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Nutrition for team and individual sport athletes

Edited by
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Nutrition for team and individual sport athletes

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Editorial: Nutrition for team and individual sport athletes

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dietary supplements, sports nutrition, nutritional periodization, athletes, sports performance

Editorial on the Research Topic
Nutrition for team and individual sport athletes

Introduction

Individual and team sports involve bursts of high-intensity activity interspersed with periods of moderate-lower intensity, but the specific patterns vary across different team sports and individual sports (1). Although it is challenging to predict the exact demands of any given disciplines, nutritional factors play a crucial role in individual or team sport performance, with growing scientific interest in developing personalized nutrition strategies for athletes. Such personalized approaches are designed to support and enhance health, body composition, exercise performance and recovery by tailoring dietary advice to align with an individual's genetic makeup (2) and lifestyle/training demands. However, when implementing personalized sports nutrition programs or incorporating dietary supplements, it is vital to take an evidence-based approach aligned with acknowledged physiological and biochemical mechanisms. Furthermore, evaluating the safety and efficacy of supplements in specific populations and examining their interactions with other components or nutrients are essential considerations. To tackle these topics, we introduced a special issue titled "*Nutrition for Team and Individual Sport Athletes*," which garnered submissions from researchers and academics experts in the field of sports nutrition. This special issue features fifteen high-quality original research articles. In this editorial, we will provide an overview of the key findings from each manuscript.

Dietary and nutritional supplements

Caffeine and beetroot juice

Caffeine and beetroot juice are dietary supplements have been extensively studied in recent years, with good to strong evidence of achieving performance benefits when used in specific scenarios (3). Mor et al., evaluated the impact of moderate-dose caffeine (i.e., 3 mg/kg) on anaerobic performance and hydration status in moderately trained male football players. The results of this study showed that caffeine improved vertical jump capacity and agility without affecting hydration status, but no differences in 30 m sprint, ball kicking speed and balance were found, suggesting caffeine is as an effective ergogenic aid for enhancing some neuromuscular performance determinants in football players. Referring to beetroot juice, Nyman et al., assessed the effectiveness of a mixed beet-based supplement (i.e., beetroot juice) vs. a placebo in reducing inflammation during recovery from sustained intensive cycling (2.25 h) in 20 male and female cyclists, using a multi-omics approach. A randomized, placebo-controlled, double-blind, crossover design was employed. Participants underwent two 2-week supplementation periods with a 2-week washout in between, followed by a 2.25 h cycling exercise at 70% $\dot{V}O_{2max}$. The exercise led to an increase in 41 of 67 detected oxygenated polyunsaturated fatty acids (i.e., oxylipins) that are bioactive molecules established as important mediators during inflammation. Beetroot juice supplementation notably raised post-exercise concentrations of anti-inflammatory oxylipins (i.e., 18-HEPE and 4-HDoHE) and proteomic analysis revealed changes in protein clusters related to inflammation. Thus, an acute 2-week intake of a beetroot juice was linked to enhanced post-exercise anti-inflammatory markers and a reduction in protein biomarkers associated with inflammation, supporting its potential in moderating exercise-induced inflammation in athletes.

Carbohydrate mouth-rinsing

Carbohydrates are the body's main source of energy for athletic events and very used for athletes in several forms (i.e., solid, liquid, mouth rinsing). Nyman et al. study explored the effects of carbohydrate mouth-rinsing on physical performance during ice hockey scrimmages in male athletes. Findings indicated that mouth-rinsing improved endurance and performance metrics during repeated high-intensity efforts on ice hockey performance. Thus, mouth rinsing with carbohydrate solutions (but not consuming) may be a valuable nutritional strategy to protect against decrements in external load with increased playing time in ice hockey without undue negative effects sometimes experienced in carbohydrates ingestions (e.g., gastrointestinal distress).

Menthol

There is also current interest in menthol consumption, which has been associated with improvements in several aspects of athletic performance including endurance, speed, strength and joint range of motion. Roriz et al. undertook a randomized, counterbalanced crossover design, in which participants consumed either a 0.01% menthol solution or a placebo noncaloric solution 45 min football protocol (i.e., Intermittent Soccer Aerobic Fitness Test). The authors analysed the effects of pre-exercise non-thermal cooling sensations on examined perceptual, physical, and physiological responses in football referees in a protocol consisting of two 45 min intermittent exercise bouts under hot and humid conditions, with physical performance measured as the total distance covered across three 15 min exercise blocks. Mouth rinsing with a menthol solution resulted in immediate improvements in thermal sensation and comfort. However, these perceptual benefits did not persist throughout the 45 min exercise protocol and did not translate into improvements in physical performance or physiological responses. Therefore, based on the findings, use of menthol solutions may be of limited impact for sustained events.

Turmeric

Acute muscle damage and subsequent inflammatory responses associated with intensive exercise has led to interest in strategies to promote rapid recovery which may be paramount to optimising subsequent performance and reducing injury risk. For this reason, Clayton et al., undertook a between-groups design study design to examine the effect of a turmeric supplement (i.e., 60 ml turmeric drink twice per day), containing high concentrations of curcumin a polyphenol proposed to reduce muscle damage and soreness in recreational athletes. The study aimed to determine whether turmeric supplementation could enhance recovery and improve neuromuscular performance [e.g., countermovement jump (CMJ) and isometric mid-thigh pull (IMTP)] and physiological markers of recovery [e.g., creatine kinase (CK) and plasma C-reactive protein (CRP)] in elite male football players. Measurements were taken immediately (0 h) and at 40–64 hours post-match following eight competitive matches showing that the curcumin-containing supplementation appeared to attenuate post-match inflammation, as indicated by reduced CRP levels. However, no significant effects were found on muscle damage markers (CK) or on neuromuscular performance measures (CMJ and IMTP). As such, further research is needed to establish the acute and chronic effects of polyphenol nutrients such as curcumin.

Other nutrients

New dietary or nutritional supplements appear in the scientific domain constantly and there is need to verify their efficacy. According to Penggalih et al., marine-derived substances, such as omega-3 fatty acids, proteins, biopeptides, carotenoids, glucosamine, and minerals, have demonstrated potential in addressing obesity-related health issues, including dyslipidaemia, diabetes, oxidative stress, and inflammation. However, their effects on sports performance are not as well-documented. For this reason, the authors of this review stress the importance of further research to investigate marine-derived proteins and the development of functional foods specifically designed for high-performance athletes. In addition, Zhou et al., undertook a systematic review and meta-analysis regarding effects of molecular hydrogen (H_2) supplementation and potential contribution in enhancing physical performance (i.e., endurance, muscular strength, and explosive power performance). Authors stated that H_2 supplementation short-term (<14 days) enhance lower limb explosive power, relieve fatigue, and improve blood lactate clearance, although they may not significantly boost aerobic endurance, anaerobic endurance, or muscular strength, being inhalation of H_2 appears to be the most effective method for enhancing physical performance, particularly lower limb explosive power, in healthy adults. Future studies with robust designs are needed to provide more definitive conclusions regarding the effects of H_2 on lower limb explosive power and muscle strength in this population.

Perception and use of supplements

The abovementioned studies support the utility of some supplements, when used in defined athletic populations (including football players), to support discrete performance outcomes. Acknowledging that research on supplements is a growing field, Abreu et al., aimed to investigate the supplement perspectives and practices of nutritionists working with elite football teams, via online questionnaire. They established that the majority of nutritionist (70.8%) agreed or strongly agreed to recommend dietary supplements to football players. Interesting, however, just 50% of nutritionist believed that supplements were effective, which may reflect findings in the literature, including within this special issue, that efficacy varies across supplements, sporting discipline, and outcome of interest.

Relationships between body composition, nutritional status/intake, health status (including RED-s), and nutritional knowledge assessment

Oukheda et al. evaluated whether the dietary practices of professional and adolescent football players in Morocco during the competitive period met international macronutrient recommendations and explored the relationship between their nutritional status and aerobic performance, as measured by the Yo-Yo IRL1 test. The authors reported that higher intake levels of carbohydrates and proteins were positively correlated with the total distance covered by the players, while higher proportion of energy derived from fats in the diet was negatively correlated with the distance covered. Therefore, optimizing carbohydrates and protein intake while managing fat consumption may be highly important for enhancing football performance. Staśkiewicz et al. assessed changes in body composition among professional football players throughout the macrocycle season, aiming to identify the correlation between nutrition knowledge and the maintenance of muscle mass. The authors assert that players' knowledge of macronutrient subcategories was significantly negatively correlated with the variability of skeletal muscle mass content. This finding establishes that nutrition knowledge impacts the stability of body composition across all analysed periods: preparatory, competitive, and transition phases highlighting the benefits of targeted strategies to improve the level of nutritional knowledge of athletes to maintain appropriate body composition. Ostensibly, the relationship between knowledge and body composition identified by Staśkiewicz et al. is mediated by dietary practices. AlKasasbeh and Akroush, in a cross-sectional study, aimed to explore the relationships between food habits, perceived barriers to healthy eating, and sports nutrition knowledge among adolescent swimmers. The study found a significant positive association between food habits and sports nutrition knowledge, determining that nutrition knowledge emerged as a significant positive predictor of healthy food habits. Nicholas and Grafenauer, were also interested in the potential value of knowledge and education. Their research aimed to establish a baseline understanding of dance students at a single pre-professional institution, using metrics focused on current health, nutrition, lifestyle, and overall wellbeing, while also assessing their knowledge of long-term health implications. Utilizing a cross-sectional study design, the Dance-Specific Energy Availability Questionnaire was adapted for Australian participants and administered online. They determined that assessing the health status and preventative health knowledge of pre-professional dancers can inform educational strategies that promote dancers' health and career longevity. The findings provide valuable insights into health knowledge and specific issues relevant to dancers, underscoring the need for tailored educational strategies to emphasize preventative health. Additionally, the study highlights the necessity for increased awareness of low energy availability and relative energy deficiency (REDs) in sport among practitioners working with dancers, as well as cultural and structural changes within the wider dance community to safeguard and enhance dancers' wellbeing. Also in the REDs field, Dvořáková et al. provide a comprehensive review of the markers used to diagnose Relative Energy Deficiency in Sport REDs and compare them to the REDs CAT2 score. This review highlights the most used diagnostic markers, such as bone mineral density, anthropometric parameters, and T3 hormone concentration. The authors emphasize the importance of standardizing methodologies in future research to assess the holistic nature of an individual's profile in determining the presence of REDs or other contributing factors as previously suggest in the literature (4).

Wider aspect of diet and nutrition

Finally, the last two articles of our special issue covered wider fields such as: the impact of nutrition on visual perceptual-cognitive performance in healthy adults; the interrelationships among food habits, sports nutrition knowledge, and perceived barriers to healthy eating in adolescent swimmers; and association between diet and sleep with internalising symptoms in young athletes. Beathard et al., examined the impact of nutritional intake on visual perceptual-cognitive performance in young, healthy adults. The authors reported that cognitive function benefits from higher dietary intake of carbohydrates, lutein/zeaxanthin, and vitamin B2. Conversely, elevated protein consumption was associated with a negative effect on visual perceptual-cognitive performance in female participants. Finally, Gao and Wang, realized a cross-sectional study investigating the association between diet, sleep, and internalizing symptoms in 758 young Chinese athletes. The Australian Athletes Diet Index was employed to evaluate dietary patterns, while sleep quality was measured using the Athletes Sleep Screening Questionnaire. Symptoms of anxiety and depression were assessed using the Generalized Anxiety Disorder 7 scale and the 9-item Patient Health Questionnaire. The findings indicated that diet mediated the relationship between chronotype and sleep quality, while sleep quality mediated the association between diet and symptoms of anxiety and depression. Furthermore, both diet and sleep quality jointly mediated the association between chronotype and symptoms of anxiety and depression.

Author contributions

AL-S: Writing – original draft, Writing – review & editing. GT: Writing – original draft, Writing – review & editing. JR: Writing – original draft, Writing – review & editing.

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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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The author(s) declare that no Gen AI was used in the creation of this manuscript.

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References

1. Mujika I, Burke LM. Nutrition in team sports. *Ann Nutr Metab*. (2011) 57(Suppl 2):26–35. doi: 10.1159/000322700

2. Guest NS, Horne J, Vanderhout SM, El-Sohemy A. Sport nutrigenomics: personalized nutrition for athletic performance. *Front Nutr*. (2019) 6:8. doi: 10.3389/fnut.2019.00008

3. Maughan RJ, Burke LM, Dvorak J, Larson-Meyer DE, Peeling P, Phillips SM, et al. Ioc consensus statement: dietary supplements and the high-performance athlete. *Br J Sports Med*. (2018) 52(7):439–55. doi: 10.1136/bjsports-2018-099027

4. Jeukendrup AE, Areta JL, Van Genechten L, Langan-Evans C, Pedlar CR, Rodas G, et al. Does relative energy deficiency in sport (REDs) syndrome exist? *Sports Med*. (2024) 54:2793–816. doi: 10.1007/s40279-024-02108-y

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ORIGINAL RESEARCH
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Carbohydrate Mouth-Rinsing Improves Overtime Physical Performance in Male Ice Hockey Players During On-Ice Scrimmages

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Purpose: This randomized, double-blind, crossover study examined the effects of mouth-rinsing (MR) with a carbohydrate (CHO) vs. a placebo (PLA) solution on external and internal loads in hydrated ice hockey players during regulation and overtime (OT) periods of an on-ice scrimmage.

Methods: Twelve skilled male hockey players (22.6 [3.4] years, 178.9 [4.7] cm, 84.0 [6.5] kg) played three 20-min regulation periods and one 12-min OT period of small-sided 3-on-3 scrimmage. Skaters repeated 2 min shift and rest intervals. Participants mouth rinsed with 25 mL of CHO or PLA solution approximately every 10 min for a total of 7 rinses. A local positioning system (LPS) tracked external load variables including speed, distance, acceleration, and deceleration. Internal load was monitored with heart rate (HR) sensors and a rating of perceived exertion (RPE).

Results: During regulation play, both the conditions developed similar fatigue, with significantly decreased high-intensity distance, average speed and decelerations, and increased RPE, from period 1 to 2 and 3. In OT, CHO MR increased the distance skated at high-intensity (224 [77], 185 [66] m, $p = 0.042$), peak speed (24.6 [1.6], 23.7 [1.3] km·h^{-1}, $p = 0.016$), number of sprints (1.9 [1.2], 1.2 [0.9], $p = 0.011$), and decreased distance skated at slow speed (300 [33], 336 [47], $p = 0.034$) vs. PLA MR. OT RPE was similar between the two conditions in spite of more work done in CHO MR.

Conclusions: CHO MR may be a valuable practice to protect against decrements in external load with increased playing time in ice hockey, and possibly allows athletes to perform more work relative to perceived levels of exertion.

Keywords: team sport, local positioning system, heart rate monitors, external load, internal load, 3-on-3 scrimmage, hydration

INTRODUCTION

Ice hockey is an intermittent high-intensity team sport that requires explosive power, speed, muscular strength, and superior anaerobic and aerobic capacities (1–6). On-ice shifts are short (30–80 s), but players perform several bouts of near maximal-intensity exercise per minute, including repeated sprints, quick direction changes, body contact, grappling, and rapid accelerations and decelerations (1–5). These bouts are interspersed with periods of low-intensity skating and gliding, and rest.

