 ng/mL, and above 29 ng/mL characterize vitamin D deficiency, insufficiency, and sufficiency, respectively[3]. Observational studies indicate a high prevalence of vitamin D insufficiency or deficiency in

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athletes[4,5], with a higher incidence during the winter season[6] and among those who train indoors[7–9]. Vitamin D is known for its role in regulating calcium homeostasis, establishing a significant link between vitamin D and bone health in non-athletic populations[5]. Vitamin D contributes to skeletal muscle function and regeneration[9], and has been shown to support muscle recovery by enhancing peak isometric force following exercise-induced damage[10]. It also modulates both innate and adaptive immune responses, helping to regulate inflammation and prevent autoimmune reactions[11]. These immunomodulatory effects may lower susceptibility to respiratory infections by improving mucosal barrier integrity and enhancing antimicrobial peptide expression[11].

Recent studies have explored the potential of vitamin D supplementation in enhancing athletic performance. Most intervention trials have been conducted during winter months, when cutaneous vitamin D synthesis is markedly reduced[12,13]. Typically, these interventions last 8–12 weeks, a duration shown to be sufficient to elicit measurable increases in serum 25(OH)D levels[14–16]. For instance, an 8-week daily 6000 IU vitamin D_3 supplementation significantly increased 25-hydroxyvitamin D levels and improved both aerobic and anaerobic performance in young, active males, suggesting that high-dose vitamin D_3 enhances physical capacity and vitamin D status[17]. Positive correlations were found between vitamin D status and relative grip strength, as well as peak power during the Wingate Anaerobic Test among junior and collegiate hockey players. However, after adjusting for training exposure and other factors, vitamin D status only significantly predicted relative grip strength[18].

Athletic training aims to disrupt homeostasis, prompting an adaptive response that enhances performance[9]. In vitro and in vivo studies suggest a positive role for vitamin D in muscle repair and remodeling[19]. A randomized controlled trial demonstrated that daily supplementation with 4000 IU of vitamin D_3, which elevated serum 25(OH)D_3 concentrations above 75 nmol/L, significantly enhanced recovery by improving peak isometric force in the lower limbs following muscle-damaging exercise consisting of high-volume, one-legged horizontal jumps on a leg press at 75% of body mass[10]. These findings indicate that adequate vitamin D can enhance the recovery of skeletal muscle strength following intense exercise[10]. Further evidence from a study supplementing for 12 weeks 2000 IU/day of oral vitamin D_3 in active males showed higher serum levels of 25[OH]D, maximum oxygen consumption (VO_2max), and average power compared to the placebo[20].

However, numerous studies have found no effect of vitamin D supplementation on strength or endurance performance. For instance, a study on vitamin D-insufficient middle-aged men engaged in resistance training showed no significant gains in muscle strength or reductions in fat mass with vitamin D supplementation, although serum 25(OH)D_3 levels increased[21]. A 12-week study involving vitamin D supplementation among vitamin D-deficient young healthy men participating in resistance training found no impact on VO_2max. However, supplementation did improve the inflammatory status[22]. Another investigation observed that vitamin D supplementation among male conscripts in the Estonian Army during winter prevented a significant decrease in serum vitamin D levels but did not enhance hand grip strength[23]. Additionally, a study found that vitamin D supplementation in indoor wheelchair athletes improved vitamin D levels, but the connection between vitamin D status and upper body performance remains unclear[24]. Notably, most previous trials either lacked a follow-up phase or monitored participants for fewer than 8 weeks after supplementation, which may have been insufficient to capture the gradual post-supplementation decline in serum vitamin D[25,26].

The identification of the vitamin D receptor (VDR) and vitamin D metabolising enzymes in immune cells such as monocytes, macrophages and neutrophils has led to recognition of the essential role that vitamin D has in the regulation of the inflammatory response and immune system[27]. Recent research indicates that immune cells, including monocytes, macrophages, dendritic cells, and lymphocytes, express VDR and enzymes that activate vitamin D, and respond to vitamin D by lowering levels of pro-inflammatory cytokines[28]. According to Jones et al., vitamin D status modulates exercise-induced alterations in innate immune defense parameters and metabolomic signatures, including indicators of inflammation and metabolic stress, suggesting that athletes' serum 25(OH)D_3 concentrations should be carefully monitored[28].

Vitamin D deficiency is a prevalent issue globally, and its impact on health and athletic performance has been a matter of extensive research. Given the significant role of vitamin D in immune function, muscle repair, and overall health, it is crucial to understand how supplementation can benefit different populations with various lifestyles, including athletes and non-athletes (i.e., rather active or sedentary).

In the study of Pegreffi et al.[29], significant differences in vitamin D status were observed between runners and non-runners during the autumn season, with runners exhibiting higher serum 25(OH)D_3 levels. Although VO_2max showed a slight positive correlation with vitamin D levels, no association was found with maximal isometric force or jump height. Additionally, the authors reported that non-runners had higher levels of neutrophils and monocytes, which were inversely correlated with vitamin D status[29].

Building upon these baseline observations, the present study represents the interventional and longitudinal phase of the same research project. While Pegreffi et al.[29] reported only the initial pre-intervention characteristics of the cohort, the current manuscript focuses on the randomized supplementation protocol and the longitudinal changes observed across T0, T1, and T2, thus providing the framework to evaluate the effects of vitamin D supplementation on performance, inflammatory markers, and hematological responses over time.

Therefore, the objective of this study was to investigate the impact of an 8-week daily 2000 IU vitamin D_3 supplementation on various health and performance parameters in both runners and non-runners. Specifically, it aimed at examining changes in vitamin D levels, leukocyte count, cardiorespiratory performance, strength, and power. The study also considered the effects of diet, training, and solar radiation exposure, compared to control groups without supplementation. It was hypothesized that vitamin D_3 supplementation would prevent the seasonal decline in serum 25(OH)D concentrations and mitigate reductions in leukocyte count, without producing a direct ergogenic effect on performance.

Materials and methods

Study design

This was an exploratory open study that aimed to investigate vitamin D levels, hematological markers associated with immune function, nutritional status, cardiorespiratory fitness, and strength performance in groups of runners and healthy non-runners, who either received an 8-week supplementation with 2000 IU Vitamin D in orodispersible film, or served as controls without supplementation.

The study was conducted between October 2023 and March 2024, corresponding to the months with minimal sunlight exposure at the investigation latitude, when endogenous vitamin D synthesis is markedly reduced[12,13]. The intervention period lasted 8 weeks, followed by a 12-week observational follow-up to monitor post-supplementation changes in serum 25(OH)D concentrations and related variables. This follow-up duration was chosen based on previous studies indicating that plasma 25(OH)D levels remain stable one month after supplementation with 2000 IU/day of vitamin D_3, and that the half-life of circulating 25(OH)D is approximately two months, allowing for a reliable assessment of the expected gradual decline after cessation of supplementation[30,31]. The daily dose of 2000 IU vitamin D_3 (cholecalciferol) was selected as a safe and effective regimen supported by prior intervention trials in athletes showing significant improvements in serum 25(OH)D with comparable doses[15,16]. Both male and female participants were included to ensure gender representation and to allow preliminary exploration of potential sex-related differences in vitamin D response.

Study population

Five local running associations were contacted and informed about the research project and its inclusion/exclusion criteria. Potential non-runner participants were reached through social media platforms and local cultural associations. Figure 1 presents the CONSORT flow diagram of participant enrolment. A total of 57 adults were initially recruited; of these, 45 completed the study: 23 amateur runners (15 males and 8 females) and 22 non-runners (10 males and 12 females). All participants were Caucasian and resided and trained at a latitude between 43.6° N and 43.9° N.

General inclusion criteria for all volunteers were being healthy (absence of acute or chronic diseases, no history of surgery in the previous year, no smoking habits, no excessive alcohol consumption (> 3 drinks/day), no Body Mass Index (BMI) > 30, and no ongoing treatments or supplementation known to affect immune, hematological, or musculoskeletal function) at the time of enrolment and aged between 20 and 45 years in order to reduce variability associated with growth, hormonal changes, or age-related decline[32]. Additionally, this age range allowed the recruitment of comparable groups of male and female amateur runners and non-runners, thereby ensuring homogeneity and balance between the intervention and control groups. For runners, eligibility additionally required at least three consecutive years of endurance running training prior to the study, a minimum training frequency of three sessions per week, and an average outdoor mileage of at least 50 km/week for men and 40 km/week for women. This information was obtained through a structured questionnaire during the screening phase that included specific items on training history such as the number of years of continuous endurance running practice, with the requirement of no training interruptions longer than one month. All runners owned GPS-enabled devices of different brands and were instructed to maintain a daily training log using a shared Google document, beginning 15 days prior to the commencement of assessments and continuing throughout the duration of the study. In this log, they recorded the type of training session (continuous running vs. interval running), the number and characteristics of intervals (including recovery and distances), and the total distance covered (km). The decision to differentiate mileage thresholds between male and female runners was based on the evidence that recreational male endurance runners report higher weekly training volumes than their female counterparts. For example, Knechtle et al.[33], demonstrated that male runners completing half-marathon, marathon, and ultra-marathon events accumulated significantly greater weekly mileage compared to female runners across training periods and preparation phases. This evidence supported our choice of applying sex-specific criteria that reflect the typical training characteristics of recreational male and female runners.