Given the high physical intensity of training and competition, carbohydrate (CHO) from muscle glycogen is the primary fuel for ice hockey (3, 7, 8). Historical research has established that intermittent periods of high-intensity skating significantly exacerbated skeletal muscle glycogen depletion relative to continuous steady-state skating (9, 10), and subsequent work confirmed significant depletion of both type I and type II muscle fibers from pre- to postgame in male hockey players (7, 11, 12). Providing greater details to the limited pool of on-ice research, Vigh-Larsen et al. recently established time-point analysis of muscle glycogen depletion and the development of fatigue in elite men (U20 International) during a controlled hockey game (3). Despite playing only 8, 1-min shifts per period, significant glycogen depletion occurred within period 1, and ~65% of fast- and slow-twitch fibers were depleted by the end of the 3 periods. These findings were associated with marked decrements in physical performance across time, including reduced repeated sprint ability and fewer accelerations and decelerations, which were attributed to the development of fatigue. The glycogen depletion and fatigue exhibited by hockey players are similar to postgame values reported in field sport athletes, but notably, are achieved with dramatically less playing time (24 vs. ~90 min) (13). From the collective findings of ice hockey and other intermittent high-intensity exercise research, it has been established that low energetic states within skeletal muscle fibers produce intolerance to repeated high-intensity exercise bouts and exacerbate fatigue (3, 13, 14).

In previous research, it was established that the consumption of CHO electrolyte solutions (CES) improved physical performance and helped reduce fatigue across time in ice hockey (15–17). This was observed as increased voluntary work performed, skating speed and time at high effort, and reduced ratings of perceived exertion (RPE) with CES ingestion. However, these results were compared to no-fluid, mild dehydration trials, and so it remains unclear whether performance enhancements were the result of mitigation of dehydration, CHO ingestion, oral exposure to CHO, or a combination of these factors.

Indeed, isolated oral exposure to CHO through mouth-rinsing (MR) has demonstrated small to moderate positive effects on exercise performance in short-term (< 60 min), steady-state high-intensity (> 75% VO_2max) running and cycling. Relative to taste-matched placebo solutions (PLA), CHO MR was observed to improve time trial (TT) performance and power production, as well as increase distances and times to exhaustion (18–20). In seminal work, direct infusion of glucose did not enhance exercise performance, despite elevated blood glucose levels and increased muscle glucose uptake (21). Thus, it was speculated that the mechanism responsible for performance improvement with CHO MR may be non-metabolic and related to central control. To test this hypothesis, Chambers et al. conducted a multipart study that examined human brain activity and exercise performance in response to isolated oral exposure to isocaloric sweet (glucose) and tasteless CHO (maltodextrin), as well as a non-caloric sweetener (saccharin) (22). MR with either CHO solution improved performance relative to the non-caloric sweet placebo. Notably, both CHO solutions, but not the PLA solution, increased brain activity in the reward, motivation, and motor control regions, independent of taste. Similar findings have been repeated with other non-caloric sweeteners (ex. sucralose), which also demonstrated a minimal effect on brain activity in these regions (23). Building on the seminal findings, later works theorized that oral sensing of CHO induces subconscious perceptions of energy availability that alter central control, and demonstrated the facilitation of increased corticomotor and sensorimotor activity with CHO MR, which are proposed to mitigate fatigue and attenuate performance decrements (24–26).

Though CHO MR has been thoroughly examined in steady-state exercise, there is mixed reporting of potential benefits in intermittent high-intensity exercise (27), and few studies have examined this practice in team sport settings (28–30). In female soccer players, CHO MR improved speed in short shuttle sprint performance (29). CHO MR did not affect repeated sprint performance in male soccer and rugby players (28), but it did prolong the onset of fatigue and reduce perceptions of effort in increasing-speed shuttle running to exhaustion in male lacrosse players (30). However, existing team sport research protocols are limited by the minimal inclusion of sport-specific exercises or scrimmages, which serve to replicate the rapid and sporadic changes in direction typical of training and competition (8). To date, there has been no CHO MR research in ice hockey.

Historically, measurement of ice hockey performance has been impaired by the difficulty of comparing off-ice to on-ice activity due to the prevalence of different surfaces (i.e., floor vs. ice), as well as the unique biomechanics facilitated by skating (31–33). The development of valid and reliable wearable technologies for indoor sports, such as local positioning systems (LPS) with Bluetooth heart rate (HR) monitors (34, 35), has enabled the collection of external and internal load data during on-ice training and competition (2, 3, 36).

External load is defined as the physical work performed during exercise and pertains to the organization, quality, and quantity of exercise (37). Examples of external load measures in team sports such as ice hockey include speed, total distance, and relative distance by speed zones, and explosive movements such as accelerations, decelerations, and changes in direction (2, 3, 5, 37, 38). Internal load represents the psychophysiological response to external load (36, 37). Existing measures of internal load in ice hockey include RPE, HR, and HR-derived training impulse (TRIMP), which combines exercise time, intensity and relative weighting of intensity (36, 39, 40).

Therefore, the present study used wearable LPS and HR microtechnology to examine the effects of MR with CHO vs. PLA solutions on external and internal loads in hydrated male ice hockey players during on-ice scrimmages with three regulation periods and one overtime (OT) period. It was hypothesized that relative to PLA, CHO MR would attenuate decreasing external load and increasing internal load: (1) as the 3 periods of regulation progressed, and (2) within the OT period.

MATERIALS AND METHODS

Subjects

Twelve skilled male ice hockey players volunteered for this study (22.6 ± 3.4 years, 178.9 ± 4.7 cm, 84.0 ± 6.5 kg, skill range: AA U18–collegiate/major junior). Participants were active (∼1.5 h/day, 5 days/week) and regularly played organized hockey. Participants were informed verbally and in writing about the study risks, before obtaining written consent. Ethical approval was obtained from the University of Guelph Research Ethics Board.

Study Design

This study had a double-blind, randomized, crossover design which included 1 familiarization trial and 4 experimental trials. Trials occurred at the same time (1:00–3:30 pm) and were a minimum of 48 h apart. Participants refrained from alcohol and strenuous exercise in the 24 h preceding each trial and were instructed to maintain the same dietary habits each trial day as they would in preparation for a game. Neither prescrimmage caffeine nor CHO intake was restricted. Pre- and post-skate measures were performed off-ice and included urine-specific gravity (USG) and body mass (BM) (**Figure 1**). USG was measured with a hand-held "pen" refractometer (ATAGO USA Inc., Bellevue, WA, USA), and values <1.020 were accepted as hydrated (17). BM was measured with participants in minimal clothing. Participants were on-ice for ∼90 min, which included 10 min of warm-up, 3 20-min regulation periods, and one 12-min overtime period of small-sided 3-on-3 scrimmage with goaltenders, and 3 2-min intermissions. During the scrimmage, skaters hydrated with water and performed a 10-s MR approximately every 10 min with 25 mL of CES (Gatorade: 6% CHO, 19 mM sodium, 11 mM chloride, 3 mM potassium), or a noncaloric, taste- and electrolyte-matched placebo solution (PLA; Gatorade Zero). Presently, there is great inconsistency across the literature regarding solution concentration, length, frequency, and number of MRs (26, 27). The procedures used herein align with the most prevalent protocols to have displayed positive effects on high-intensity exercise performance.

Familiarization and experimental trials had the same structure, except familiarization included the additional protocol of measuring participant sweat loss. Skaters voided their bladder for the pre-skate urine sample. On-ice, skaters drank water *ad-libitum* from assigned bottles that were weighed pre- and post-skate. Sweat amount was equal to [(pre-BM – post-BM) + (fluid intake – urine output)]. This information was used to establish individual fluid intake parameters necessary to prevent mild dehydration in experimental trials. Within the latter, players were provided with two bottles with a volume of fluid equivalent to their expected sweat amount and were given frequent encouragement to drink, with the goal of finishing one bottle by the end of the second period, and the other at the end of OT.

Methodology

On-Ice Scrimmages

Scrimmages were played across the width of an Olympic-sized rink (1.5 ± 0.9°C, 56.4 ± 4.2% humidity) between the blue line and end boards (**Figure 2**). Nets were set up ∼2 m from the boards on the midline of the area. The space between the two blue lines (neutral zone) was used as a rest area for players. Research occurred during the COVID-19 pandemic, so a 3-on-3 scrimmage structure was adopted over 5-on-5 to abide by public health gathering guidelines. Each team had 6 players to permit rotation of 2 lines. A similar "small-sided game" set-up had previously been observed to elicit high-intensity game play which mimicked conventional 5-on-5 competition (41). The entire scrimmage included three 20-min regulation periods and one 12-min OT period, separated by 2-min intermissions. Regulation and OT were treated as separate events, as the score was always reset in the latter to imitate the tied score that would necessitate the additional "winner-take-all" OT period in a professional hockey competition (42). Within periods, skaters repeated 2-min shift and rest intervals, with changes made on a whistle. During the rest phases skaters remained seated. Lines were matched on the basis of skill to ensure fair gameplay (assessed during familiarization scrimmage and initial screening questionnaire). Six players with the highest skill level were put into group A and were randomly divided into two lines of 3 ("A Lines"). The remaining 6 players were put into group B and randomly divided into two groups of 3 ("B Lines"). The two lines in group A competed only against each other throughout the study as did the two lines in B. To encourage a high-level of competition, monetary awards were granted to players of the winning team for regulation and for OT.

MR Protocol

Mouth-rinse was performed at rest, preceding the first shift of every period (regulation and OT), and at roughly the mid-point of each regulation period, for a total of 7 rinses (**Figure 1**). Rinse aliquots were premeasured (25 mL) and administered orally *via* a plastic syringe. MR involved vigorous swishing of solution around the mouth for 10 s before expectorating the entire volume into a bucket. Participants were instructed to refrain from drinking any water in the remaining rest time after each rinse to prevent accidental ingestion of the solution. Individuals were assigned one MR condition per trial, and CHO and PLA conditions were randomized within teams and lines by a blinded researcher as to not confound scrimmage outcome. Duplicate experimental trials were completed for each condition. Blinding effectiveness was assessed *via* a postskate questionnaire that asked participants whether they felt they knew which rinse condition they had received. If the participants answered "yes" to this question, they were asked to identify the condition and explain their reasoning.

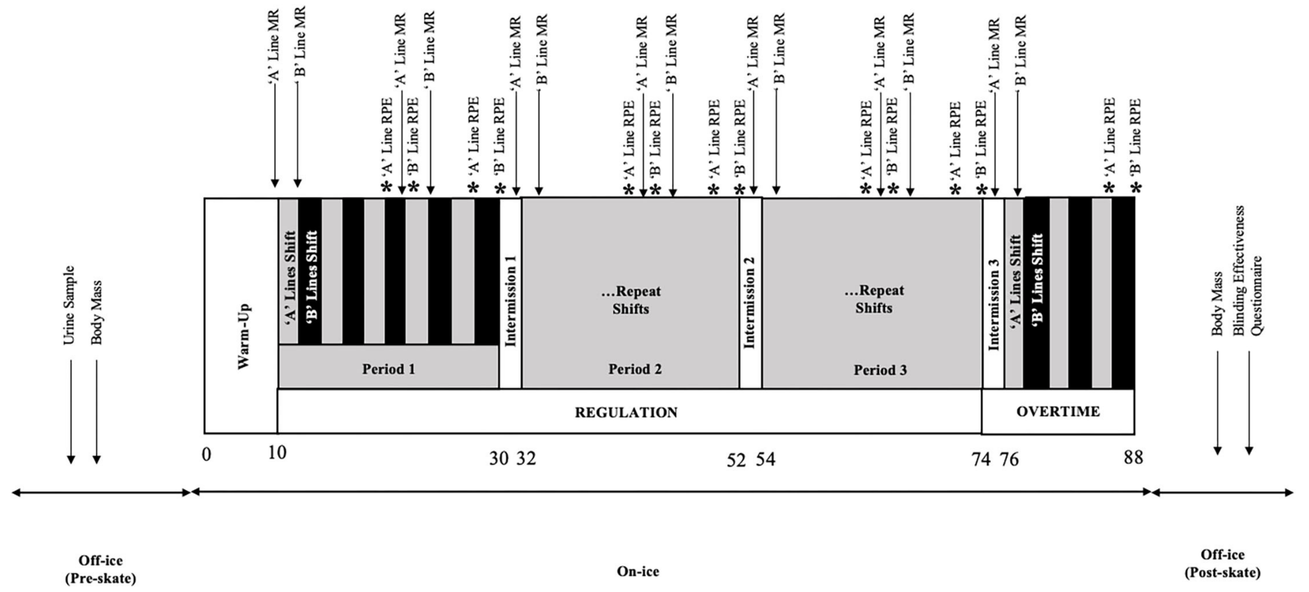

FIGURE 1 | Experimental trial timeline. Numbers are in minutes. MR, mouth-rinsing; RPE, rating of perceived exertion.

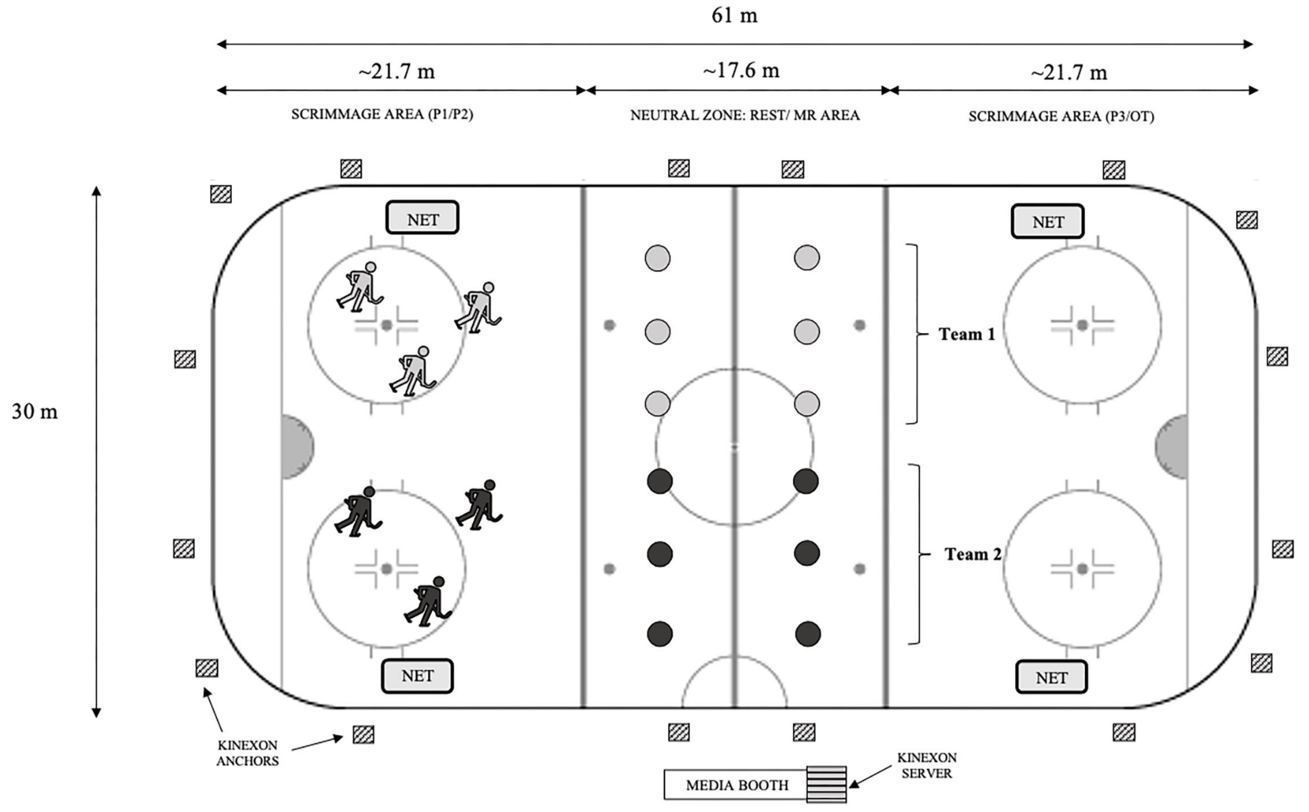

FIGURE 2 | Schematic diagram outlining the ice-rink set up for small-sided 3-on-3 scrimmage and the location of one server and 16 anchors of an ultrawideband local positioning system (LPS; Kinexon, Munich, Germany) used to measure on-ice movements of skaters during scrimmage. Circles represent player rest/MR stations. MR, mouth-rinsing; P1, period one; P2, period two; P3, period three; OT, overtime.

Athlete Monitoring

On-ice external loads were tracked using a tri-axial LPS (Kinexon Sports & Media, New York, NY, USA) which operated through specific local network access, one Power over Ethernet switch, one server, 16 anchors (secured to the arena rafters), and individual player sensors (**Figure 2**). Unpublished observations from our group have confirmed the validity and reliability of this system for use in ice hockey. Briefly, LPS variables including time on ice, skating distance, peak and average speed, and peak acceleration and deceleration demonstrated intersensor reliability (coefficient of variation (CV) <10%) when comparing two sensors worn by the same player. Also, when compared against a previously validated robotic sprint device (1080 Sprint, 1080 Motion, Lidingo, Sweden), near perfect correlations were observed between systems for instantaneous speed ($r = 0.892$, $p < 0.001$), peak speed ($r = 0.989$, $p < 0.001$), and acceleration ($r = 0.968$, $p < 0.001$), with very low CVs for all (<3%).

In the present study, sensors were secured to the exterior shoulder pads, positioned between the scapulae. LPS sensors included accelerometers to quantify linear motion in all directions, gyroscopes to measure angular motion and rotation, and magnetometers to measure direction and orientation of body position (44). To measure on-ice internal loads, players wore HR sensors (Polar OH1, Polar Electro OY, Kempele, Finland) secured in a band around the mid-bicep to avoid interference with protective equipment. These data were transmitted *via* Bluetooth to LPS sensors. Ultrawideband (UWB) channels (3244.88–4742.40 MHz) allowed for communication between LPS sensors and anchors, enabling real-time collection of data (20 Hz), transmitted to the server *via* hardwired connection. Data were stored on the LPS platform and could be retrieved on a secure computer for live or retrospective analysis. Commencement and cessation of data collection occurred automatically as players entered and exited the ice surface.

External load variables were derived from spatial and temporal LPS data and included distances, speeds, and explosive movements (accelerations, decelerations, sprints). Definitions of these metrics were adapted from One & Media—KNX ONE Hockey Metrics (Kinexon, Munich, Germany). Distance skated was categorized according to 6 speed zones: zone 1–very slow = 1.0–10.9 $km{\cdot}h^{-1}$, zone 2–slow = 11.0–13.9 $km{\cdot}h^{-1}$, zone 3–moderate = 14.0–16.9 $km{\cdot}h^{-1}$, zone 4–fast = 17.0–20.9 $km{\cdot}h^{-1}$, zone 5–very fast = 21.0–24.0 $km{\cdot}h^{-1}$, and zone 6–sprint = >24.0 $km{\cdot}h^{-1}$ (5). Distances traveled at low-intensity (<17 $km{\cdot}h^{-1}$) and high-intensity (≥17 $km{\cdot}h^{-1}$) speeds were also reported (3). Average and peak speeds ($km{\cdot}h^{-1}$) were determined from instantaneous changes in position and time. Accelerations were defined by positive rates of change of velocity (>2.0 $m{\cdot}s^{-2}$), while decelerations were defined by negative rates of change of velocity (<-2.0 $m{\cdot}s^{-2}$). A sprint was an action ≥22 $km{\cdot}h^{-1}$, maintained for at least 1 s.

Physiological indicators of internal load were measured across each period and included peak, average HR, and TRIMP (27, 45). RPE (Borg-10 scale) provided a psychophysiological measure of internal load and was recorded immediately following players' third and last shifts in periods 1–3, and the last shift in OT (**Figure 1**).

Statistical Analysis

All participant data were from duplicate trials and were reported as mean (SD). Data from regulation and OT were treated as separate events. Data were confirmed to present a normal Gaussian distribution with a Shapiro–Wilk test. Data from regulation periods 1–3 were analyzed with two-way repeated measures ANOVAs (condition × period). When a significant F ratio was found, a *post-hoc* analysis was performed using Tukey's multiple comparisons test with adjusted p-values. Data collected in OT were analyzed using paired t-tests. Effect sizes (ES) were reported as partial η^2 ($\eta_p{}^2$) for ANOVA (small = 0.01– 0.059, moderate = 0.06–0.139, large = ≥ 0.14) and Cohen's d for pairwise comparisons (small = 0.2–0.59, moderate = 0.6–1.19, large = 1.2–1.99, very large = ≥ 2.0) (45). All statistical analyses were performed with GraphPad Prism 9.1.0 for Mac (Graphpad Software, LLC, San Diego, CA). Significance was accepted at $p \leq 0.05$, with confidence intervals (CI) of 95%.

RESULTS

Blinding Effectiveness

There were 10/48 trials where players ($n = 5$) were confident they knew which MR solution they received. However, they were only correct 50% of the time, such that the correct MR solution was identified 5/48 times.

Hydration Status

The average pre-scrimmage USG for all trials was 1.007 ± 0.006. Average player sweat loss was 1.38 ± 0.31 L and sweat rate was 0.97 ± 0.22 $L{\cdot}h^{-1}$. Participants maintained hydration during the CHO and PLA trials, losing only 0.32 ± 0.01 and 0.33 ± 0.01% BM, respectively.

Performance in Regulation Play

External Load

There was no difference in total distance skated between MR conditions (CHO: 6,176 ± 287 m, PLA: 6,292 ± 402 m). For both conditions, there was a main effect of period [$F_{(2, 22)} = 6.45$, $p = 0.006$, $\eta_p{}^2 = 0.71$], and distance in period 1 (2,130 ± 19 m) was greater than periods 2 (2,062 ± 11 m, $p = 0.008$) and 3 (2,072 ± 29 m, $p = 0.024$).

In zone 1 there was a significant two-way interaction between period and condition [$F_{(2, 22)} = 5.07$, $p = 0.015$, $\eta_p{}^2 = 0.46$; **Figure 3A**]. Within period 2, significantly less distance was skated with CHO (695 ± 108 m) compared to PLA MR (737 ± 92 m, $p = 0.005$). For PLA MR only, significantly greater distance was skated in period 2 (737 ± 92 m) compared to period 1 (677 ± 108 m, $p < 0.001$) and period 3 (701 ± 116 m, $p = 0.21$). There was a main effect of period on distance in zones 2 [$F_{(2, 22)} = 4.58$, $p = 0.022$, $\eta_p{}^2 = 0.93$], 3 [$F_{(2, 22)} = 3.58$, $p = 0.045$, $\eta_p{}^2 = 0.53$], and 4 [$F_{(2, 22)} = 9.15$, $p = 0.001$, $\eta_p{}^2 = 0.79$]. Across both conditions, greater distance was skated in period 1 compared to period 2 in zones 2 ($p = 0.018$) and 3 ($p = 0.037$), and period 1 compared to period 3 in zone 4 ($p < 0.001$) (**Figures 3B–D**).

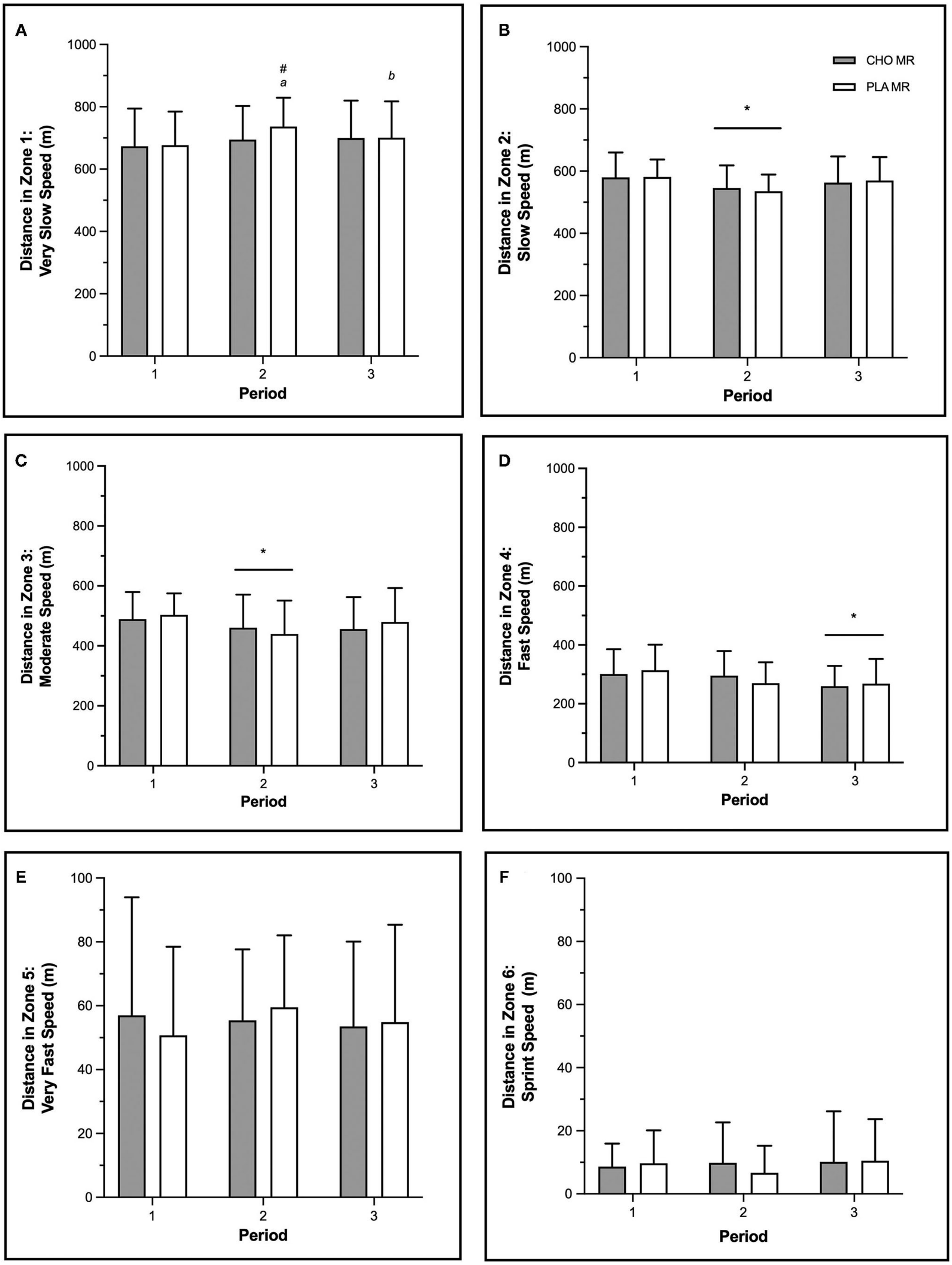

FIGURE 3 | Distance traveled by male high-level hockey players ($n = 12$) in **(A)** zone 1–very slow (1.0–10.9 km·h^{-1}), **(B)** zone 2–slow (11.0–13.9 km·h^{-1}), **(C)** zone 3–moderate (14.0–16.9 km·h^{-1}), **(D)** zone 4–fast (17.0–20.9 km·h^{-1}), **(E)** zone 5–very fast (21.0–24.0 km·h^{-1}), and **(F)** zone 6–sprint (>24.0 km·h^{-1}) speed zones when mouth-rinsing with carbohydrate (CHO) or placebo (PLA) solution during three 20-min regulation periods of small-sided 3-on-3 ice hockey scrimmage. Data are mean (SD). *Main effect of period, significantly lower than period 1. [a]Significantly greater than period 1, same condition. [b]Significantly lower than period 2, same condition. [#]Signifcantly greater than other MR condition, same period. Significance accepted at $p \leq 0.05$.

There were no differences in the distances of zones 5 and 6 (**Figures 3E,F**).

There was a main effect of period for distance at high-intensity speed [$F_{(2, 22)} = 6.04$, $p = 0.008$, $\eta_p^2 = 0.40$], and the distance for both conditions in period 3 (328 ± 7 m) was less than period 1 (370 ± 5 m, $p = 0.006$). There was a main effect of period on average speed [$F_{(2, 22)} = 5.73$, $p = 0.010$, $\eta_p^2 = 0.41$], which decreased in both conditions from period 1 to periods 2 ($p = 0.012$) and 3 ($p = 0.041$) (**Table 1**).

There was a main effect of period on number of decelerations [$F_{(2, 22)} = 17.39$, $p < 0.001$, $\eta_p^2 = 1.93$], which decreased in both conditions from period 1 to periods 2 and 3 ($p < 0.001$ for both) (**Table 1**).

Internal Load

Peak HR displayed a main effect of time [$F_{(2, 22)} = 11.94$, $p < 0.001$, $\eta_p^2 = 0.79$], and was lower in both conditions in period 1 (183.6 ± 1.2 bpm) compared to period 2 (187.5 ± 0.4 bpm, $p < 0.001$) and 3 (186.9 ± 1.7 bpm, $p = 0.002$). Average HR exhibited a significant two-way interaction between period and MR condition [$F_{(2, 22)} = 5.09$, $p = 0.015$, $\eta_p^2 = 0.46$], and within both conditions, period 1 (CHO: 147.8 ± 11.1 bpm, PLA: 145.6 ± 11.1 bpm) was lower than periods 2 (CHO: 155.3 ± 10.3 bpm, PLA: 156.9 ± 10.4 bpm, $p < 0.001$ for both) and 3 (CHO: 155.0 ± 10.9 bpm, PLA: 157.4 ± 11.3 bpm, $p < 0.001$ for both). Across regulation for CHO and PLA MR, peak HR values were 94 ± 4% and 94 ± 4% of age-predicted maximum and average HR vales were 77 ± 5% and 78 ± 6% of age-predicted maximum, respectively.

There was a significant two-way interaction between period and condition for TRIMP [$F_{(2, 22)} = 4.11$, $p = 0.030$, $\eta_p^2 = 0.37$]. Within CHO and PLA conditions, period 1 (CHO: 37 ± 11 AU; PLA: 35 ± 12 AU) was lower than periods 2 (CHO: 43 ± 13 AU, $p = 0.002$; PLA: 45 ± 12 AU, $p < 0.001$) and 3 (CHO: 42 ± 15 AU, $p = 0.016$, PLA: 46 ± 15 AU $p < 0.001$). TRIMP values across regulation were not different between conditions (CHO: 122 ± 37 AU, PLA: 126 ± 38 AU).

Ratings of perceived exertion displayed a main effect of period [$F_{(5, 55)} = 8.28$, $p < 0.001$, $\eta_p^2 = 1.01$], and in both conditions average values were lower in period 1 vs. 2 ($p = 0.001$) and 3 ($p = 0.007$). In periods 2 and 3 there was a non-significant trend for RPE to be lower with CHO (RPE values: period 1 = 6.7, period 2 = 7.2, and period 3 = 6.9) compared with PLA (RPE values: period 1 = 6.7, period 2 = 7.4, and period 3 = 7.4).

Performance in Overtime Play

External Load

There was no difference in total distance skated between CHO and PLA MR in OT (1,229 ± 78, 1,225 ± 99 m). In zone 2, greater distance was skated with PLA compared to CHO MR ($p = 0.034$, ES = 0.89; **Figure 4**). Distance skated was comparable between MR conditions in the other 5 speed zones. Distance at high-intensity speed was significantly greater with CHO relative to PLA MR ($p = 0.042$, ES = 0.54; **Figure 5**).

Peak speed was higher with CHO over PLA MR ($p = 0.016$, ES = 0.62; **Figure 6**), but there was no difference in average speed between conditions. Average number of sprints performed was higher with CHO [1.9 ± 1.2] opposed to PLA [1.2 ± 0.9, $p = 0.011$, ES = 0.66]. There were no differences between CHO and PLA MR for number of accelerations (8.5 ± 3.5, 7.7 ± 2.1) and decelerations (9.7 ± 3.5, 10.3 ± 2.8), or peak acceleration (3.4 ± 0.4, 3.5 ± 0.4 m·s^{-2}) and peak deceleration (−4.4 ± 0.5, −4.0 ± 0.5 m·s^{-2}).