Non-runners were defined as individuals who had not engaged in regular running practice or other structured endurance or strength programs during the previous six months, although the majority reported engaging in light physical activities, such as walking during their daily commute or participating in occasional recreational activities. The physical activity status was assessed in both groups using the International Physical Activity Questionnaire (IPAQ). The mean IPAQ values were 2,622 ± 1,382 MET·min/week in non-runners and 6,446 ± 3,928 MET·min/week in runners. Despite not being involved in systematic training, according to the IPAQ scoring protocol, non-runners were classified as "active".

Exclusion criteria were: smoking; consumption of more than three alcoholic drinks per day (Beer: 280–330 mL; Wine: 100–120 mL; Spirits: 30–40 mL; Liqueur: 60–80 mL); BMI > 30; presence of acute or severe chronic diseases; surgery in the past year; use of medications interfering with muscle recovery or musculoskeletal performance; intake of supplements such as vitamin D, calcium, iron, or immune-stimulating complexes containing zinc or Echinacea; hypersensitivity to any of the ingredients in the vitamin D supplement and, for female participants, a diagnosis of early menopause.

All participants gave written informed consent (signed in September) after a medical screening consisting of a detailed medical history, pulmonary function tests, and a resting electrocardiogram[34]. The study protocol was approved by the Ethics Committee of the University of Urbino "Carlo Bo", Italy (54_24gennaio2023_running D+) and was carried out in accordance with the Declaration of Helsinki.

Study interventions

Data collection was conducted across three time points: mid-autumn 2023 (third week of October, T0), late autumn 2023 (third week of December, T1), and late winter 2024 (second week of March, T2). After enrollment, participants first visited an analysis laboratory for blood serum analysis to determine vitamin D, leukocyte, and

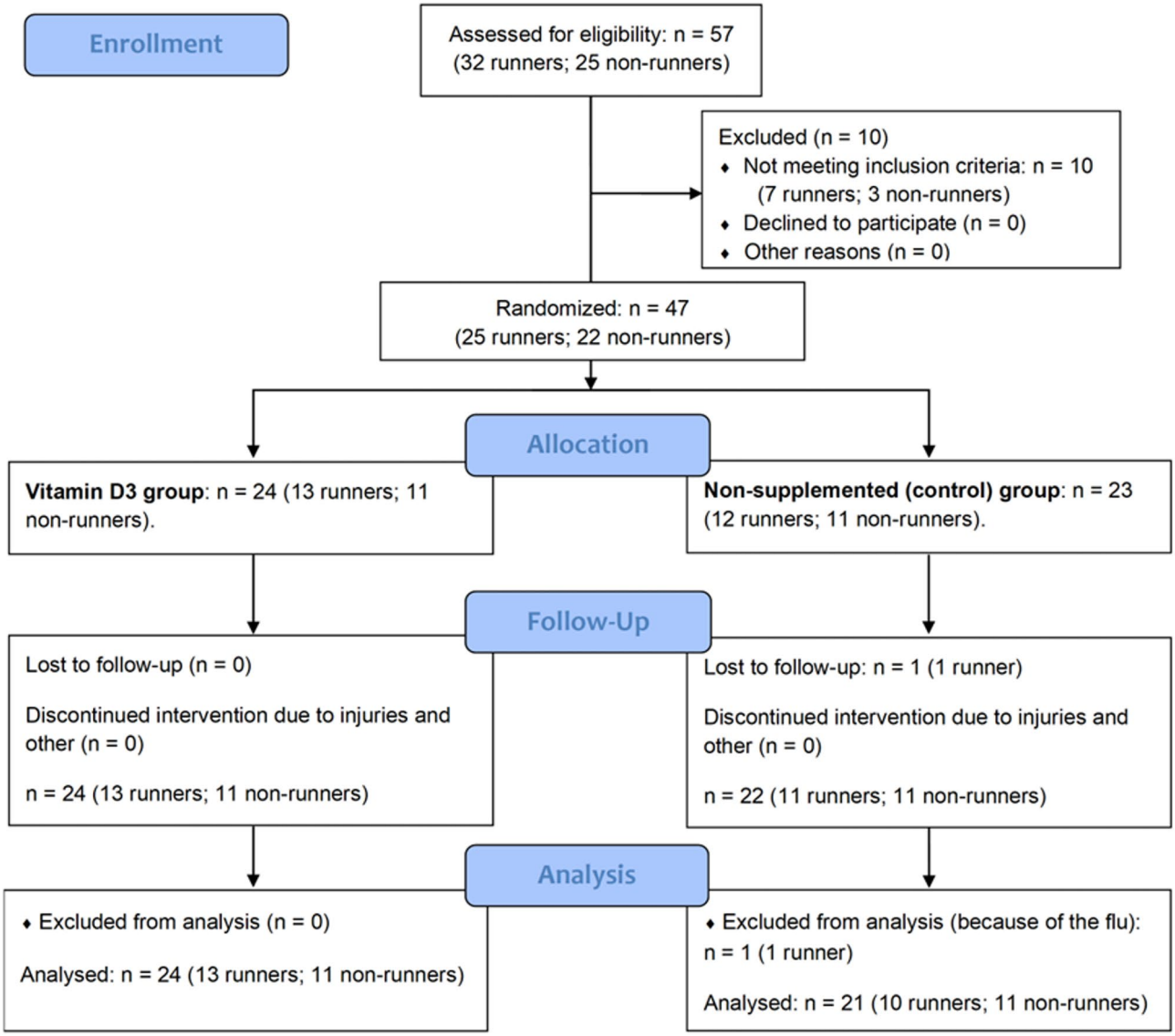


Fig. 1. Consolidated standards of reporting trials (CONSORT) flow diagram of the study participant enrollment.

calcium levels. They proceeded then to the laboratories of the Sport Sciences Center at Urbino University (Italy) where they followed a standardized routine, that included: (i) completion of the Physical Activity Rating (PA-R) 0–15 scale questionnaire[35,36]; (ii) anthropometric assessments (weight, height); (iii) a warm-up phase with 5 min of walking/running treadmill followed by 5 min of mobilization exercises; (iv) a maximal isometric strength test using a sensor-controlled leg press; (v) a 5-minute rest followed by a countermovement jump test using a force platform; (vi) graded exercise testing (GXT) for runners to assess maximal oxygen consumption[21], while non-runners performed a submaximal CustomTM test that provided a scientifically valid estimation of maximal oxygen consumption[36]. All performance tests were conducted in the afternoon, between 3:00 PM and 6:00 PM. A comprehensive description of the testing procedures is provided in the 'Performance Tests' section.

Participants were then randomly assigned into four groups: non-runners non-supplemented (NON-RUN-CON), non-runners supplemented (NON-RUN-SUPPL), runners non-supplemented (RUN-CON) and runners supplemented (RUN-SUPPL). Groups were balanced by gender and activity (in Table 1 demographics and anthropometrics data of randomized participants are reported). The supplemented groups received an oral daily dose of 2000 IU vitamin D_3 (orodispersible films, IBSA Farmaceutici Italia, Lodi, Italy), while the non-supplemented groups received no supplement. Supplemented participants received daily reminders to take the

	NON-RUN-CON ($n=11$)	NON-RUN-SUPPL ($n=11$)	RUN-CON ($n=10$)	RUN-SUPPL ($n=13$)	p-value
Age (y)	**28.3 ± 6.0**	**29.3 ± 7.5**	**34.8 ± 8.6**	**33.4 ± 7.5**	**0.138**
Height (m)	1.68 ± 0.11	1.70 ± 0.08	1.73 ± 0.06	1.70 ± 0.07	0.584
Weight (kg)	64.2 ± 10.4	65.3 ± 11.7	62.0 ± 8.2	62.5 ± 8.6	0.850
BMI (kg/m^2)	22.6 ± 1.8	22.4 ± 2.9	20.6 ± 1.6	21.5 ± 1.9	0.131

Table 1. Demographics and anthropometrics data of randomized participants according to running activities and supplementation.