TABLE 1 | Speed and explosive movement external load variables of hydrated male high-level hockey players (n = 12) when mouth-rinsing with carbohydrate (CHO) or placebo (PLA) solution during three 20-min regulation periods of small-sided 3-on-3 ice hockey scrimmage.

Variable	Condition	Period 1	Period 2	Period 3
Peak speed (km·h^{-1})	*CHO MR*	24.3 (1.3)	24.2 (1.3)	24.4 (1.6)
	PLA MR	24.5 (1.2)	24.4 (1.4)	24.6 (1.7)
Average speed (km·h^{-1})	*CHO MR*	12.6 (0.9)	12.4 (0.9)*	12.3 (0.7)*
	PLA MR	12.8 (0.7)	12.3 (0.9)*	12.6 (1.0)*
Sprints (number)	*CHO MR*	2.7 (1.9)	2.5 (1.5)	2.4 (1.6)
	PLA MR	1.9 (1.1)	2.4 (1.1)	2.3 (1.2)
Peak acceleration (m·s^{-2})	*CHO MR*	3.5 (0.3)	3.4 (0.3)	3.3 (0.3)
	PLA MR	3.5 (0.2)	3.6 (0.2)	3.6 (0.2)
Accelerations (number)	*CHO MR*	12.8 (4.8)	12.0 (3.9)	11.5 (3.9)
	PLA MR	13.3 (4.6)	12.4 (3.7)	12.5 (4.4)
Peak deceleration (m·s^{-2})	*CHO MR*	4.7 (0.5)	4.6 (0.5)	4.4 (0.4)
	PLA MR	4.6 (0.4)	4.7 (0.3)	4.4 (0.5)
Decelerations (number)	*CHO MR*	19.3 (4.2)	15.6 (4.1)*	15.7 (3.8)*
	PLA MR	19.9 (5.9)	16.0 (3.5)*	15.8 (5.2)*

*A sprint is a movement at any speed ≥ 22 km·h^{-1}, maintained for at least 1 s. Accelerations (> 2.0 m·s^{-2}). Decelerations (< −2.0 m·s^{-2}). Values are presented as mean (SD). *Main effect of period, significantly lower than period 1 (p ≤ 0.05).*

Internal Load

There were no differences between CHO or PLA MR for peak HR (183.3 ± 9.1, 185.7 ± 9.0 bpm), average HR (153.7 ± 11.8, 155.2 ± 11.3 bpm), TRIMP (24.9 ± 8.5, 26.4 ± 8.2 AU), or RPE (7.7 ± 0.9, 7.5 ± 0.9). For CHO and PLA MR, peak HR values were 93 ± 4% and 94 ± 4% for age-predicted maximum, and average HR values were 78 ± 6% and 79 ± 5% of age-predicted maximum, respectively.

DISCUSSION

This research examined the effects of CHO MR on external and internal loads in hydrated male ice hockey players during three regulation periods and one OT period of a small-sided 3-on-3 on-ice scrimmage. This is the first study to examine CHO MR in ice hockey, and the first MR research to use an entirely team sport-specific exercise protocol. The principle findings of this study were: (1) in regulation, there was a similar development of fatigue across both MR conditions from period 1 to 2 and 3, observed as decreases in external load metrics of total distance, high-intensity distance, number of decelerations, and average speed, and increases in internal load metrics of peak HR, average HR, TRIMP, and RPE, (2) CHO MR did not attenuate the decreases in external load and increases in internal load during regulation play, (3) in OT, peak speed, high-intensity distance, and number

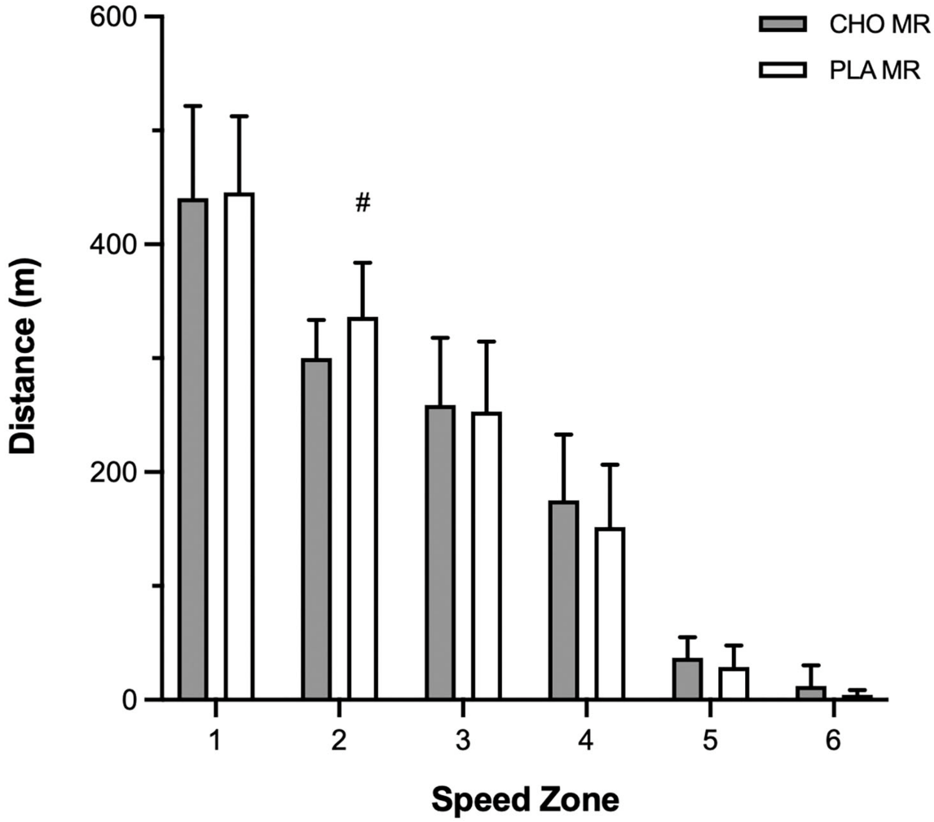

FIGURE 4 | Distance traveled by male high-level hockey players ($n = 12$) in zone 1–very slow (1.0–10.9 km·h^{-1}), zone 2–slow (11.0–13.9 km·h^{-1}), zone 3–moderate (14.0–16.9 km·h^{-1}), zone 4–fast (17.0–20.9 km·h^{-1}), zone 5–very fast (21.0–24.0 km·h^{-1}), and zone 6–sprint (>24.0 km·h^{-1}) speed zones when mouth-rinsing (MR) with carbohydrate (CHO) or placebo (PLA) solution during one 12-min overtime period of small-sided 3-on-3 ice hockey scrimmage. Data are mean (SD). #Signifcantly greater than other MR condition, same zone ($p \leq 0.05$).

of sprints were greater with CHO over PLA MR, but (4) there were no differences in HR, TRIMP, or RPE between conditions in OT, despite elevated external loads with CHO MR. These results demonstrate that CHO MR improved physical performance in OT of an ice hockey scrimmage, and allowed athletes to perform more work relative to perceived levels of exertion.

Regulation

There are limited recent descriptions of physical performance in ice hockey, and only two studies have included analysis of 3-on-3 play, despite its frequent occurrence in high-level competition (42). Lachaume and colleagues used small-sided 3-on-3 game, however the playing field was oriented differently, playing duration was shorter, the population was younger, and external loads were not monitored, severely limiting comparability to the present work (41). Alternatively, Lignell's group examined 3-on-3 full-ice competition, but only during an OT period (5). The present protocol has previously been used in other hockey research, but for analyzing goaltender performance (17). Therefore, the current analysis of player external and internal loads across time in small-sided 3-on-3 hockey scrimmage is novel.

The external loads observed herein were comparable to elite men's 5-on-5 full-ice hockey, where no change in distance was reported in the top speed zones between periods, but skaters displayed difficulty maintaining high-speed and performing explosive efforts across time (3, 5). Within the latter, marked skating fatigue was associated with the significant depletion of muscle glycogen, which occurred at the greatest rate in period 1 where players traveled ∼900 m of high-intensity distance (3). During regulation in the present study, skaters traveled ∼1,000 m of high-intensity distance, and accumulated similar total distance (∼6,200 m) to 5-on-5 players (∼6,000 m). Since anaerobic metabolism is the primary source of energy for high-intensity skating, transitions to higher speeds, and explosive efforts, it is proposed that late-scrimmage fatigue in small-sided 3-on-3 scrimmage was at least partly due to significant depletion of glycogen within skeletal muscle fibers (3, 14).

Average and peak HR values recorded in small-sided 3-on-3 scrimmage were similar to values reported in elite adolescent male small-sided 3-on-3 (41), but were slightly lower than university and elite men's 5-on-5 competition (3, 46). The lack of disparity between MR conditions is not surprising, as consumption of CES did not attenuate increases in HR

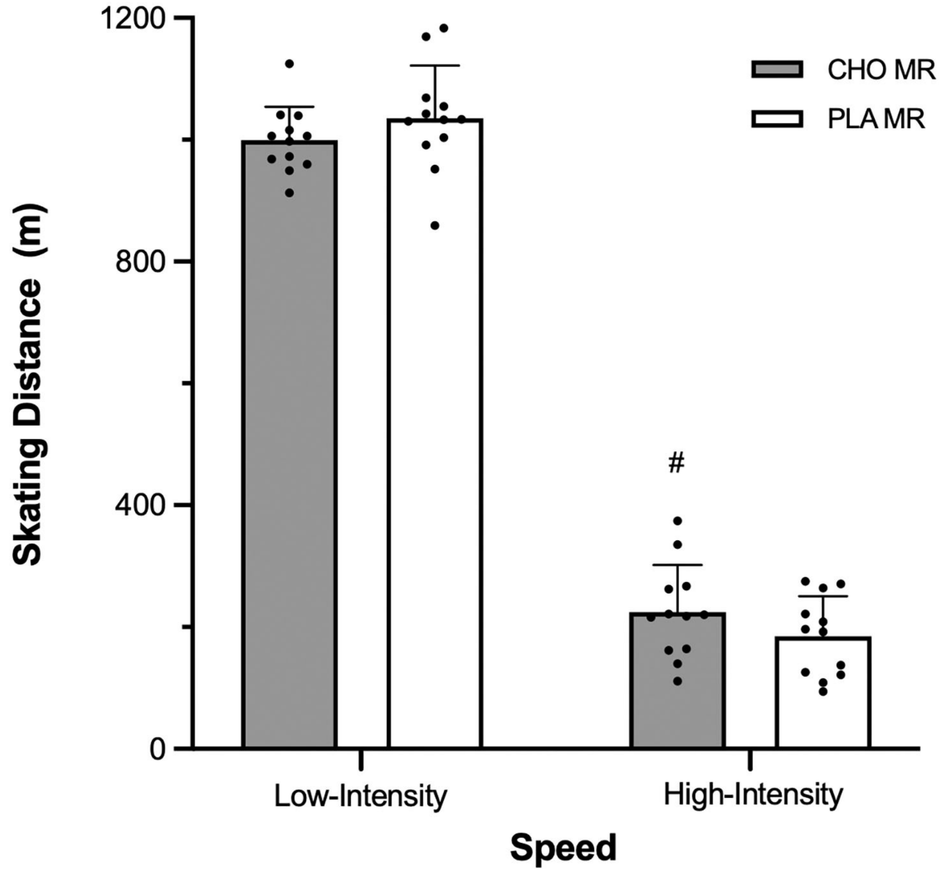

FIGURE 5 | Distance traveled by male high-level hockey players ($n = 12$) at low-intensity (<17 km·h^{-1}) and high-intensity (≥17 km·h^{-1}) speeds when mouth-rinsing with carbohydrate (CHO) or placebo (PLA) solution during one 12-min overtime period of small-sided 3-on-3 ice hockey scrimmage. Data are mean (SD). #Signifcantly greater than other MR condition, same speed ($p \leq 0.05$).

in simulated or on-ice 5-on-5 scrimmage in recreational men (15, 16). Previous studies demonstrated reduced perceptions of fatigue with CES consumption in hockey, but no effects were observed presently. However, the former were compared to no-fluid trials, and therefore it is proposed that the maintenance of euhydration in the present protocol may have overpowered any additional benefit of oral CHO sensing on RPE in regulation. TRIMP values attained herein were greater than the values of university males in both regular season (95 ± 54 AU) and postseason (104 ± 68 AU) competitions (36). This is likely the result of far fewer players per team and increased active playing time in small-sided 3-on-3.

Overtime

Despite similar total distance covered, players traveled lesser distance at slow speed, and more distance at high-intensity speed when MR with CHO was compared to PLA. Enhanced physical performance with CHO MR in OT is further highlighted by faster peak speeds and a greater number of sprints. Nonetheless, the external loads depicted within CHO and PLA MR conditions were both comparable to those reported in full-ice 3-on-3 OT by National Hockey League (NHL) players (5). The current players reached peak speeds (CHO MR: 24.6 ± 1.7 km·h^{-1}, PLA MR: 23.7 ± 1.3 km·h^{-1}) similar to NHL sprint speeds (24.5 ± 0.1 km·h^{-1}), and actually traveled greater high-intensity distances [CHO MR: 224 ± 77 m, PLA MR: 185 ± 66 m] than NHL skaters [118 ± 17 m].

This is the first study to examine CHO MR in ice hockey, which adds to the paucity of information regarding MR and team sport performance. There are mixed findings within the existing literature, which provide limited evidence that CHO MR has small positive effects on speed and perceptions of fatigue in field sport athletes (28–30). The present findings exhibit the most favorable performance responses to CHO MR in intermittent high-intensity sport to date. Moreover, these results demonstrate that performance improvements observed in earlier hockey research with ingestion of exogenous CHO, as compared to no-fluid ingestion, may have been partially related to oral exposure to CHO (15–17).

For over a decade, the existence of oral receptors that respond to the energy content of CHO and increase the excitability of reward, motivation, and motor control brain regions has been known (21). Subsequent research demonstrated that oral sensing of CHO manipulated efferent outputs during voluntary

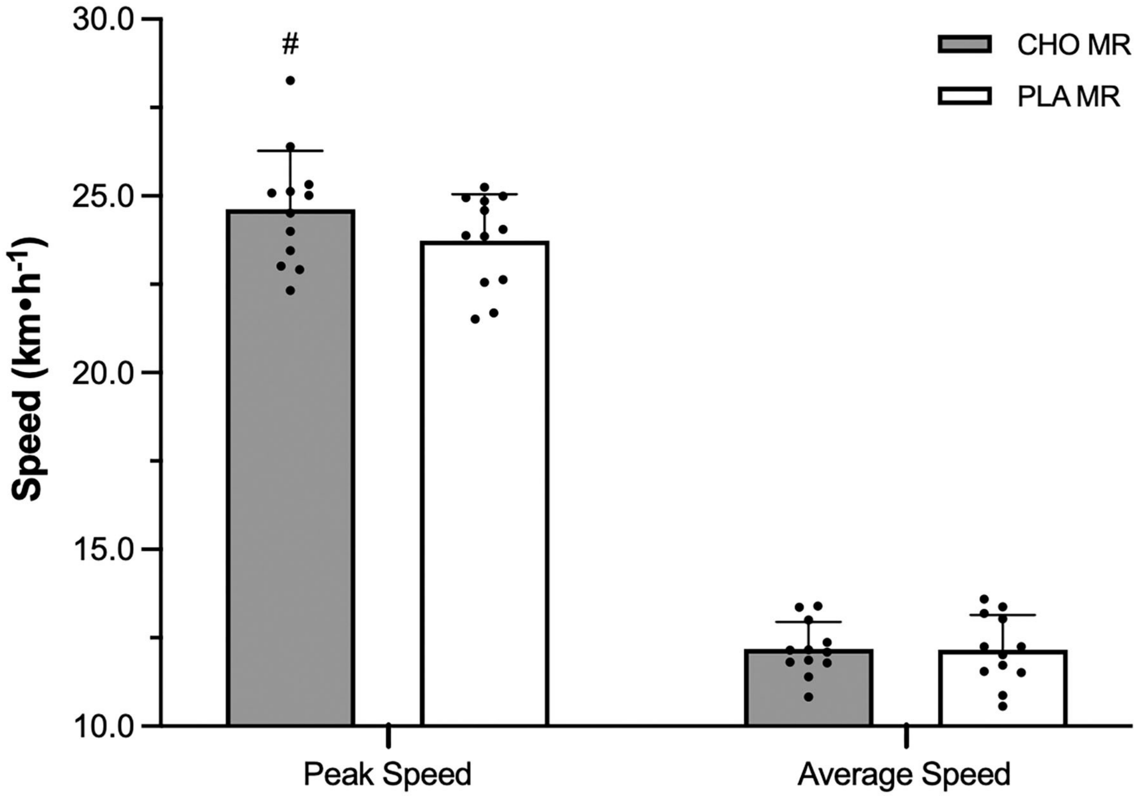

FIGURE 6 | Peak and average speeds of male high-level hockey players when mouth-rinsing with carbohydrate (CHO) or placebo (PLA) solution during one 12-min overtime period of small-sided 3-on-3 ice hockey scrimmage. Data are mean (SD). #Signifcantly greater than other MR condition, same variable ($p \leq 0.05$).

contraction and task-specific actions, revealing positive physical responses to the perception of forthcoming energy (24, 25). Thus, it is proposed that during ice hockey, CHO MR might increase sensorimotor cortex activity and attenuate declines in motor function and neural drive to contracting muscles that are associated with fatigue, prolonging the quality of physical performance relative to the PLA condition. Notably, brain and sensorimotor responses to oral CHO exposure appeared to be greatest when energy status is low, which might explain why significant performance improvements in the present study were not observed until OT (24, 25).

Despite greater external loads with CHO MR, internal loads were not different between conditions. The absence of diverging RPE values between CHO and PLA MR with the development of physical fatigue opposes previous CHO-ingestion ice-hockey research (15–17). However, this observation has been frequently repeated in existing CHO MR exercise research (18–20). The present findings along with others demonstrate that instead of reducing perceptions of effort and fatigue, oral sensing of CHO permits maintenance of these perceptions despite greater work performed.

Practical Applications

Carbohydrate MR is a simple, minimally disruptive practice that can be added to player nutrition and hydration regimes to facilitate heightened physical performance in situations where skaters are fatigued, such as OT. OT is a frequent occurrence in high-level hockey and is used to decide a winner when there is a tied score at the end of the three 20-min regulation periods (42). Within OT, the game is ended and won by the team that scores first. Most leagues have adopted a full-ice 3-on-3 structure, which facilitates increased space per skater and higher scoring chances. Therefore, the capacity to maintain speed and high-intensity actions in OT would be particularly advantageous.

This study demonstrated that CHO MR alone, without CHO ingestion, can produce beneficial effects on physical performance in hydrated male ice hockey players. Expectoration of MR solutions may be a favorable practice to attain the benefits of oral CHO exposure in athletes who prefer to consume minimal to no CHO during training and competition due to fear of experiencing gastrointestinal discomfort (47). However, swallowing after MR may be a more realistic practice for athletes in real-world competition. Beyond oral CHO exposure, drinking a CES throughout high-intensity sport can help prevent or alleviate the negative effects of dehydration by replenishing fluids and electrolytes lost through sweat (8). Ideally, carbohydrate supplementation practices should be customized based on individual preferences and designed to meet the needs of each athlete.

Limitations and Future Directions

Strategic game-play in small-sided 3-on-3 scrimmage may have varied from 5-on-5 competition, which possibly altered external load variables (3, 5, 41). The smaller playing field may have forced players to rely more on technical skills vs. skating to generate offensive chances, and could have also prevented athletes from achieving true maximum speed. Together these factors may have reduced the distance and duration of high-intensity actions. Future research should replicate standard competition by including full-ice 5-on-5 play, followed by 3-on-3 OT, which could be shortened to 5 min (5). Adjustments to player work-to-rest ratios could further improve this protocol, as the current shift length (2 min) was longer than shifts in elite male 5-on-5 competition (30–80 s) (1–3, 5).

Though the present MR design was novel to ice hockey, future trial parameters should expand to include combinations of MR and ingestion. This would determine if previously observed performance benefits with CHO ingestion were due to oral exposure to CHO alone, or if there might be an additive effect with MR and ingestion (15–17). Additionally, special attention should be paid to nutritional control in forthcoming research, as it is possible that the absence of pre-trial nutritional restrictions in the present work may have impacted the findings herein.

Future research on ice hockey and MR would also benefit from the inclusion of female participants. Currently, there is one soccer MR study that included female participants (29), and a small number of general hockey studies that characterized female performance (38, 43). Therefore, a gap in the literature exists pertaining to the use of performance-enhancing strategies in female ice hockey players.

CONCLUSIONS

This is the first study to examine CHO MR in ice hockey, and the only study to demonstrate multiple significant physical performance enhancements with CHO MR in a team sport-specific protocol. The positive effects of CHO MR on external loads were most prevalent with considerable development of fatigue. There were no effects in regulation, but in OT CHO MR improved physical performance, with less distance traveled at slow speed and increased distance traveled at high-intensity speeds, higher peak speed, and a greater number of sprints relative to PLA MR. It is proposed that these improvements were potentially due to manipulation of motor function stemming from alteration of central control through activation of oral CHO receptors.

Despite greater external load in OT with CHO MR, there were no differences in physiological or psychophysiological measures of internal load between conditions. This suggests that CHO MR allowed athletes to maintain perceptions of effort while performing relatively higher workloads. In conclusion, the results of this study indicate that CHO MR may be a valuable practice in ice hockey to protect against decrements in external load with increased playing time, and further, offers an alternative performance-enhancing solution for athletes who choose to avoid CHO ingestion during competition. Nonetheless, future work with CHO ingestion and MR trials would aid in understanding how to optimally administer exogenous CHO to enhance ice hockey performance.

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 human participants were reviewed and approved by the University of Guelph Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DN and LS designed the study, interpreted the data, and wrote the manuscript. DN organized the trials and analyzed all data. DN, AG, JB, LB, AV, and LS collected on-ice data. AG and JB provided expertise with local positioning system data collection. All authors contributed substantially to revision and approved the final submission.

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REFERENCES

1. Brocherie F, Girard O, Millet GP. Updated analysis of changes in locomotor activities across periods in an international ice hockey game. *Biol Sport.* (2018) 35:261–7. doi: 10.5114/biolsport.2018.77826
2. Douglas AS, Kennedy CR. Tracking in-match movement demands using local positioning system in world-class men's ice hockey. *J Strength Cond Res.* (2020) 34:639–46. doi: 10.1519/JSC.0000000000003414
3. Vigh-Larsen JF, Ermidis G, Rago V, Randers MB, Fransson D, Nielsen JL, et al. Muscle metabolism and fatigue during simulated ice hockey match-play in elite players. *Med Sci Sports Exerc.* (2020) 52:2162–71. doi: 10.1249/MSS.0000000000002370

4. Cox MH, Miles DS, Verde TJ, Rhodes EC. Applied physiology of ice hockey. *Sports Med.* (1995) 19:184–201. doi: 10.2165/00007256-199519030-00004
5. Lignell E, Fransson D, Krustrup P, Mohr M. Analysis of high-intensity skating in top-class ice hockey match-play in relation to training status and muscle damage. *J Strength Cond Res.* (2018) 32:1303–10. doi: 10.1519/JSC.0000000000001999
6. Stanula A, Roczniok R, Maszczyk A, Pietraszewski P, Zajac A. The role of aerobic capacity in high-intensity intermittent efforts in ice-hockey. *Biol Sport.* (2014) 31:193. doi: 10.5604/20831862.1111437
7. Akermark C, Jacobs I, Rasmusson M, Karlsson J. Diet and muscle glycogen concentration in relation to physical performance in Swedish elite ice hockey players. *Int J Sports Nutr.* (1996) 6:272–84. doi: 10.1123/ijsn.6.3.272
8. Williams C, Rollo I. Carbohydrate nutrition and team sport performance. *Sports Med.* (2015) 45:13–22. doi: 10.1007/s40279-015-0399-3
9. Green HJ. Glycogen depletion patterns during continuous and intermittent ice skating. *Med Sci Sports.* (1978) 10:183–7.
10. Green HJ, Daub BD, Painter DC, Thomson JA. Glycogen depletion patterns during ice hockey performance. *Med Sci Sports.* (1978) 10:289–93.
11. Montpetit RR, Binette P, Taylor AW. Glycogen depletion in a game-simulated hockey task. *Can J Appl Sport Sci.* (1979) 4:43–5.
12. Simard C, Tremblay A, Jobin M. Effects of carbohydrate intake before and during an ice hockey game on blood and muscle energy substrates. *Res Q Exerc Sport.* (1988) 59:144–7. doi: 10.1080/02701367.1988.10605492
13. Krustrup P, Mohr M, Steensberg A, Bencke J, Kjaer M, Bangsbo J. Muscle and blood metabolites during a soccer game: implications for sprint performance. *Med Sci Sports Exerc.* (2006) 38:1165–74. doi: 10.1249/01.mss.0000222845.89262.cd
14. Ørtenblad N, Westerblad H, Nielsen J. Muscle glycogen stores and fatigue. *J Physiol.* (2013) 591:4405–13. doi: 10.1113/jphysiol.2013.251629
15. Linseman ME, Palmer MS, Sprenger HM, Spriet LL. Maintaining hydration with a carbohydrate-electrolyte solution improves performance, thermoregulation, and fatigue during an ice hockey scrimmage. *Appl Physiol Nutr Metab.* (2014) 39:1214–21. doi: 10.1139/apnm-2014-0091
16. Palmer MS, Heigenhauser G, Duong M, Spriet LL. Ingesting a sports drink enhances simulated ice hockey performance while reducing perceived effort. *Int J Sports Med.* (2017) 38:1061–9. doi: 10.1055/s-0043-119874
17. McCarthy DG, Wickham KA, Vermeulen TF, Nyman DL, Ferth S, Pereira JM, et al. Impairment of thermoregulation and performance via mild dehydration in ice hockey goaltenders. *Int J Sports Physiol Perf.* (2020) 15:833–40. doi: 10.1123/ijspp.2019-0464
18. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc.* (2004) 36:2107–11. doi: 10.1249/01.MSS.0000147585.65709.6F
19. Rollo I, Williams C, Gant N, Nute M. The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run. *Int J Sports Nutr Metab.* (2008) 18:585–600. doi: 10.1123/ijsnem.18.6.585
20. Rollo I, Cole M, Miller R, Williams C. Influence of mouth rinsing a carbohydrate solution on 1-h running performance. *Med Sci Sports Exerc.* (2010) 42:798–804. doi: 10.1249/MSS.0b013e3181bac6e4
21. Carter JM, Jeukendrup AE, Mann CH, Jones DA. The effect of glucose infusion on glucose kinetics during a 1-h time trial. *Med Sci Sports Exerc.* (2004) 36:1543–50. doi: 10.1249/01.MSS.0000139892.69410.D8
22. Chambers ES, Bridge MW, Jones DA. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol.* (2009) 587:1779–94. doi: 10.1113/jphysiol.2008.164285
23. Van Opstal AM, Hafkemeijer A, van den Berg-Huysmans AA, Hoeksma M, Mulder TP, Pijl H, et al. Brain activity and connectivity changes in response to nutritive natural sugars, non-nutritive natural sugar replacements and artificial sweeteners. *Nutr Neurosci.* (2021) 24:395–405. doi: 10.1080/1028415X.2019.1639306
24. Gant N, Stinear CM, Byblow WD. Carbohydrate in the mouth immediately facilitates motor output. *Brain Res.* (2010) 1350:151–8. doi: 10.1016/j.brainres.2010.04.004
25. Turner CE, Byblow WD, Stinear CM, Gant N. Carbohydrate in the mouth enhances activation of brain circuitry involved in motor performance and sensory perception. *Appetite.* (2014) 80:212–9. doi: 10.1016/j.appet.2014.05.020
26. Burke LM, Maughan RJ. The Governor has a sweet tooth–mouth sensing of nutrients to enhance sports performance. *Eur J Sports Sci.* (2015) 15:29–40. doi: 10.1080/17461391.2014.971880
27. Peart DJ. Quantifying the effect of carbohydrate mouth rinsing on exercise performance. *J Strength Cond Res.* (2017) 31:1737–43. doi: 10.1519/JSC.0000000000001741
28. Dorling JL, Earnest CP. Effect of carbohydrate mouth rinsing on multiple sprint performance. *J Int Soc Sports Nutr.* (2013) 10:1–8. doi: 10.1186/1550-2783-10-41
29. Pribyslavská V, Scudamore EM, Johnson SL, Green JM, Stevenson Wilcoxson MC, Lowe JB, et al. Influence of carbohydrate mouth rinsing on running and jumping performance during early morning soccer scrimmaging. *Eur J Sports Sci.* (2016) 16:441–7. doi: 10.1080/17461391.2015.1020345
30. Dolan P, Witherbee KE, Peterson KM, Kerksick CM. Effect of carbohydrate, caffeine, and carbohydrate + caffeine mouth rinsing on intermittent running performance in collegiate male lacrosse athletes. *J Strength Cond Res.* (2017) 31:2473–9. doi: 10.1519/JSC.0000000000001819
31. Burr JF, Jamnik RK, Baker J, Macpherson A, Gledhill N, McGuire EJ. Relationship of physical fitness test results and hockey playing potential in elite-level ice hockey players. *J Strength Cond Res.* (2008) 22:1535–43. doi: 10.1519/JSC.0b013e318181ac20
32. Stetter BJ, Buckeridge E, Nigg SR, Sell S, Stein T. Towards a wearable monitoring tool for in-field ice hockey skating performance analysis. *Eur J Sports Sci.* (2019) 19:893–901. doi: 10.1080/17461391.2018.1563634
33. Thompson KM, Safadie A, Ford J, Burr JF. Off-ice resisted sprints best predict all-out skating performance in varsity hockey players. *J Strength Cond Res.* (2020). doi: 10.1519/JSC.0000000000003861
34. Van Iterson EH, Fitzgerald JS, Dietz CC, Snyder EM, Peterson BJ. Reliability of triaxial accelerometry for measuring load in men's collegiate ice hockey. *J Strength Cond Res.* (2017) 31:1305–12. doi: 10.1519/JSC.0000000000001611
35. Hoppe MW, Baumgart C, Polglaze T, Freiwald J. Validity and reliability of GPS and LPS for measuring distances covered and sprint mechanical properties in team sports. *PLoS One.* (2018) 13:e0192708. doi: 10.1371/journal.pone.0192708
36. Bigg, JL, Gamble ASD, Spriet LL. Internal load of male varsity ice hockey players during training and games throughout an entire season. *Int J Sports Physiol Perform.* (2021). doi: 10.1055/a-1555-3484
37. Impellizzeri FM, Marcora SM, Coutts AJ. Internal and external training load: 15 years on. *Int J Sports Physiol Perform.* (2019) 14:270–3. doi: 10.1123/ijspp.2018-0935
38. Douglas A, Johnston K, Baker J, Rotondi MA, Jamnik VK, Macpherson AK. On-ice measures of external load in relation to match outcome in elite female ice hockey. *Sports.* (2019) 7:173. doi: 10.3390/sports7070173
39. McLaren SJ, Macpherson TW, Coutts AJ, Hurst C, Spears IR, Weston M. The relationships between internal and external measures of training load and intensity in team sports: a meta-analysis. *Sports Med.* (2018) 48:641–58. doi: 10.1007/s40279-017-0830-z
40. Ulmer JG, Tomkinson GR, Short S, Short M, Fitzgerald JS. Test-retest reliability of TRIMP in collegiate ice hockey players. *Biol Sport.* (2019) 36:191–4. doi: 10.5114/biolsport.2019.84670
41. Lachaume CM, Trudeau F, Lemoyne J. Energy expenditure by elite midget male ice hockey players in small-sided games. *Int J Sports Sci Coach.* (2017) 12:504–13. doi: 10.1177/1747954117718075
42. Rosenberg SI, Baker HP, Rizzi A, Terry MA, Tjong VK. Frequency of overtime and consecutive games and their impact on injury rate in the National Hockey League (NHL). *Phys Sportsmed.* (2021) 49:100–5. doi: 10.1080/00913847.2020.1782715
43. Douglas A, Rotondi MA, Baker J, Jamnik VK, Macpherson AK. On-ice physical demands of world-class women's ice hockey: from training to competition. *Int J Sports Physiol Perform.* (2019) 14:1227–32. doi: 10.1123/ijspp.2018-0571
44. Banister EW, Calvert TW. Planning for future performance: implications for long term training. *Can J Appl Sports Sci.* (1980) 5:170–6.
45. Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc.* (2009) 41:3–12. doi: 10.1249/MSS.0b013e31818cb278
46. Jackson J, Snydmiller G, Game A, Gervais P, Bell G. Investigation of positional differences in fitness of male university ice hockey players and the frequency,

time spent and heart rate of movement patterns during competition. *Int J Kin Sports Sci.* (2017) 5:6–15. doi: 10.7575/aiac.ijkss.v.5n.3p.6