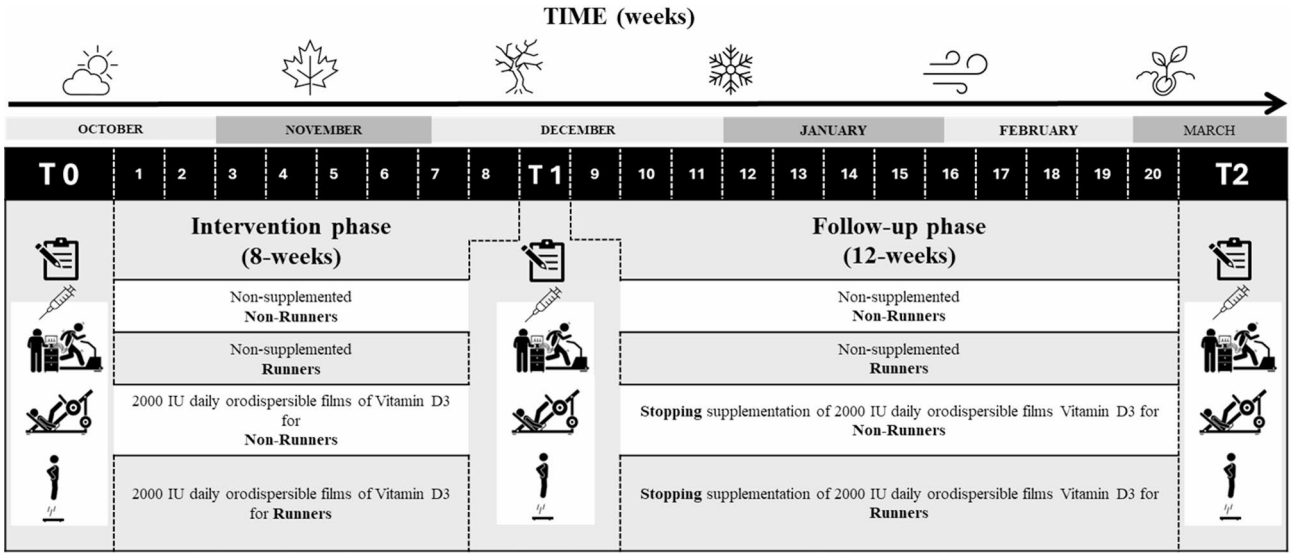


Fig. 2. Schematic representation of the experimental study design.

supplement before bedtime. All participants completed a baseline physical activity questionnaire (T0) and began dietary monitoring through a 24-hour food diary. Runners also recorded their daily running distance.

Air temperature was measured with a Rotronic MP103A probe (Rotronic, Milan, Italy) using a PRT100 platinum sensor, delivering a linear 0–1 V output mapped to − 40 to + 60 °C with ± 0.3 °C accuracy. Global solar irradiance was sampled every second with a Delta OHM LPPYRA02 thermopile pyranometer (Senseca Italy Srl, Italy) and the signal was logged as total amount in kJ/m². Data on temperature, average daily solar radiation and daily sunshine duration were provided by the "Alessandro Serpieri" Meteorological Observatory, Department of Pure and Applied Sciences of the University of Urbino Carlo Bo (Italy).

Values are presented as mean ± SD. Differences among groups were assessed using one-way ANOVA. The p-values refer to comparisons across the four groups for each variable. RUN-SUPPL: supplemented runner participants; RUN-CON: non-supplemented runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; NON-RUN-CON: non-supplemented non-runner control participants; BMI: Body mass index.

After 8 weeks of supplementation, all participants repeated the dietary monitoring, hematological analyses, and physical tests in the same order. The supplemented group ceased supplementation at this point (T1), while the control group continued without supplementation. After an additional 12 weeks (T2), participants repeated all hematological tests, VO_2max, MIF, and jump tests, as well as diet and training monitoring. In Fig. 2a schematic representation of the experimental study design is reported. The experimental activities concluded in March with the final physical tests.

Performance tests

Part of the methodological procedures described in the present study, including baseline performance testing, hematological assessments, and dietary monitoring, were previously reported in a cross-sectional investigation by Pegreffi et al.[29] conducted on the same cohort, from which the T0 data were derived and are therefore cited for methodological transparency.

Jump test

A force platform (MuscleLab™ system, PFMA 3010e, Ergotest Innovation AS, Norway) with a sampling frequency of 100 Hz was used to measure vertical peak force (N) during a bilateral countermovement jump (CMJ) without arm swing. Each trial began with the participant standing still with knees fully extended, arms on the hips, and feet at shoulder width. Participants then performed a downward movement to approximately 90° of knee flexion

before initiating the upward phase as explosively as possible. Proper technique and positioning were monitored before and during each jump, and only valid trials were considered.

After completing 3–5 warm-up jumps, participants executed three maximal efforts, with a 1-minute rest between attempts. Jump performance was quantified based on jump height (m), calculated using the impulse–momentum method[37], and the highest one was used for subsequent analyses.

Isometric leg press test

A uniaxial load cell (AIP, Varese, Italy) interfaced with an analog-to-digital converter (Muscle Lab, Rome, Italy) with a sampling frequency of 100 Hz was used to measure peak force (N) generated during maximal isometric leg press contractions. The transducer was integrated in line with the sliding mechanism of a horizontal leg press apparatus (Technogym, Gambettola, Italy), allowing direct acquisition of the applied force signal. To ensure mechanical rigidity, the two chains anchoring the load cell to the machine were pre-tensioned before each trial, after which the system was zeroed to eliminate any residual preload generated by the chains. Calibration of the dynamometer was routinely verified using ISO-certified reference weights.

Participants were positioned supine on the leg press with the backrest inclined at 30° relative to the horizontal plane. The knee angle was targeted at 100 degrees, and its positioning was guided using an electronic goniometer (Muscle Lab, Rome, Italy). Due to the discrete adjustment slots of the leg press machine, it was not possible to set the knee angle with continuous precision. Therefore, the goniometer was used to identify the slot that yielded a knee angle as close as possible to 100 degrees, ensuring consistency across participants. Prior to maximal testing, participants completed two to three submaximal isometric contractions to familiarize themselves with the task and to provide a specific warm-up. They then performed three maximal isometric contractions, each consisting of a 5-second effort during which they were instructed to exert force as hard and fast as possible. A recovery period of 2 to 3 min was provided between trials. A minimum rest period of 2 min was enforced, and participants were instructed to extend the rest up to 3 min only if they did not feel mentally prepared to perform the next maximal effort. This approach aimed to reduce variability due to perceived fatigue and improve the ecological validity of the protocol. Since the participants were not specifically trained in this type of contraction, this flexible recovery period allowed them to regain both neuromuscular and psychological readiness before each attempt. Strong verbal encouragement was provided throughout testing. The highest peak force value recorded across the three trials was retained for analysis as maximal isometric force (MIF)[38].

Maximal oxygen consumption tests (VO_2max)

For the GXT protocols, the initial running/walking speed was individually set according to each participant's estimated peak velocity (Vpeak), determined through a two-step estimation procedure based on the Physical Activity Rating (PA-R) questionnaire[35]. Equation 1 provided a non-exercise estimate of VO_2max using PA-R, age, BMI and sex, according to the regression model proposed by Jamnick et al.[35]. Equation 2, derived from the linear relationship between oxygen uptake and running speed originally described by Léger and Mercier[39] and then revisited by Strom et al.[36] was then used to convert the estimated VO_2max into an estimated peak running velocity (Vpeak) on level ground.

$$\begin{aligned} &Estimated\,VO_2max\ (mL/kg/min) \\ &= 56.363 + (1.921 \times PA-R) - (0.381 \times Age) - (0.754 \times BMI) + (10.987 \times Gender) \end{aligned} \tag{1}$$

$$\text{Estimated}Vpeak\ (m/min) = \frac{(Estimated\,VO_2max - 3.5)}{0.2} \tag{2}$$

where PA-R is the Physical Activity Rating questionnaire (0 to 15 scale); Age is participants' age (years); BMI is the Body Mass Index (kg/m^2); Gender is 0 for females and 1 for males; and Vpeak is the peak running velocity (meters per minute or m/min)[36]. Subsequently, since the ergometers operated in km·h^{-1}, Vpeak values were converted to the same unit. The GXT began with a 5-minute warm-up at 13 km·h^{-1} less than each athlete's estimated Vpeak, followed, without pause, by increments of 1 km·h^{-1} per minute until exhaustion (the incline level of the treadmill was set to 1%). The oxygen consumption (VO_2), heart rate (HR), and rate of perceived effort (RPE) were continuously monitored through a metabolimeter (K5, Cosmed, Rome, Italy), a Polar H10 heart rate sensor (Polar Electro Oy, Kempele, Finland) and the 6–20 Borg scale[35,36,40]. The K5 system provides real-time breath-by-breath gas analysis using a turbine flowmeter and electrochemical and infrared sensors to measure ventilation and gas concentrations. Its validity and reliability for VO_2 measurement in breath-by-breath mode have been previously demonstrated[41]. Maximal effort criteria[35,36,40] were considered to verify the outcomes, for which participants had to meet at least three of the following: (i) failure of HR to increase with further increases in exercise intensity; (ii) a plateau in VO_2 (or failure to increase VO_2 by 150 mL·min − 1) with increased workload; (iii) a respiratory exchange ratio (RER) ≥ 1.10; (iv) an RPE > 17 on the 6–20 scale. The maximum VO_2 (VO_2max) was defined as the highest value of a 15-second rolling average of breath-by-breath oxygen consumption data calculated across the entire duration of the cardiopulmonary exercise test. This method is consistent with standard CPET procedures, which recommend using short-period moving averages (e.g., 15–30 s) to account for breath-by-breath variability and to better capture transient peaks in oxygen uptake[42]. In all participants, VO_2max was observed either during the final or the penultimate workload stage. Maximum heart rate was defined as the highest single HR value recorded during the GXT[35,36,40]. If verified, individuals' physiological parameters were determined and considered for subsequent analysis.

The non-runners participants underwent a CustomTM protocol, consisting of two consecutive 3-min stages (i.e., 6-min total test duration), whereby the first and second stages were set at 35 and 70% of the estimated Vpeak (Eqs. 1–2), respectively. Similarly to the GXT protocol, VO_2 and HR data were recorded continuously. The

speed associated with the age-predicted heart rate maximum was extrapolated from the submaximal HR values (x-axis) relative to the actual speeds (y-axis) via linear regression[36].

Dietary and training monitoring

Participants' dietary intake and training routines were monitored during the two weeks preceding each blood sampling session (T0, T1, and T2). They were instructed to maintain both a food and training log, while an experienced nutrition researcher conducted daily 24-hour recalls to ensure compliance and accuracy. Detailed guidance was provided on how to complete the food records, including standardized approaches for estimating portion sizes. For each monitoring period, participants were asked to document all foods and beverages consumed over 14 consecutive days as precisely as possible, including the date, time, and weight (in grams) of every item consumed. Adherence to the supplementation protocol was verified through daily evening phone calls by the nutrition researcher. During these calls, participants confirmed the intake of the prescribed vitamin D dose, and any miss was promptly addressed with reminders.

Food diary data were processed using WinFood nutritional analysis software (version Pro 3.37.3; Medimatica S.u.r.l., Teramo, Italy). Analyses included macro- and micronutrient intake, with particular attention to average calcium and vitamin D consumption. Macronutrient intake (proteins, fats, and carbohydrates) was expressed in grams (g), percentage (%), and grams per kilogram of body weight (g/kg/day). Micronutrients were reported in milligrams (mg) and micrograms (µg).

Athletes were instructed to complete a structured training diary after each session. The diary recorded the type of training (continuous or interval running), the total distance covered (km), the duration of the session (min), and the session rating of perceived exertion (sRPE) using the CR-10 scale[43]. For interval training sessions, participants were additionally required to specify the workout format (e.g., 10 × 400 m with 1-min recovery) and to report the split times achieved in each repetition. From these records, the weekly training volume (hours per week) and the distribution of continuous and interval-based training were computed for each athlete.

Prior to the intervention, athletes were also asked to indicate their habitual training time. Most runners (16 out of 23) reported a preference for training during daylight hours before dusk, whereas the remaining participants alternated morning and evening sessions according to work schedules.

Hematological tests

Seven days after completing the anthropometric and performance assessments, participants attended a certified blood collection facility (Biolab s.r.l., Pesaro, Italy) for venipuncture. They were instructed to arrive by 8:30 a.m. after an overnight fast of at least 8 h. All laboratory procedures were conducted under blinded conditions, meaning the technician was unaware of each participant's group allocation (runner or non-runner). Participants were seated in a semi-reclined position, and blood was drawn from the antecubital vein using a butterfly needle. Serum concentrations of 25(OH)D3 were measured using a chemiluminescent immunoassay (Beckman Coulter Inc., A98856, Brea, CA, USA), which demonstrated this intra-laboratory precision: SD ≤ 1.5 ng/mL for values ≤ 15.0 ng/mL and CV ≤ 10.0% for values > 15.0 ng/mL (37.5 nmol/L). Additional hematochemical parameters were determined with a Beckman Coulter DxH 800 automated analyzer (Beckman Coulter Inc., Brea, CA, USA). The variables analyzed included total leukocyte count, lymphocytes, monocytes, neutrophil granulocytes, and calcium (Ca).

Statistical analysis

A priori sample size estimation was performed using G*Power (v3.1.9.7) for a one-way ANOVA with four groups (supplementation × running status). The expected large effect size (Cohen's d ≈ 1.25, corresponding to f ≈ 0.625) was derived from previous vitamin D_3 supplementation studies reporting mean increases of 10–25 ng/mL in serum 25(OH)D after 8–12 weeks[44,45]. With $\alpha = 0.05$ and power $(1 - \beta) = 0.80$, the analysis estimated that a total sample size of 36 participants (approximately nine per group under equal allocation) would be sufficient. Our final sample of 45 participants, with 10 to 13 individuals per group, therefore exceeded the a priori requirement for group-specific sample size. All statistical analyses were performed using JASP (version 0.18.2.0, Department of Psychological Methods, University of Amsterdam, Amsterdam, The Netherlands, https://jasp-stats.org/). Statistical significance was set at a level of 0.05. The distribution of our data was tested using the Kolmogorov-Smirnov test, the Shapiro-Wilk test, and the Q-Q plot. Data were presented as mean ± standard deviation (SD).

One-way ANOVA with Dunnett's post-hoc test was used to compare mean solar radiation (kJ/m^2) and temperature (°C) across months from October to March. A Multivariate Analysis of Variance (MANOVA) was conducted to evaluate the differences in the dependent variables according to the group (supplemented vs. non-supplemented) and time (within Time*Supplemented/Non-supplemented; Time*Runner/Non-runners; Time*Supplemented/Non-supplemented*Runners/Non-runners). MANOVA was chosen to account for the potential correlations between multiple dependent variables and to provide a more comprehensive understanding of the group differences. The percentage variation of the different variables in T1 and T2 with respect to T0 were calculated using the formula $\%\Delta = ((Tx - T0) / T0) * 100$, where T0 represents the baseline value (T0 = 0%) and Tx the post-intervention value at a given time point (T1 or T2). This approach allowed to express all changes as relative variations from baseline.

Post-hoc comparisons were performed using Tukey's HSD test to identify specific group differences when significant effects were found. Partial eta squared (η^2_p) was calculated as the effect size for all ANOVA tests and interpreted according to conventional thresholds (small = 0.01, medium = 0.06, large = 0.14)[46,47].

Results

Variation of serum vitamin D levels and solar radiation

Baseline (T0) vitamin D was measured in mid-October (Fig. 2). As shown in Fig. 3, weather conditions were generally sunny, with a mean temperature of 18.1 ± 2.7 °C, mean daily solar radiation of 12,119.4 ± 3861.4 kJ/m², and a maximum of 17,439 kJ/m². The second (T1) and third (T2) vitamin D measurements were obtained in mid-December and mid-March, respectively (Fig. 2). Compared with October, mean temperature was 10.1 °C lower (95% CI, 8.2 to 11.9; $p < 0.001$) in December and 8.1 °C lower (95% CI, 6.2 to 9.9; $p < 0.001$) in March. Solar radiation similarly declined from October to December (mean difference, 6414 kJ/m²; 95% CI, 3955 to 8874; $p < 0.0001$) and returned to October levels by March (mean difference, 103.4 kJ/m²; 95% CI -2376 to 2583; $p > 0.05$).

The ANOVA revealed a significant main effect of time on serum level of vitamin D ($p < 0.001$, $\eta^2_p = 0.605$), as well as a significant time × supplementation interaction ($p < 0.001$, $\eta^2_p = 0.258$) and a significant time × running status interaction ($p = 0.025$, $\eta^2_p = 0.095$). There were significant changes in vitamin D levels from T0 to T1 in both supplemented and non-supplemented groups, with notable differences between runners and non-runners. Specifically, in the RUN-SUPPL group, vitamin D levels increased by 20.82%, from 30.45 ± 7.0 to 35.35 ± 8.9 ng/mL ($p < 0.001$). In contrast, the RUN-CON group did not show a significant change at T1, with levels decreasing slightly from 29.7 ± 6.6 ng/mL to 28.3 ± 5.9 ng/mL. However, the NON-RUN-SUPPL group at T1 experienced a 28.78% increase in vitamin D, from 25.0 ± 8.7 to 30.2 ± 9.7 ng/mL ($p < 0.001$). In the NON-RUN-CON group, there was a -32.23% reduction in vitamin D, from 25.93 ± 6.6 to 17.8 ± 7.3 ng/mL ($p < 0.001$). After the follow-up period at T2, the RUN-SUPPL group experienced a significant reduction in vitamin D levels, from 35.35 ± 8.9 to 23.52 ± 5.6 ng/mL ($p < 0.001$). A decrease was also evident in the RUN-CON group, with levels falling from 29.7 ± 6.6 to 22.5 ± 6.3 ng/mL ($p < 0.001$). For the NON-RUN-SUPPL group, vitamin D levels decreased significantly from 30.2 ± 9.7 to 20.8 ± 5.0 ng/mL ($p < 0.001$), while the NON-RUN-CON group experienced a further drop from 17.8 ± 7.3 to 15.2 ± 4.2 ng/mL ($p < 0.001$). Figures 3 and 4 illustrate the variations over time between the supplemented and non-supplemented groups, as well as between runners and non-runners, along with their respective percentage changes.