47. Whitham M, McKinney J. Effect of a carbohydrate mouthwash on running time-trial performance. *J Sports Sci.* (2007) 25:1385–92. doi: 10.1080/02640410601113676

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Changes in body composition during the macrocycle of professional football players in relation to sports nutrition knowledge

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Professional football players are obligated to meet the physical demands and maintain the best possible performance throughout the whole macrocycle. It is important to assess the players' nutrition knowledge, identify areas that require increased nutrition awareness and identify the impact of knowledge on changes in body composition as this can affect the players' health and performance. This study aimed to assess changes in the body composition of professional football players during the macrocycle of the spring round of the football championship and to identify the correlation between nutrition knowledge and maintaining body composition. The study included 38 football players. The players' body compositions were analyzed 6 times during the macrocycle consisting of preparatory, competitive, and transition periods using the Direct Segmental Multi-Frequency Bioelectrical Impedance Analysis method. Athletes completed the Nutrition for Sport Knowledge Questionnaire to assess their nutrition knowledge. During the preparatory period, a statistically significant negative correlation was demonstrated between the players' knowledge about the subsections of micronutrients in the diet and the dispersion of the adipose percentage tissue content ($r = -0.36$, $p = 0.03$). In the competitive period, there was a statistically significant negative correlation between the players' knowledge of sports nutrition and the dispersion of lean body mass ($r = -0.46$, $p = 0.004$), and skeletal muscle mass ($r = -0.36$, $p = 0.03$). During the transition period, a statistically significant negative correlation between the players' knowledge of weight control and the dispersion of body mass ($r = -0.47$, $p = 0.00$) and BMI values ($r = -0.48$, $p = 0.00$) was identified. The player's knowledge about the subsection of macronutrients significantly negatively correlated with the dispersion of skeletal muscle mass content ($r = -0.33$, $p = 0.05$). Nutrition knowledge has an impact on the stability of body composition during all analyzed periods: preparatory, competitive, and transition periods.

KEYWORDS

body composition, nutrition knowledge, football, professional athletes, anthropometry

Introduction

Football[1] players are required to meet physical demands and maintain top physical condition throughout the whole macrocycle. For this reason, constant assessment of players' physical condition plays a key role in the success of any team (1). Anthropometric characteristics and body composition were proven to have a significant impact on players' performance (2–4), and studies report changes in them over the course of the football training periods (5, 6).

In football, depending on a calendar of competitions, the preparation of players occurs in one-cycle, two-cycle, or three-cycle models (7). The two-cycle model of the training process takes into consideration competition in a spring and fall system. One round constitutes a 6-month macrocycle, referred to as a round. In one round, we distinguish three periods: preparatory, competitive, and transition periods, individual periods of the macrocycle are successive stages of managing the development of the sport form (7, 8). The preparatory period is designed to develop athletic prowess in preparation for the competitive period (9). Training during this period is geared toward rebuilding athletes' physical fitness after the transition period (10–12). The competitive period is difficult to plan because training loads should be adjusted to maximize physiological adaptations and, at the same time, to avoid overtraining and injuries of players. This period consists in maximizing the result of the sport during matches based on the players' performance and skills dispositions obtained during the preparatory period (8, 13). The transition period is characterized by a complete cessation or significant reduction of training. Athletes may be engaged in recreational sporting activities or voluntary, non-periodic training during this time (14). Cessation of physical activity, the reduction in training, and the fitness level of the players will modulate the kinetics of changes in body weight and physiological functions (14–17). This period should be regarded by athletes as an opportunity to recover before the next season (18). There are few scientific research reports on how the body composition is shaped during periods in the football season (6, 19–22). It is extremely important to evaluate body composition during the entire macrocycle (preparatory, competitive, and transition periods) due to the differences in training intensity, care of the coaching staff, amount of match play, and amount of free time during each period. One training macrocycle (spring round) was included in the study to analyse each period (13, 20, 22).

One of the most common causes of inadequate diets amongst athletes is poor nutrition knowledge derived from inappropriate sources (23). To improve nutrition knowledge, it is required to know the areas that are characterized by the lowest nutritional awareness in the group of athletes and to identify the correlation between them and body weight composition (24). Poor knowledge of nutrition among professional football players can lead to bad eating behaviors. This results in an energy imbalance, weight gain or loss, decreased exercise performance, or increased risk of injury and illness (25, 26). Therefore, adequate nutrition knowledge and optimal body composition are important for the athletic performance of professional football players (26).

This study aimed to assess the body composition of professional football players during the preparatory, competitive, and transition periods and to identify the influence of their nutrition knowledge on body composition modification.

Materials and methods

Study design

The research was conducted during the spring round of the PKO BP Ekstraklasa football championship (the highest league of football competitions in Poland) between 7 January 2021 and 23 July 2021. Participating athletes were players of two Silesian football clubs belonging to PKO BP Ekstraklasa 2020/2021 (the highest league of football competitions in Poland). The study was conducted with the approval of the Bioethics Committee of the Silesian Medical University in Katowice (PCN/0022/KB/68/I/20).

Body composition analyses were conducted in three periods of the spring round (preparatory, competitive, and transition

1 Football is used to describe association football, the sport most commonly referred to in the English-speaking world as "football" or "soccer".

periods) of the PKO BP Ekstraklasa 2020/2021 football championship. The days on which the analyses were conducted were planned to take into account league and cup games, ensuring adequate time for post-match regeneration.

All players' nutritional knowledge of sports nutrition was assessed in the study. Due to the presence of athletes in the study group who did not speak Polish, nutritional consultations were conducted in Polish or English.

The next step was to analyse the correlation of differences between the maximum and minimum measurements of body composition in the studied period and nutritional awareness. The contribution of nutrition knowledge to the stability of body composition (measured by the difference between maximum and minimum measurements) during the three periods was assessed (Figure 1). The following were taken into account:

- preparatory period (1–2 measurements)
- competitive period (2–5 measurements)
- transition period (5–6 measurements).

Participants

The study included 58 athletes aged 20–31. The participants in the study were of different nationalities (36 Polish, six Slovakian, three Spanish, three Portuguese, two Greek, two Slovenian, one Czech, one Austrian, one Danish, one Hungarian, one Ghanaian, and one Gambian). The classification was made in terms of function on the field: forward, midfielder, defender, and goalkeeper. Considering the inclusion and exclusion criteria, 228 complete body composition measurements were finally obtained from 38 athletes.

Inclusion criteria in the study were defined as follows: professional training of football in the clubs included in the study, giving consent to participate in the study, and no injury requiring long-term treatment that would exclude players from training and playing matches during the period of the study, i.e., 7 months.

Exclusion criteria for the study included: not speaking Polish or English at a communicative level, exclusion of players from training and playing matches for at least consecutive 14 days due to injury, illness, quarantine, or isolation related to COVID-19 pandemic, transfer to another football club during the study period, i.e., 7 January 2021 to 23 July 2021, absence from at least one of the six measurements for a reason other than the above.

The study included first-team players as well as reserve players. However, in both clubs, players who did not play in the championship match were obliged to participate in the additional match to maintain the match rhythm. Therefore, the players' game loads may be comparable.

BMI

Height measurements were taken immediately before the first body composition measurement, while body weight was measured during the body composition analysis.

Height (cm) and body mass (kg) were measured to the nearest 0.1 cm (SECA 756, Seca GmbH & Co. KG, Hamburg, Deutschland) and 0.1 kg (InBody 770, InBody USA, California, USA), respectively, with the subject wearing underwear and no shoes. Body mass index (BMI) was calculated as body mass (kg) divided by height (m) squared. The results formed the basis for assessing height–weight ratios in relation to the standards of the European population and WHO (World Health Organization) recommendations (27).

Body composition analysis

Body composition was assessed using a DSM-BIA (Direct Segmental Multi-Frequency Bioelectrical Impedance Analysis) (InBody 770, InBody USA, California, USA). The DSM-BIA technique is based on assumption that the human body is composed of five interconnecting cylinders and takes direct impedance measurements from the various body compartments. A tetrapolar eight-point tactile electrode system is used, which separately measures the impedance of the subject's torso, arms, and legs at six different frequencies (1, 5, 50, 250, 500, and 1,000 kHz) for each of the body segment. The analyser allows for obtaining a complete body composition in about 60 s. The device works with the use of a current of 80 uA (28, 29). At the molecular level, total body water (TBW) consists of fat mass (FM) and fat-free mass (FFM). Fat-free mass is in turn divided into compartments: lean body mass (LBM) and bone mineral mass (BMC). Lean body mass (LBM) is the sum of body water, total protein, carbohydrates, fat-free lipids, and soft tissue, excluding fat mass and bone mineral mass.

Body composition parameters were obtained using the Lookin'Body Software version 120.3.0.0.6. The measurements were conducted according to a standard protocol as recommended by the device manufacturer. Before each testing session, the analyser was checked with a calibration circuit of known impedance (resistance = 500.0 Ω; reactance = 0.1 Ω; 0.9% error). The participants were on an empty stomach and did not consume alcohol or caffeine for at least 24 h before the examination. The measurement was performed at a regular time, after defecation, at least 24 h after the end of intensive physical activity, without shoes and socks, in underwear, with clean and dried feet and hands without applied cream and lotion. The participants had to step on the foot electrodes barefoot and maintain an evenly distributed weight on the measurement platform while holding a pair of electrodes fixed to the display unit. Then, the participants extended their arms

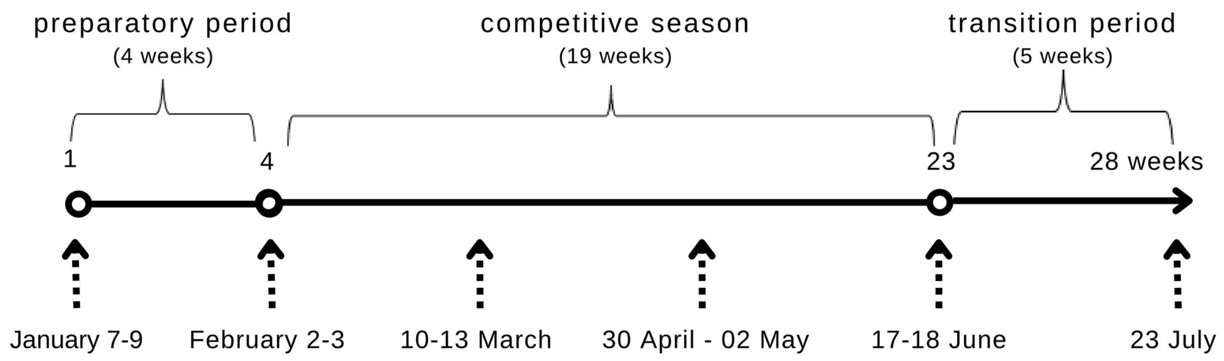

FIGURE 1
Procedure for the anthropometric measurements.

in front of the chest, maintaining a steady position until the measurements were completed.

Recently, BIA technology has developed significantly, allowing more accurate estimation of TBW, FFM, LBM, FM, and other results. Dual-energy X-ray absorptiometry (DEXA) is considered the reference method for assessing body composition, but there are some limitations. DXA is not portable, is expensive, and often requires training by a licensed technician due to its low radiation exposure. Bioelectrical impedance analysis has evolved to use multiple frequencies and impedance measurements to improve the validity and reliability of body composition estimates. Compared to other methods, BIA is relatively simple, quick, and non-invasive. The DSM-BIA method used in the InBody 770 compared to the reference DEXA method is valid and reliable in populations of healthy athletes who are clinically and physically inactive (30–33).

Nutrition knowledge

To assess the nutrition knowledge of the study group, a survey method was applied using the Nutrition for Sport Knowledge Questionnaire (NSKQ) (34, 35). Assessment of the nutrition knowledge of the study group was carried out using the original version of the questionnaire in English and the Polish version. The NSKQ questionnaire developed by Trakman et al. was validated using an eight-item methodology. The researchers performed a reliability assessment using PerSepIndex, a summary statistic created by RUMM 2030, which is analogous to Cronbach's α. Both PerSepIndex and Cronbach's α is based on multiple split-half reliability assessments; ≥0.7 is an accepted measure of internal reliability (35). The authors of the questionnaire gave their consent for its use in the study. For this study, a pilot study was conducted using the Polish version of the questionnaire on a group of 30 people. The pilot study was conducted to validate the Polish version of the questionnaire and to check the accuracy and acceptability of the questions included in it. The original version of the survey instrument was translated into Polish by two independent English translators. For both, their native language was Polish. Based on the two translations, a Polish-language version of the NSKQ questionnaire was created, then all the sentences of the translation were discussed until the opinions were consistent. These procedures resulted in a version that met the condition of semantic consistency for each response. Two back-translations were then created by other independent translators. These were reviewed for consistency along with the original version by a person whose native language is English. The next step was to adapt the questionnaire, which maintained consistency in terms of graphics (font, text size, distribution of questions and answers, and amount of text per page) with the original version.

Cronbach's α coefficient for sample normalization was 0.83, indicating the high reliability of the questions. The pilot study allowed to validate the questions included in the Polish language questionnaire. The PerSepIndex coefficient for the actual part of the study was estimated at 0.8, which is the same value as the obtained Cronbach's α coefficient in the pilot study (34, 35). The questionnaire contained 87 questions divided into six subsections: weight control ($n = 12$), macronutrients ($n = 30$), micronutrients ($n = 13$), sports nutrition ($n = 12$), supplementation ($n = 12$), and alcohol ($n = 8$). Each section in the questionnaire used is one-dimensional; therefore, individual sections can be used independently to assess nutrition knowledge in each area (36). The test time was 25 min. One point was awarded for each question answered correctly, and the correct answer to all 87 questions resulted in a score of 100%. Nutrition knowledge was quantified using the scoring established by Trakman et al. (34).

The questionnaire was expanded to include questions about club membership, age, education, medical conditions, medications taken, dietary advice, source of knowledge about proper nutrition, preparation and type of meals consumed, and adjustment of diet to physical activity. The questionnaire was

TABLE 1 General physical characteristics and body composition variables—averages (mean ± SD).