NON-RUN-CON: non-supplemented non-runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; RUN-CON: non-supplemented runner control participants; RUN-SUPPL: supplemented runner participants. *** Statistically significant from T0 to T1 ($p < 0.001$); ### Statistically significant from T0 to T2 ($p < 0.001$); ††† Statistically significant from T1 to T2 ($p < 0.001$).

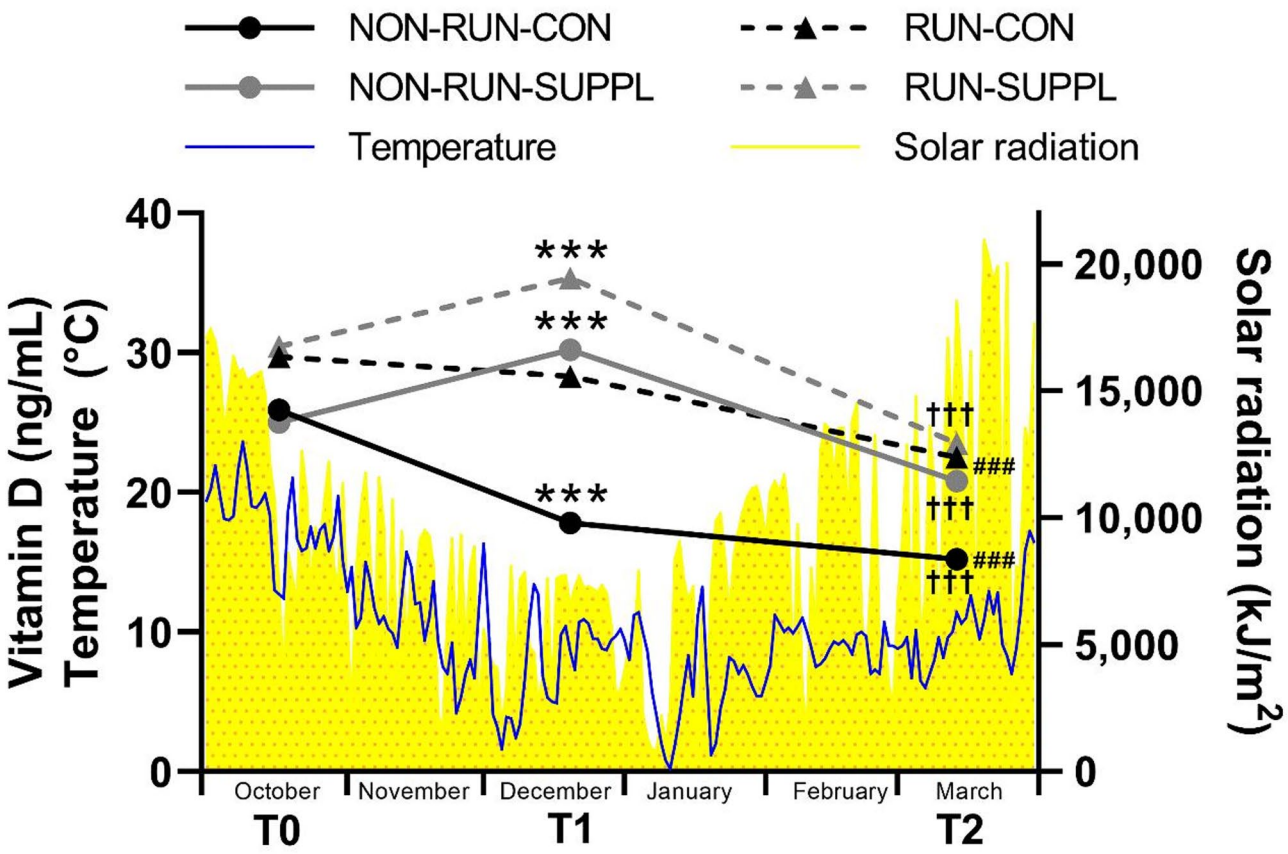


Fig. 3. Variation in 25(OH)D_3 levels between supplemented and non-supplemented groups, solar radiation and temperature during the experimental period (from T0 in October to T2 in March).

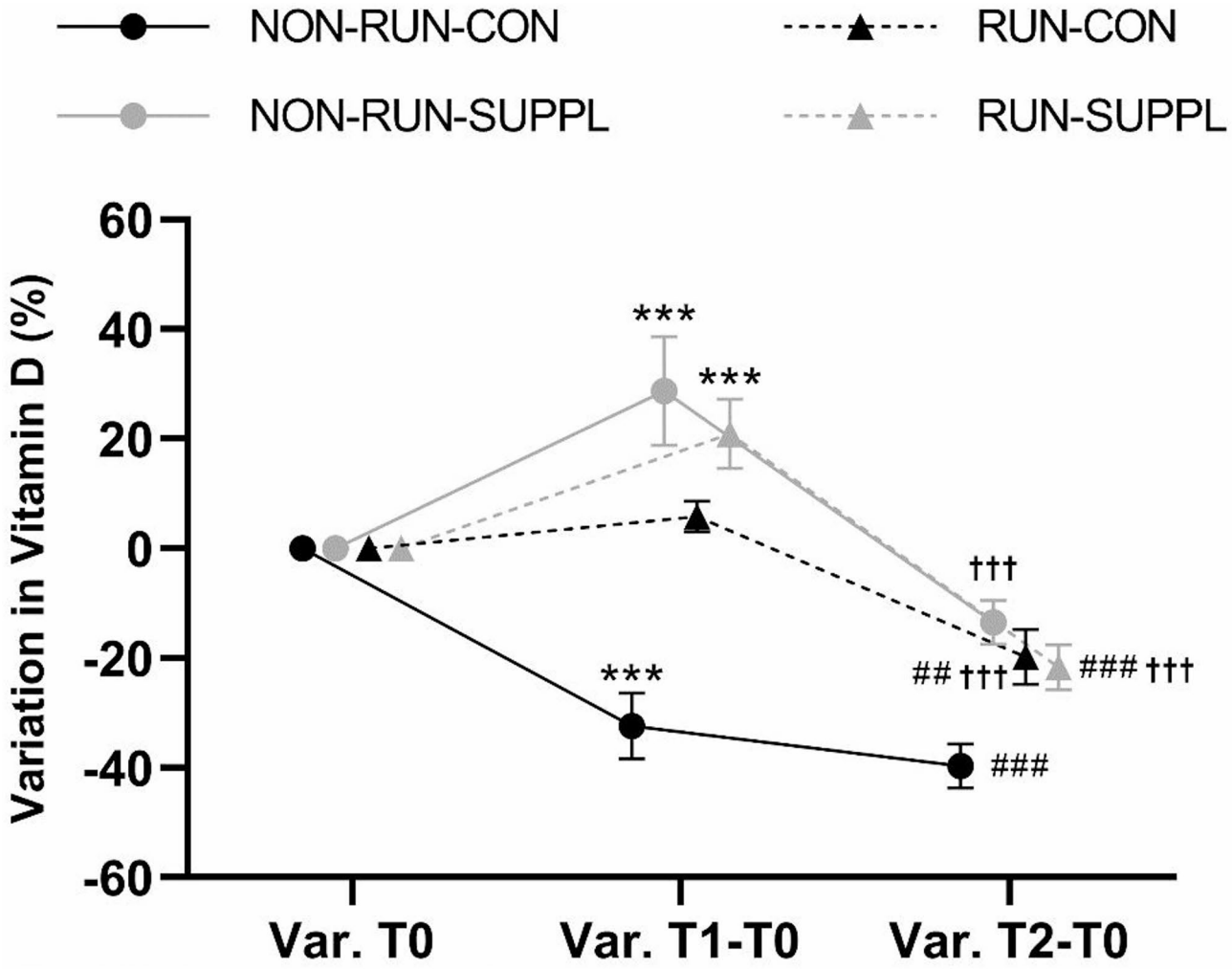


Fig. 4. Variation in 25(OH)D_3 levels between supplemented and non-supplemented groups as percentage variation (%) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2). Error bars represent the standard error of the mean (SEM).

NON-RUN-CON: non-supplemented non-runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; RUN-CON: non-supplemented runner control participants; RUN-SUPPL: supplemented runner participants. *** Statistically significant from T0 to T1 ($p < 0.001$); ## Statistically significant from T0 to T2 ($p < 0.01$); ### Statistically significant from T0 to T2 ($p < 0.001$); ††† Statistically significant from T1 to T2 ($p < 0.001$).

Training characteristics of runners and diet monitoring

Training data are reported separately for male and female runners to provide a clearer descriptive overview of habitual training volume and intensity, although statistical analyses were not stratified by sex. No significant differences in training characteristics were observed over time ($p > 0.05$). On average, male runners completed 5.8 ± 0.8 sessions per week, accumulating 5.9 ± 1.7 h of training per week, and covering 48.2 ± 11.2 km of continuous running (sRPE = 3.4 ± 1.0) and 10.6 ± 1.8 km of interval training (sRPE = 7.4 ± 1.0). Female runners performed 4.7 ± 0.8 sessions per week, corresponding to 4.4 ± 1.4 h of weekly training, with 33.6 ± 10.5 km of continuous running (sRPE = 3.1 ± 1.1) and 7.2 ± 0.7 km of interval training (sRPE = 6.9 ± 0.4).

Overall adherence to the supplementation protocol was high, with participants reporting > 95% compliance across the study period. Missed doses were rare and were promptly corrected following reminders.

Macronutrient intake was assessed in all four groups. Carbohydrate consumption was significantly higher in runners compared to non-runners ($p = 0.026$), with mean values ranging from 248 to 253 g/day versus 191 to 208 g/day across the three time points. Specifically, runners consumed 248.1 ± 55.5 g/day, 253.3 ± 61.4 g/day, and 246.5 ± 65.0 g/day before T0, T1, and T2, respectively, while non-runners consumed 208.3 ± 45.3 g/day, 203.9 ± 52.4 g/day, and 190.9 ± 46.0 g/day before T0, T1, and T2, respectively. No significant changes over time were observed for carbohydrate intake. Protein and lipid intakes were comparable between groups and remained stable across time points (protein: 75–82 g/day; lipids: 68–72 g/day; both $p > 0.05$). Vitamin D intake from food sources was also monitored through dietary records and showed no significant differences among groups or

across time points (runners: 2.9 ± 1.6 µg at T0, 2.3 ± 1.1 µg at T1, and 3.0 ± 1.8 µg at T2; non-runners: 2.9 ± 1.7 µg at T0, 2.4 ± 1.1 µg at T1, and 2.9 ± 1.2 µg at T2; $p > 0.05$).

Performance tests results

Maximum oxygen consumption tests (VO_2max)

No significant differences in VO_2max were observed between RUN-SUPPL and RUN-CON groups or between NON-RUN-SUPPL and NON-RUN-CON groups ($p > 0.05$). Supplemented runners maintained stable VO_2max values of 59.7 ± 6.8, 60.1 ± 6.7, and 59.5 ± 6.9 mL·kg^{-1}·min^{-1} at T0, T1, and T2, respectively ($p > 0.05$). Similar stability was observed in the RUN-CON group (60.7 ± 6.7, 61.1 ± 7.1, and 60.4 ± 6.9 mL·kg^{-1}·min^{-1}, $p > 0.05$). Among non-runners, estimated VO_2max remained unchanged over time (NON-RUN-CON: 41.3 ± 6.7, 41.5 ± 6.8, and 41.1 ± 7.1, $p > 0.05$; NON-RUN-SUPPL: 39.6 ± 7.1, 39.5 ± 7.1, and 39.8 ± 7.1 mL·kg^{-1}·min^{-1}, $p > 0.05$). These trajectories are shown in Fig. 5a.

For the runner groups, no significant differences were found in maximum heart rate (HRmax) or Vpeak across time or between groups ($p > 0.05$). Mean HRmax values were 185 ± 11, 185 ± 11, and 182 ± 12 bpm at T0, T1, and T2, respectively, while Vpeak values were 18.1 ± 2.3, 18.2 ± 2.5, and 17.7 ± 2.5 km·h^{-1}.

NON-RUN-CON: non-supplemented non-runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; RUN-CON: non-supplemented runner control participants; RUN-SUPPL: supplemented runner participants.

Countermovement jump (CMJ) and maximum isometric force (MIF) performance

Regarding the countermovement jump (CMJ) test, no significant differences were observed in any of the groups over time ($p > 0.05$). Similarly, for maximal isometric force (MIF), the interaction between time and

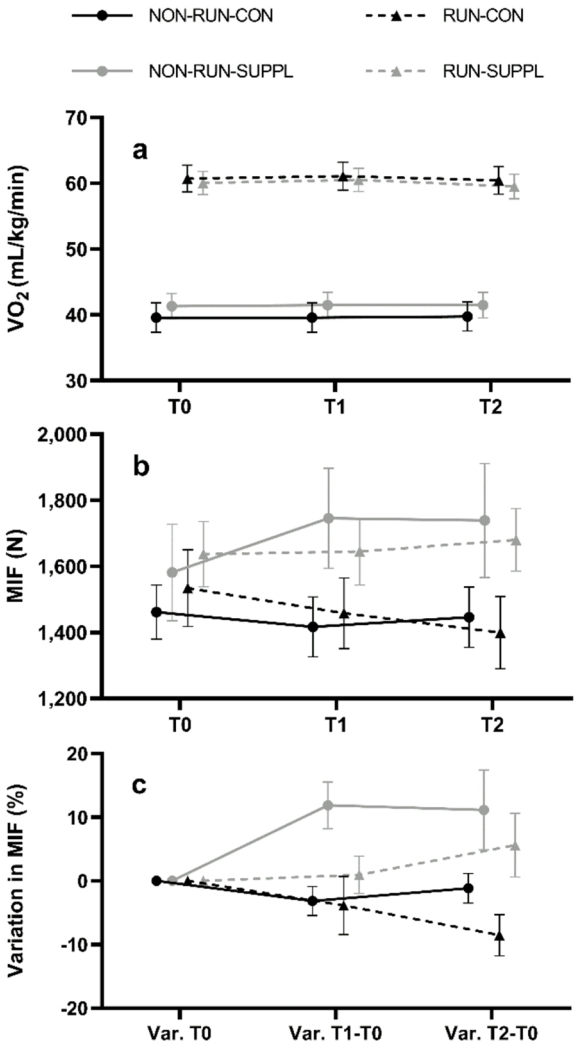


Fig. 5. **a**) Variation in VO_2max Levels (mL/O_2/kg) in all groups and time tested (from T0 to T2). **b**) Variation in MIF levels (N) in all groups and time tested (from T0 to T2). c). Percentage variation in MIF levels (N) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2). Error bars represent the standard error of the mean (SEM).

supplementation status approached significance ($p = 0.056$). Detailed descriptive values for CMJ and MIF across groups and time points are reported in Table 2. Since MIF values normalized to body mass closely overlapped with the absolute values and did not affect the interpretation of the results, only absolute values were reported to avoid redundancy. Figure 5b and c show the trends of MIF and the percentual variation in MIF, respectively.

Values are presented as mean ± SD for the four groups across the three measurement time points (T0, T1, T2). Differences across time and between groups were assessed using repeated-measures ANOVA. For each outcome, the analysis evaluated the main effect of time, the main effect of group, and the time × group (or time × supplementation) interaction. For CMJ, none of the ANOVA effects reached statistical significance (main effect of time: $p > 0.05$; main effect of group: $p > 0.05$; time × group interaction: $p > 0.05$); therefore, no post-hoc analyses were performed. For MIF, the time × supplementation interaction approached significance ($p = 0.056$), while all other effects were non-significant ($p > 0.05$). RUN-SUPPL: supplemented runner participants; RUN-CON: non-supplemented runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; NON-RUN-CON: non-supplemented non-runner control participants; CMJ: Countermovement jump; MIF: Maximal isometric force.

Hematological tests results

Serum calcium levels

A significant variation in calcium levels was observed over time ($p < 0.001$, $\eta^2_p = 0.250$), with no significant interactions between time and supplementation status ($p = 0.593$), time and runner status ($p = 0.754$), or the three-way interaction ($p = 0.771$). No significant between-group differences were found for supplementation ($p = 0.101$), runner status ($p = 0.621$), or their interaction ($p = 0.542$). Post-hoc analyses revealed a significant decrease in calcium levels from T1 to T2 ($p < 0.001$) and from T0 to T2 ($p = 0.002$), while no difference was observed between T0 and T1 ($p = 1.000$). Calcium levels in the RUN-SUPPL group at T0, T1, and T2 were 9.95 ± 0.26, 9.88 ± 0.35, and 9.81 ± 0.33 mg/dL, respectively. In the NON-RUN-SUPPL group, the levels were 9.90 ± 0.39, 9.86 ± 0.32, and 9.65 ± 0.44 mg/dL, corresponding to a − 2.53% change from T0 to T2 and − 2.13% from T1 to T2. In the RUN-CON group, levels were 9.70 ± 0.28, 9.80 ± 0.33, and 9.56 ± 0.29 mg/dL, with a − 2.45% change from T1 to T2. Finally, in the NON-RUN-CON group, calcium levels were 9.73 ± 0.37, 9.78 ± 0.46, and 9.59 ± 0.46 mg/dL, showing a − 1.44% change from T0 to T2 and − 1.94% from T1 to T2. Across all groups, values remained stable or slightly increased from T0 to T1, followed by a consistent and statistically significant decline at T2.

Total leukocyte, neutrophil, lymphocyte and monocyte levels

Total leukocyte levels showed a significant time × supplementation interaction ($p = 0.026$, $\eta^2_p = 0.096$), while no other effects were significant. In the NON-RUN-CON group, leukocyte count decreased continually, dropping from 7,733 ± 2,489 µl/L to 6,558 ± 2,637 µl/L ($p = 0.0205$, − 15.2%) at T1, and further to 6,348 ± 1,754 µl/L ($p = 0.0066$, − 17.9%) at T2, as illustrated in Fig. 6a and b.