Variable		Age (year)	Height (cm)	Body mass (kg)	BMI (kg/m^2)	FFM (kg)	SMM (kg)	%BF	FM (kg)
Total (n = 38)		25.89 ± 5.22	182.59 ± 5.45	79.51 ± 7.16	23.82 ± 1.25	71.20 ± 5.77	41.18 ± 3.53	10.16 ± 2.44	8.14 ± 2.39
Field position	F	25.50 ± 5.89	182.50 ± 5.32	79.70 ± 7.55	23.96 ± 1.25	71.65 ± 6.40	41.45 ± 4.06	10.06 ± 1,52	8.02 ± 1.68
	M	24.13 ± 4.69	179.63 ± 4.60	75.10 ± 6.48	23.24 ± 1.24	68.02 ± 5.72	39.08 ± 3.37	9.47 ± 2.28	7.12 ± 1.93
	D	27.50 ± 4.50	183.88 ± 5.11	83.34 ± 5.71	24.63 ± 0.85	74.47 ± 4.50	43.02 ± 2.69	10.68 ± 3.00	8.99 ± 2.82
	G	27.80 ± 7.19	188.50 ± 3.50	83.35 ± 5.60	23.45 ± 1.30	72.37 ± 3.76	42.74 ± 2.30	11.07 ± 2.40	9.30 ± 2.58
	p	0.32	**0.01****	**0.01****	**0.03***	**0.01***	**0.01***	0.5	0.14
			M vs. G	**M vs. D**	**M vs. D**	**M vs. D**	**M vs. D**		

F, forward; M, midfielder; D, defender; G, goalkeeper; FFM, fat free mass; SMM, skeletal muscle mass; %BF, percentage of body fat; FM, fat mass.
* = p < 0.05; ** = p < 0.01. The bolding is to emphasize statistical significance.

completed between the 5th and 6th measurements under the supervision of an interviewer during an individual consultation to avoid incomplete answers.

Statistical analysis

The obtained data were developed using Statistica v.13.3 (Stat Soft Polska) and the R v. 4.0.0 package (2020) under the GNU GPL license (The R Foundation for Statistical Computing).

To present quantitative data, mean values and standard deviations were calculated - X ± S. For qualitative data, percentage notation was used. Qualitative data were expressed as numerical values determined by mathematical methods to make statistical inferences.

Compliance with the normal distribution was checked using the Shapiro-Wilk test. The evaluation of the significance of differences between the means in groups due to the position on the field (goalkeeper, defender, midfielder, and forward) was made using the ANOVA analysis of variance.

For distributions deviating from the normal distribution, their compliance for multiple groups was checked using the Kruskal-Wallis test. For comparisons between the groups, appropriate *post-hoc* tests were performed—Tukey's HSD test for parametric analysis and Dunn's test for non-parametric analysis. Calculations were made for the averaged measurement over the entire period (expressed as the arithmetic mean of the six measurements) and in relation to all individual measurements taken.

To perform a comparative analysis for anthropometric measurements taken at different times, an ANOVA analysis of repeated measurements or a non-parametric Friedman test was performed, depending on the compliance of the distributions with the normal distribution. For comparisons between the groups, appropriate *post-hoc* tests were performed—the HSD Tukey test or the *post-hoc* test for the Friedman test.

An analysis of the level of nutrition knowledge (poor, average, good, and excellent) was also performed in groups, taking into consideration the study group's education (elementary—vocational education and secondary—high education), use of a dietitian (yes and no), and position on the field (goalkeeper, defender, midfielder, and forward). To assess the dependence, the χ^2 test with variations depending on the sample size or the Fischer test for nxm tables was used.

Spearman's *R*' correlation coefficient with its significance test was used in the correlation analysis of the averaged anthropometric measurements obtained during the study periods in relation to the results obtained in the nutrition knowledge survey.

A value of $p < 0.05$ was used as a criterion for statistical significance.

Results

A total of 228 body composition measurements were obtained in six different measurements during the spring round of the PKO BP Ekstraklasa 2020/2021 championship, constituting the complete research material. The players were divided into groups according to their function on the field: 5 (13.2%) goalkeepers, 12 (31.6%) defenders, 15 (39.5%) midfielders, and 6 (15.8%) forwards.

Taking education into account, the majority of the respondents had secondary education ($n = 25$; 65.8%). The rest declared elementary education ($n = 8$; 21.1%), vocational education ($n = 2$; 5.3%), and higher education ($n = 3$; 7.9%).

The participants did not report any chronic diseases or ongoing pharmacotherapy (except for one person taking Fostex, a drug used to treat asthma, containing two active substances: beclometasone dipropionate and formoterol fumarate dihydrate).

Physical characteristics and body composition

The age, physical characteristics (height, body mass, and BMI), and body composition variables (fat-free mass, skeletal muscle mass, and fat mass) in relation to positions on the field are shown in Table 1.

During the measurements, statistically significant differences were found in the prevalence of overweight expressed by BMI. The highest number of athletes with a BMI classified as overweight was found at the 6th measurement, which took place after the end of the transition period, while the lowest number was found at the 1st measurement, which took place at the beginning of the preparatory period, and at the 3rd measurement at the beginning of the competitive period (Figure 2).

Statistically significant differences between players performing different functions on the field were found in body mass and BMI measurements. Statistically significantly lower values of analyzed parameters concerned midfielders ($p < 0.05$). In addition, a statistically significant increase in their body mass and BMI values between the 5th and 6th measurements was found ($p < 0.05$). The details are shown in Figure 3.

Statistically significant differences in lean body mass and skeletal muscle mass were found in midfielders, they were characterized by lower content of the above-mentioned body mass components ($p < 0.05$).

An increase in lean body mass content was shown in this group between the 1st and 2nd measurements ($p < 0.01$). The midfielders and defenders groups showed statistically significant increases in skeletal muscle mass content between the 1st and 2nd as well as the 1st and 6th measurements ($p < 0.05$; $p < 0.01$). There were statistically significant increases in the percentage of body fat between the 1st and 6th, the 2nd and 6th, the 3rd and 6th, and the 3rd and 5th measurements in the midfielders group ($p < 0.05$) as well as between the 2nd and 6th measurement in the defender's group ($p < 0.05$). Statistically significant increases in fat mass were shown between the 2nd and 6th, the 3rd and 6th, and the 3rd and 5th measurements in the midfielders group and between the 2nd and 6th measurements in the defenders group ($p < 0.05$; $p < 0.05$). The details are shown in Figure 4.

Nutrition knowledge

The study groups' knowledge of nutrition was significantly obtained from professional sources. The majority (71%; $N = 27$) of participants received advice from a dietitian, and 44.7% ($N = 17$) of the players at the time of the study. Dietary advice was the primary source of knowledge in this area for 60.5% ($N = 23$). A small group ($N = 12$) of the respondents obtained nutrition knowledge from a coach, as many as 71.1% ($N = 27$) from the Internet, and 34.2% ($N = 13$) from other people. In a question about where meals were eaten, 60.5% ($N = 23$) of the respondents declared eating at home, while 33.3% ($N = 12$) used catering services. Almost half of the athletes prepared their meals (44.7%; $N = 17$), and all players confirmed the adaptation of nutrition to their needs and considered their nutrition to be healthy.

Figure 5 shows the average scores and nutrition knowledge divided by categories. On average, the athletes obtained 51.49% of correct answers and their nutrition knowledge was assessed as average. The nutrition knowledge of the studied players regarding micronutrients in the diet, and supplementation was at a poor level, while the nutrition knowledge regarding body weight control, macronutrients in the diet, sports nutrition, and alcohol consumption was at an average level.

Figure 6 shows the percentage distribution of nutritional knowledge of the athletes from each sub-section.

Nutrition knowledge was also verified by taking into account the age of the participants. There was no statistically significant difference in the knowledge in relation to age ($p > 0.05$).

The analysis of nutrition knowledge with regard to the players' level of education was performed similarly. Only in the case of the knowledge of body weight control ($p = 0.03$) and sports nutrition ($p = 0.03$), a higher level of knowledge was found amongst athletes with secondary or higher education.

There was no statistically significant variation in nutrition knowledge amongst athletes under the care of a dietitian ($p > 0.05$).

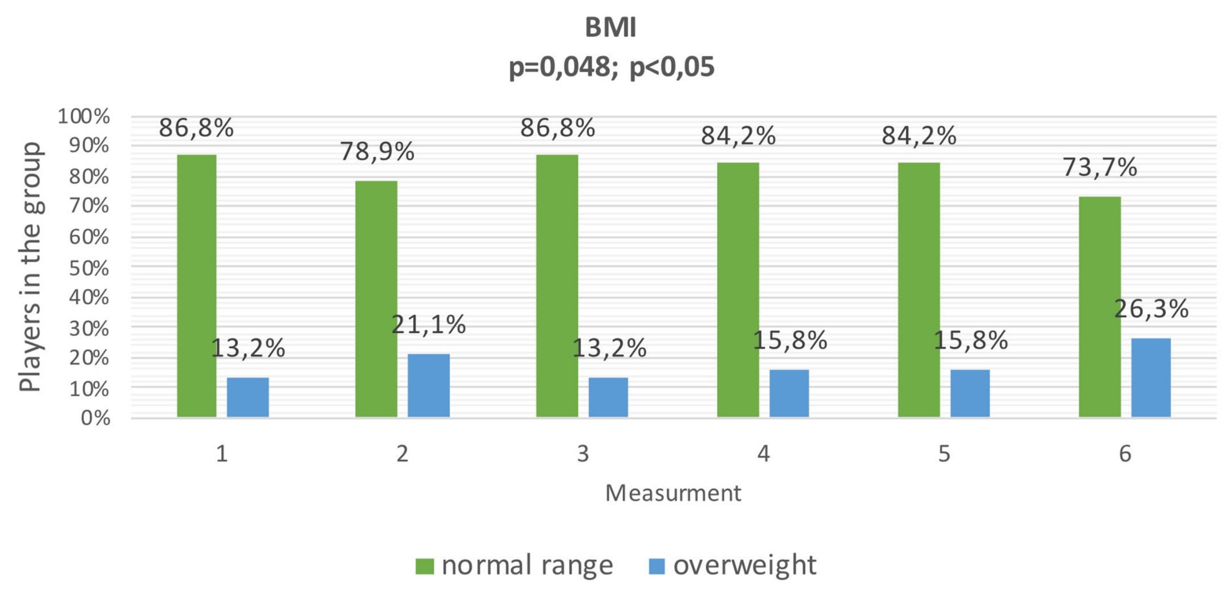

FIGURE 2
Change in nutritional status of football players assessed by BMI (n = 38).

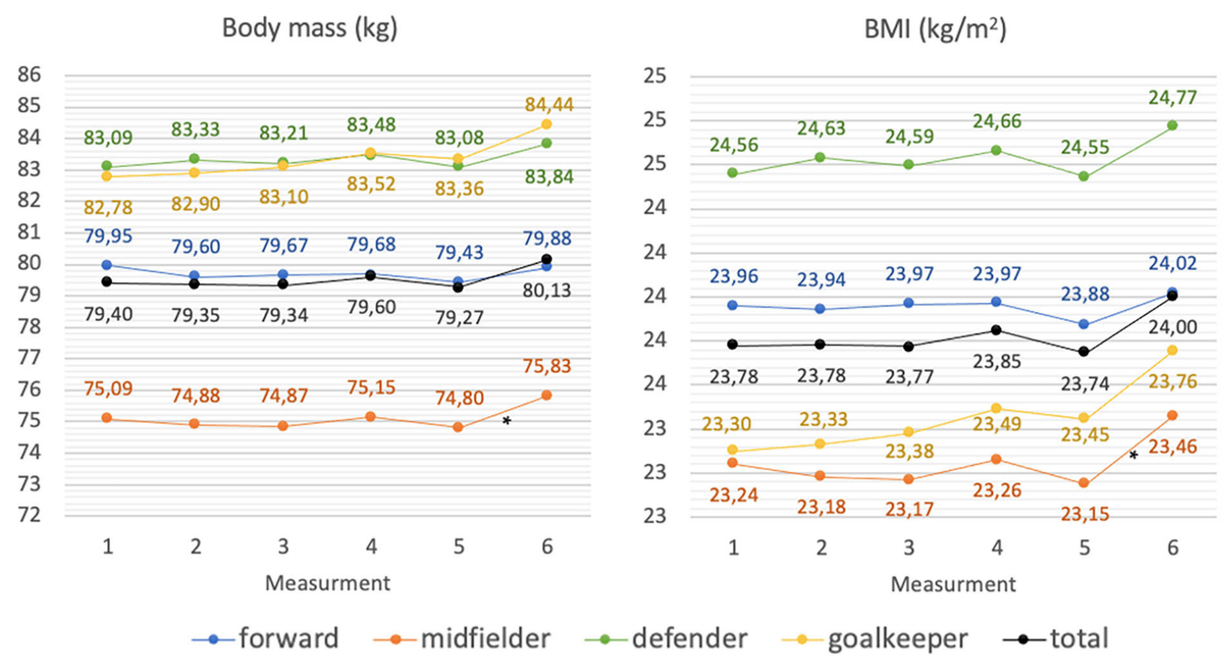

FIGURE 3
Changes in body mass, and BMI of football players during the spring round of the 2020/2021 football season (average values); *significantly different from (p < 0.05).

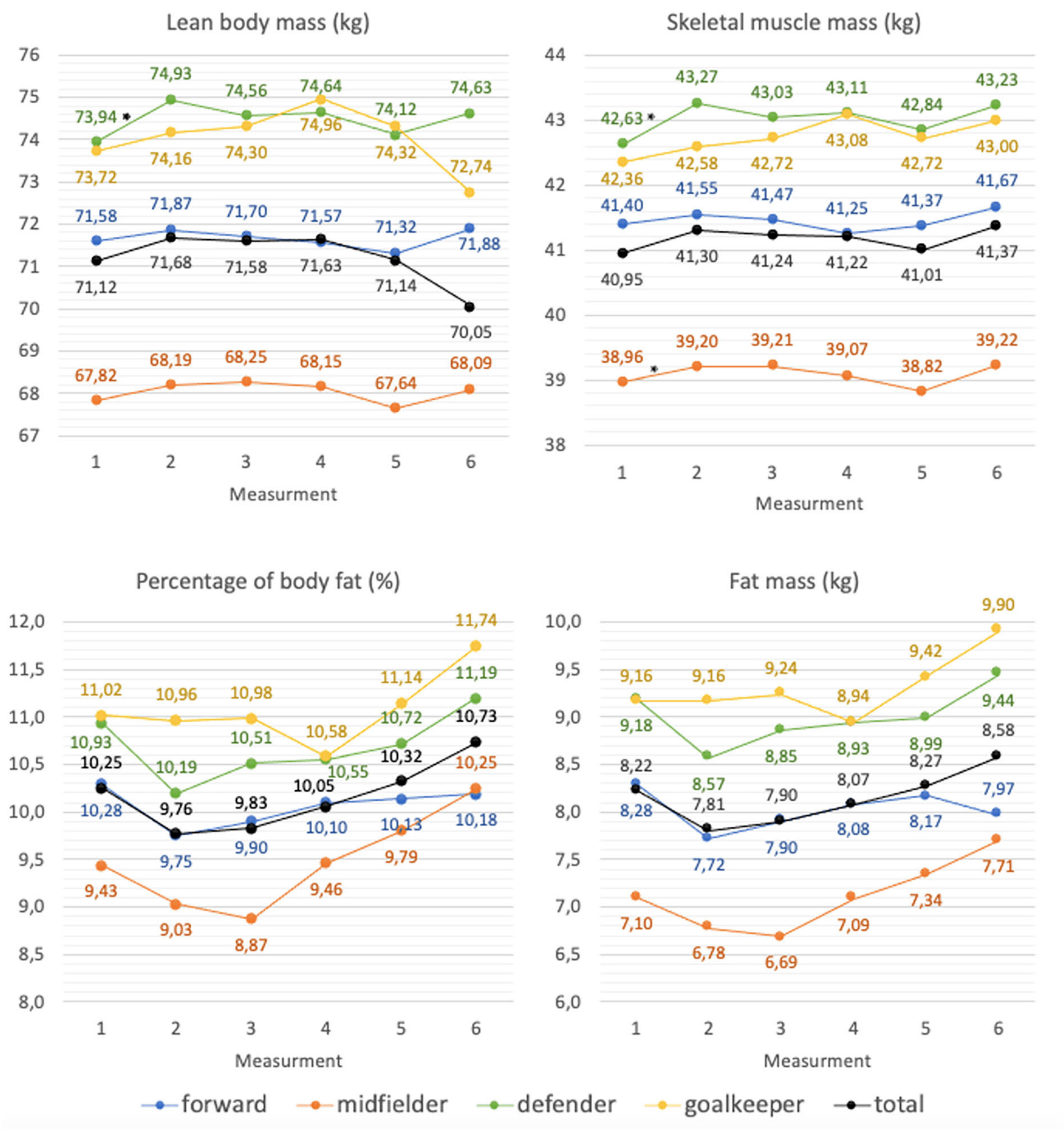

FIGURE 4
Changes in body composition of football players during the spring round of the 2020/2021 football season (average values); *significantly different from ($p < 0.05$).