Neutrophil count showed a significant effect of time ($p < 0.001$, $\eta^2_p = 0.208$) and a time × supplementation interaction ($p = 0.026$, $\eta^2_p = 0.099$). In the NON-RUN-CON group, values dropped from 4.15 ± 1.72 to $3.35 \pm 1.31 \times 10^3/mm^3$ ($p = 0.0343$, − 19.4%) at T1, and then to $2.83 \pm 0.77 \times 10^3/mm^3$ ($p = 0.0003$, − 31.8%) at T2, as shown in Fig. 6c and d. The RUN-SUPL group showed a significant decrease only from T1 to T2, going from $3.50 \pm 1.37 \times 10^3/mm^3$ to $2.68 \pm 0.82 \times 10^3/mm^3$ ($p = 0.0415$, − 13.9%).

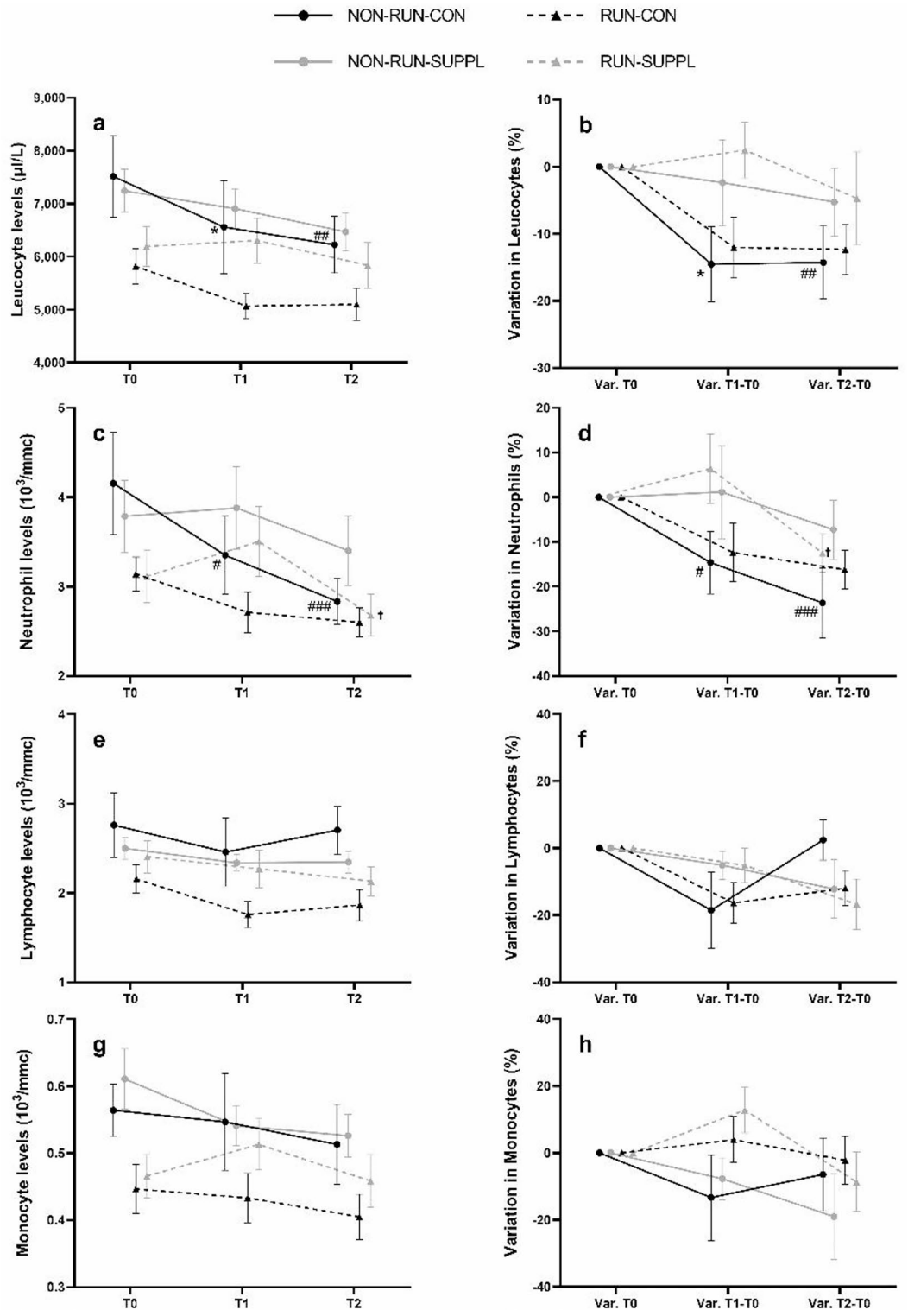
No significant differences over time or between groups were observed for lymphocyte or monocyte levels (all $p > 0.05$), whose trajectories are presented in Fig. 6e, f, g and h.

Discussion

This study investigated the effects of a 2-month daily 2000 IU vitamin D supplementation, administered via orodispersible films, on serum vitamin D status, performance, and leukocyte parameters in healthy runners and non-runners, compared to control groups without supplementation (T1), with a subsequent 3-month follow-up (T2). The main findings of the study were that at T1 supplementation induced a significant increase in vitamin D, whereas levels remained stable in non-supplemented runners and decreased in non-runners. By T2, all groups exhibited a decline. Neutrophil count was reduced in non-supplemented subjects, while VO_2max and CMI were unaffected.

Variable	Time	NON-RUN-CON	NON-RUN-SUPPL	RUN-CON	RUN-SUPPL
CMJ (m)	T0	0.260 ± 0.04	0.277 ± 0.07	0.282 ± 0.06	0.266 ± 0.06
	T1	0.269 ± 0.05	0.275 ± 0.07	0.277 ± 0.05	0.266 ± 0.05
	T2	0.270 ± 0.04	0.282 ± 0.08	0.273 ± 0.06	0.271 ± 0.05
MIF (N)	T0	1401.4 ± 235.4	1581.6 ± 506.9	1534.5 ± 385.9	1637.2 ± 370.9
	T1	1416.9 ± 286.8	1745.8 ± 523.7	1458.4 ± 353.6	1645.2 ± 378.9
	T2	1446.5 ± 289.3	1738.9 ± 598.9	1399.5 ± 363.4	1680.4 ± 352.0

Table 2. Countermovement jump (CMJ) and maximal isometric force (MIF) values (mean ± SD) across groups and time points.



The enrolled subjects were well-characterized, and the study groups were as homogeneous as possible in terms of subject characteristics. Notably, the supplemented groups exhibited very high compliance rates (> 95%) throughout the study period.

As previously reported by Pegreffi et al.[29], the baseline (T0, October) data from the same subjects indicated that the runners had slightly insufficient vitamin D levels (< 30 ng/mL), although these levels were still higher than those observed in non-runners. Following randomization and the experimental procedures, the RUN-CON group maintained relatively stable vitamin D levels from T0 to T1, likely due to continued outdoor training and associated sun exposure, while the RUN-SUPPL group showed a significant increase in vitamin D levels over the same period. However, during the follow-up period (T1 to T2, March), both runner groups experienced significant declines.

◀**Fig. 6**. **a**) Total leukocyte levels (µl/L) in all groups and tested over time; **b**) Variation in total leukocyte levels (µl/L) between supplemented and non-supplemented groups as percentage variation (%) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2); **c**) Total neutrophil levels (10^3/mmc) in all groups tested over time; **d**) Variation in total neutrophil levels (10^3/mmc) between supplemented and non-supplemented groups as percentage variation (%) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2); **e**) Total lymphocyte levels (10^3/mmc) in all groups tested over time; **f**) Variation in total lymphocyte levels (10^3/mmc) between supplemented and non-supplemented groups as percentage variation (%) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2); **g**) Total monocyte levels (10^3/mmc) in all groups tested over time; **h**) Variation in total monocyte levels (10^3/mmc) between supplemented and non-supplemented groups as percentage variation (%) in all groups and time tested (Var. T1-T0: variation between T0 and T1; Var. T2-T0: variation between T0 and T2). Error bars represent the standard error of the mean (SEM). *P*-values correspond exclusively to the analysis of total values. The variation charts are provided for illustrative purposes only. NON-RUN-CON: non-supplemented non-runner control participants; NON-RUN-SUPPL: supplemented non-runner participants; RUN-CON: non-supplemented runner control participants; RUN-SUPPL: supplemented runner participants. * Statistically significant from T0 to T1 ($p < 0.05$); # Statistically significant from T0 to T2 ($p < 0.05$); ## Statistically significant from T0 to T2 ($p < 0.01$); ### Statistically significant from T0 to T2 ($p < 0.001$); † Statistically significant from T1 to T2 ($p < 0.05$).

At T0, the NON-RUN-CON group already exhibited insufficient vitamin D levels, which further declined significantly at T2; in contrast, the NON-RUN-SUPPL group reached sufficient levels after supplementation, although this improvement was not sustained at T2, mirroring the post-supplementation decline seen in the RUN-SUPPL group.

Therefore, it seems meaningful to recommend adequate and continuous vitamin D supplementation in combination with regular physical activity, which would also help counteract seasonal variations in serum $25(OH)D_3$ levels in the non-active population. In particular, for runners, it appears crucial to take vitamin D after December, as the protective effect (likely due to increased sun exposure in warmer conditions when minimal clothing is required) seems to diminish dramatically. Indeed, in non-runner subjects who do not take vitamin D, the reduction in serum vitamin D levels appears to follow the decline in solar irradiation until January. However, despite the increase in solar exposure after January, serum vitamin D levels continue to decrease significantly, likely due to persistently low temperatures, which force individuals to spend more time indoors and remain heavily covered.

In terms of diet, vitamin D intake was consistent across all time points and between runners and non-runners, with no significant differences observed ($p > 0.05$). However, the analysis of macronutrient intake revealed that runners consumed significantly more carbohydrates than non-runners ($p = 0.026$) while protein and lipid intakes did not differ significantly between groups or over time. These findings suggest that during the overall training regimen, their vitamin D consumption was stable; while runners' higher carbohydrate intake likely reflects their greater energy expenditure during the training[48].

The European Food Safety Authority (EFSA)[49] reports that vitamin D is primarily synthesized by our body, accounting for approximately 80% of the 600 IU daily requirement. The remaining 20% is obtained through dietary intake.

In this study, the proportions of vitamin D derived from dietary sources are consistent between runners and non-runners. Given the comparable dietary intakes of vitamin D in both groups, the difference in serum vitamin D levels between runners and non-runners might be attributed to the endogenous vitamin D produced from 7-dehydrocholesterol through sunlight exposure[49]. Since the runners exercised in sunny weather conditions during daylight hours, it is likely that the observed differences were due to outdoor exercise. Sunlight exposure and dietary intake alone, however, seem to be insufficient in most individuals to maintain an optimal vitamin D status. Several factors, beyond nutrition and sun exposure, can influence both circulating vitamin D levels and its biological effects; these include genetic variants in key genes involved in vitamin D synthesis, transport, and metabolism[50], skin pigmentation, and anthropometric indices such as overweight and obesity[51]. A causal relationship between obesity and impaired vitamin D status has been established[52]; moreover, body mass index (BMI) may modulate the response to vitamin D supplementation, with individuals who are overweight or obese exhibiting reduced responsiveness[53,54].

Vitamin D deficiency has also been associated with gut dysbiosis and low-grade inflammation. Emerging evidence suggests that vitamin D supplementation can beneficially influence the composition and function of the gut microbiota, while, conversely, specific microbial signatures may contribute to interindividual variability in the response to vitamin D[53,54].

Currently, there is no international consensus on the optimal level for vitamin D supplementation. Recommendations differ in many countries and range from 400 to 2000 IU daily (10–50 µg). The EFSA recommends staying below 4000 IU/day (100 µg). Most countries have prudently set the safe upper level at 50 µg daily (2000 IU) for adults[55].

A 2000 IU vitamin D supplementation, even if not administered for therapeutic purposes, supports many metabolic reactions and processes. Supplements are easily accessible to anyone, easy to take, and are characterized by a high compliance, and could help to effectively counteract physiological vitamin D insufficiency or deficiency during the transition to winter, when sunlight exposure and endogenous synthesis decrease. Moreover, this orodispersible film form of administration was already positively tested and validated in clinical studies, being the effect of increasing serum $25(OH)D_3$ levels tangible[56,57].

Since vitamin D is a crucial immunomodulatory micronutrient, it regulates the innate immune system by driving monocyte differentiation into macrophages, enhancing their phagocytic and chemotactic activity, enabling efferocytosis, and limiting immunopathology[58]. Based on this evidence, the present study also aimed to examine the relationship between vitamin D levels and specific hematological immune parameters, such as monocyte count, which appear to play an important role in muscle function[59]. Interestingly, neutrophil levels significantly increased from T0 to T1 in the supplemented group, whereas a significant decrease was noted in the non-supplemented group. Between T1 and T2, both groups experienced a significant decrease in neutrophil levels. In contrast, monocyte levels exhibited similar patterns at T0, T1, and T2, with changes that were not statistically significant. Vitamin D plays a key role in regulating both innate and adaptive immunity in vertebrates[60]. Neutrophils are critical components of the first line of defense against invading microbes. For instance, studies in zebrafish have demonstrated that exogenous vitamin D enhances granulopoiesis in larvae, whereas adult zebrafish with a cyp2r1 mutation (affecting the capacity to 25-hydroxylate vitamin D) show lower intestinal neutrophil count. Furthermore, in gnotobiotic zebrafish larvae, the impact of vitamin D on granulopoiesis persisted, suggesting that vitamin D may regulate neutrophil production throughout early development independent of the microbiota[60].

Although Pegreffi et al.[29] identified monocyte count as a significant predictor of vitamin D status, a 2-month 2000 IU vitamin D supplementation did not significantly affect monocyte count. Current literature on this topic remains limited; however, both in vitro and in vivo non-clinical studies indicate that vitamin D contributes to immune-related processes, such as inflammation, and is vital for muscle tissue by influencing inflammatory responses, protein synthesis, and skeletal muscle function. This is achieved via two proposed mechanisms: first, the direct binding of $25(OH)D_3$ to vitamin D receptors (VDRs) in muscle cells, which may trigger genetic effects that alter myosin synthesis and increase muscle fiber dimensions[28]; and second, the modulation of calcium transport in the sarcoplasmic reticulum, though this mechanism requires further investigation[61].

At a clinical level, Jones et al.[28] investigated the influence of vitamin D status on exercise-induced changes in parameters of innate immune defense and metabolomic signatures, finding that vitamin D levels may play a significant role in modulating innate immune responses to a single, extended period of intense endurance exercise[28].

Regarding VO_2max, no significant differences were observed between the supplemented and non-supplemented groups or in the interaction between supplementation and running status. Maximal isometric force (MIF) values approached significance ($p = 0.056$) in the interaction between time and group, suggesting a trend toward higher strength values in the supplemented group at T1, although this did not reach statistical significance.

Overall, in this study, a 2000 IU vitamin D supplementation did not produce an ergogenic effect on strength, as no significant differences were found over time, despite the supplemented group tending to exhibit higher strength values at T1.

This study has several strengths. It was conducted across multiple time points and seasons (autumn and winter), allowing for an accurate assessment of seasonal changes in vitamin D status. The protocol included well-defined inclusion and exclusion criteria, close monitoring of training, diet, and supplementation compliance, and the use of validated tools for hematological and performance measurements. The randomized group allocation, high adherence rate, and inclusion of both runners and non-runners further enhanced the robustness and ecological validity of the findings.

However, several limitations should be acknowledged. Although the sample sizes exceeded the minimum required for adequate power, the relatively small number of participants in each group may still limit the ability to detect subtle differences in performance outcomes. Although participants were instructed to refrain from strenuous exercise for the previous 48 h and to avoid caffeine on the day of the physical tests, factors such as actual caffeine consumption immediately prior to CMJ and MIF assessments were not strictly controlled. Moreover, the proportion of men and women varied across groups, and sex-specific analyses were not performed due to limited subgroup size. While serum calcium was measured, ionized calcium was not assessed, and this may limit the interpretation of calcium homeostasis.

Future research should aim to address these limitations by including larger samples to reduce variability, considering stricter dietary standardization or specific dietary interventions where relevant, and ensuring rigorous control of pre-test behaviors such as exercise and caffeine intake. Additionally, studies should explore potential sex-specific responses and evaluate the effects of higher daily doses of vitamin D (greater than 2000 IU) to investigate possible ergogenic benefits. Further studies should also explore the mechanistic links between vitamin D status and immune or performance outcomes, including the role of ionized calcium, gut microbiota, and genetic variability in vitamin D metabolism and responsiveness.

Conclusions

1) A 2-month oral vitamin D supplementation in orodispersible film (2000 IU) was capable of achieving plasma levels > 29.00 µg/mL. An adequate vitamin D supplementation seems to be effective to counteract seasonal insufficiency and deficiency over a mid-term period. While non-runners should begin a daily intake of 2000 IU vitamin D in October, runners ought to initiate supplementation by December to prevent insufficiency or deficiency that may result in immune alterations and associated pathologies.

2) This supplementation did not promote any ergogenic effect, as determined in terms of cardiovascular performance or explosive power (lower limb jump test).

3) Vitamin D intake seemed to prevent the decrease of leukocyte levels (particularly neutrophils, as observed in non-supplemented subjects) independently of running activity.

4) Participants in the present study did not demonstrate a significant effect of vitamin D (2000 IU/day) supplementation on jump height or maximal isometric leg extensor force.

Future studies should examine potential interactions between vitamin D status and bone turnover markers in athletic populations, and assess whether these relationships differ across age groups, including adolescent and master/veteran athletes.

Data availability

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

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Declarations

Competing interests

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Institutional review board statement

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