Changes in body composition with regard to nutrition knowledge

During the preparatory period, there was a statistically significant negative correlation between knowledge from the subsection of micronutrient content in the diet and the dispersion of body fat content (%) ($r = -0.36$).

In the competitive period, there was a statistically significant negative correlation between knowledge from the subsection of sports nutrition and the dispersion of lean body mass (kg) ($r = -0.46$), and skeletal muscle mass (kg) ($r = -0.36$).

During the transition period, a statistically significant negative correlation between knowledge from the subsection

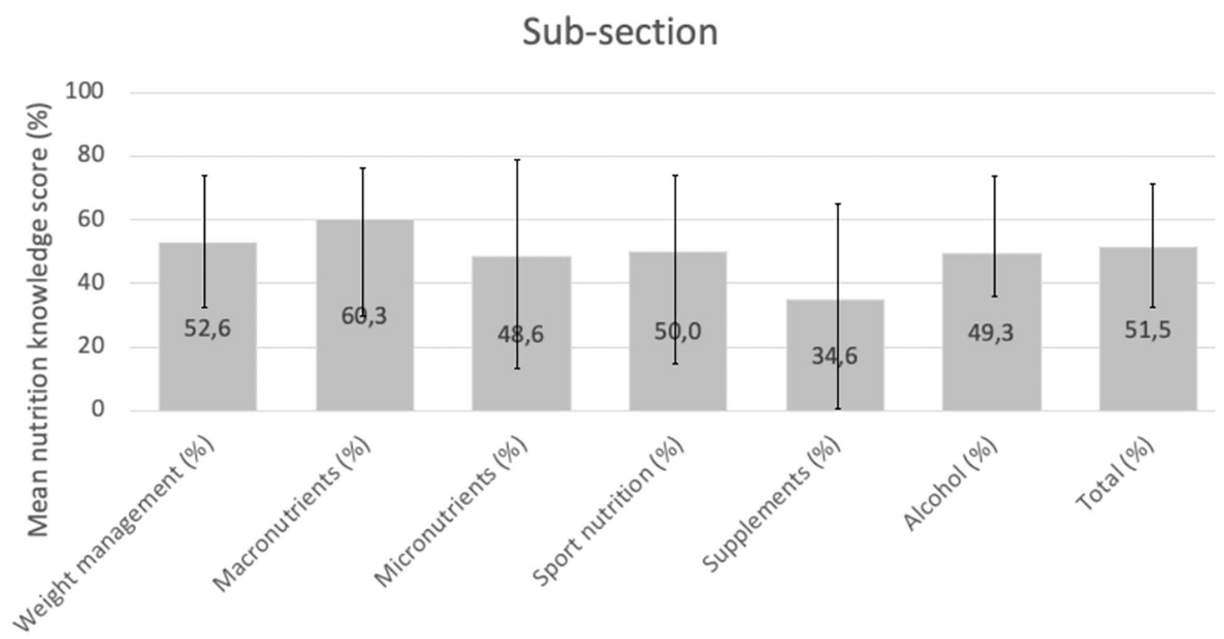

FIGURE 5
NSKQ scores in football players ($n = 38$). Mean and minimum and maximum percentages are indicated.

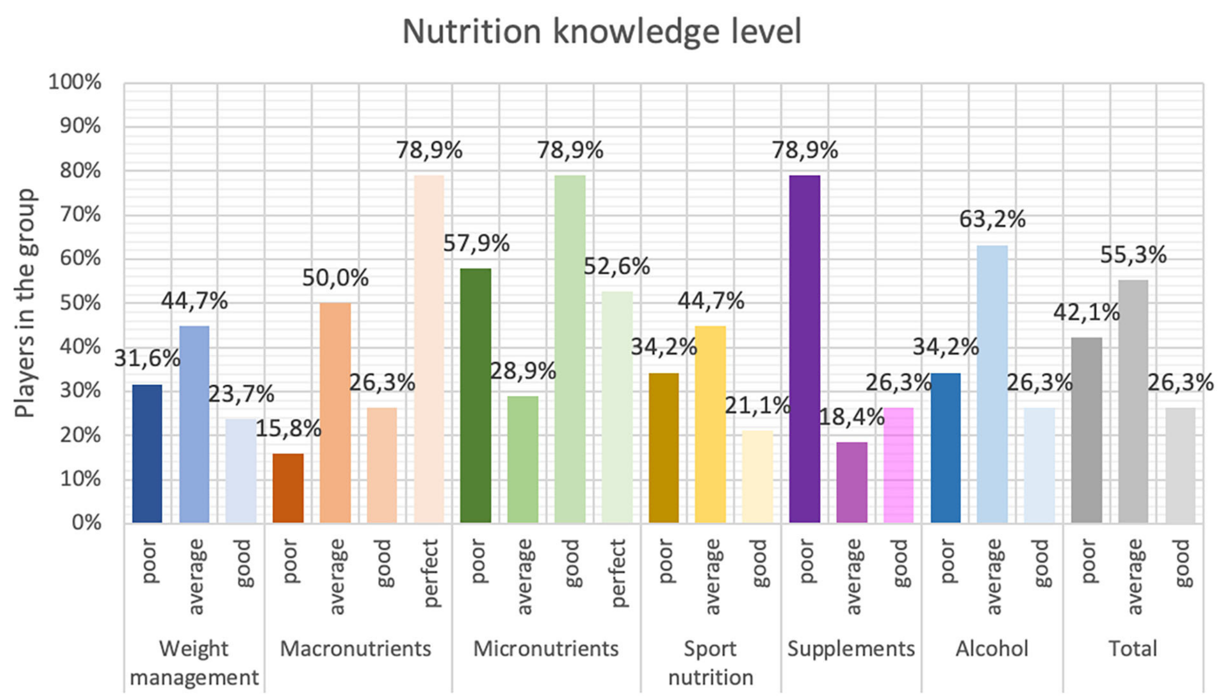

FIGURE 6
Nutrition knowledge of the football players participating in the study (n=38).

of weight control and the dispersion of body mass (kg) ($r = -0.47$) and BMI values (kg/m^2) ($r = -0.48$) was identified. Knowledge of the macronutrient subsection is significantly negatively correlated with the dispersion of skeletal muscle mass content (kg) ($r = -0.33$). The results of the analyses are presented in Table 2.

Discussion

Scientific research focuses on the contribution of variables, such as age, sex, eating habits, and training load to maintain the optimal body weight composition of athletes (37, 38). There are few reports indicating how the body composition is shaped during the football periods (6, 19–22).

The changes in body composition are determined by a properly designed training programme and proper nutrition. The training process is planned by the club's training staff, it is adjusted to the possibilities and goals of the team and modified according to individual players' preferences. The coaches, assistants, and physical trainers supervise the correctness of the individual exercises during training, while the activity and participation in games are verified by the coaching staff. Proper nutrition is a highly important aspect of body composition control. Dietitians working in a football club do not usually have regular contact with players, their consultations being limited to occasional meetings. There are about 30 players on the team, and nutrition education for each of them is time-consuming; moreover, the tasks of a dietitian include other responsibilities, e.g., preparing a menu for training camps or assessing of nutritional status of players. Due to the limited contact between the dietitian and the players and the fact that most of the players' meals are consumed outside the club, it is important to assess the players' nutrition knowledge, identify areas that require increased nutrition awareness, and identify the impact of knowledge on changes in body composition during the periods.

Players in various positions on the field have different anthropometric and performance characteristics. Goalkeepers and defenders are usually characterized by higher height and weight than other players (39). Defenders are characterized by higher height and weight compared to midfielders and forwards. Midfielders represent the lowest group of players with the lowest weight (40). The variation in absolute values of lean body mass between players with different roles may be due to differences in body size rather than body composition, so it is important to consider relative values when comparing the body composition of football players (40). Differences between players' body composition are due to the different physiological and metabolic demands of playing football depending on the function on the field (41–43).

In the current research, during the preparatory period, the number of players with a BMI value diagnosed as overweight increased from 13.2% ($N = 5$) at the beginning to 21.1% ($N = 8$) at the end of this period. Analyzing the percentage of body fat over the same period, it was shown that the number of players with a percentage of body fat below the norm has increased. At the beginning of the preparatory period, 51.4% ($N = 20$) of the players had below-normal body fat percentage, while at the end of the preparatory period is applied to 62.2% ($N = 24$) of the respondents. The above-mentioned results confirm that the BMI is not a suitable tool for assessing the body weight of professional athletes, and it is not an appropriate tool for monitoring changes that occur in body weight composition during the preparatory period.

Analyzing the body compositions of the players, an increase in the skeletal muscle mass content was shown on average from 40.95 ± 3.48 kg to 41.3 ± 3.53 kg during the preparatory period. The increase in the content of skeletal muscle mass during this period resulted in a misinterpretation of the BMI index. This study's results do not confirm a significant reduction in the content of adipose tissue in the analyzed period. Results from other studies describe fat reduction with a concomitant increase in lean body mass during the preparation period (20, 44).

Analyzing the authors' own research on the preparatory period, with regards to players' position on the field, a significant increase in lean body mass in the defender's group was shown from 73.94 ± 4.67 kg to 74.93 ± 4.16 kg and skeletal muscle mass from 42.63 ± 2.79 kg to 43.27 ± 2.53 kg. Moreover, in the midfielders group, skeletal muscle content increased from 38.96 ± 3.42 kg to 39.2 ± 3.39 kg.

The preparatory period is followed by the competitive period. Professional football players should adapt their nutrition to the training loads occurring during this period, thus ensuring proper body composition throughout the competitive period (14, 45). In the current study, evaluating the BMI index showed a variable number of overweight athletes over the competitive period. Before the competitive period, 21.1% ($N = 8$) of the athletes were overweight, at the beginning of this period the lowest number of over-weight players was found, only 13.3% ($N = 5$), then this value slightly increased to 15.8% ($N = 6$) in the middle of the period and remained until the end of the period. When analyzing body fat percentage before the start of the competitive period, 62.2% ($N = 24$) of the athletes had below normative body fat percentage, it was the highest number of athletes amongst all measurements. At the beginning of the competitive period, the number of athletes with a below-normal body fat percentage decreased slightly and reached 62.2% ($N = 24$) again by the middle of the competitive period. During the 5th measurement, which was taken at the end of the competitive period, 54.1% ($N = 21$) of the athletes had a below-normal body fat percentage. Similar to the preparatory period, BMI misrepresented changes in body composition during the competitive period in professional athletes. Analysis of body

TABLE 2 The strength of correlation of differences between maximum and minimum body composition measurements and scores on the nutrition knowledge assessment expressed by Spearman's R′ correlation coefficient.

Variable	Nutrition knowledge sub–section	Weight management 0–12 points	Macronutrients 0–30 points	Micronutrients 0–13 points	Sport nutrition 0–12 points	Supplements 0–12 points	Alcohol 0–8 points	Total 0–87 points
Preparatory period	Body mass (kg)	$r = -0.14$ $p = 0.42$	$r = 0.08$ $p = 0.65$	$r = -0.09$ $p = 0.60$	$r = -0.18$ $p = 0.28$	$r = 0.01$ $p = 0.94$	$r = -0.19$ $p = 0.26$	$r = -0.11$ $p = 0.52$
	BMI (kg/m^2)	$r = -0.06$ $p = 0.72$	$r = 0.06$ $p = 0.73$	$r = -0.12$ $p = 0.46$	$r = -0.15$ $p = 0.38$	$r = 0.04$ $p = 0.80$	$r = -0.20$ $p = 0.23$	$r = -0.10$ $p = 0.55$
	LBM (kg)	$r = -0.06$ $p = 0.72$	$r = 0.19$ $p = 0.25$	$r = 0.01$ $p = 0.94$	$r = 0.14$ $p = 0.41$	$r = 0.04$ $p = 0.82$	$r = -0.28$ $p = 0.09$	$r = 0.11$ $p = 0.52$
	SSM (kg)	$r = 0.16$ $p = 0.34$	$r = 0.18$ $p = 0.29$	$r = -0.03$ $p = 0.86$	$r = 0.20$ $p = 0.23$	$r = 0.11$ $p = 0.49$	$r = -0.20$ $p = 0.22$	$r = 0.18$ $p = 0.28$
	%BF	$r = 0.24$ $p = 0.15$	$r = -0.08$ $p = 0.65$	**$r = -0.36$** **$p = 0.03$***	$r = 0.00$ $p = 0.99$	$r = 0.11$ $p = 0.51$	$r = -0.17$ $p = 0.30$	$r = -0.11$ $p = 0.53$
	FM (kg)	$r = 0.19$ $p = 0.25$	$r = -0.01$ $p = 0.94$	$r = -0.24$ $p = 0.14$	$r = -0.03$ $p = 0.84$	$r = 0.22$ $p = 0.19$	$r = -0.16$ $p = 0.35$	$r = -0.03$ $p = 0.85$
Competitive season	Body mass (kg)	$r = -0.01$ $p = 0.94$	$r = 0.00$ $p = 0.99$	$r = 0.19$ $p = 0.26$	$r = -0.12$ $p = 0.49$	$r = -0.06$ $p = 0.72$	$r = -0.13$ $p = 0.42$	$r = 0.02$ $p = 0.91$
	BMI (kg/m^2)	$r = 0.01$ $p = 0.95$	$r = -0.01$ $p = 0.96$	$r = 0.18$ $p = 0.27$	$r = -0.10$ $p = 0.55$	$r = -0.06$ $p = 0.72$	$r = -0.13$ $p = 0.45$	$r = 0.02$ $p = 0.90$
	LBM (kg)	$r = -0.17$ $p = 0.31$	$r = -0.05$ $p = 0.79$	$r = 0.13$ $p = 0.43$	**$r = -0.46$** **$p = 0.004$***	$r = -0.13$ $p = 0.43$	$r = -0.23$ $p = 0.16$	$r = -0.20$ $p = 0.23$
	SSM (kg)	$r = -0.13$ $p = 0.45$	$r = -0.13$ $p = 0.45$	$r = 0.06$ $p = 0.73$	**$r = -0.36$** **$p = 0.03$***	$r = -0.13$ $p = 0.45$	$r = -0.28$ $p = 0.08$	$r = -0.22$ $p = 0.19$
	%BF	$r = -0.11$ $p = 0.49$	$r = -0.06$ $p = 0.73$	$r = 0.03$ $p = 0.88$	$r = -0.11$ $p = 0.50$	$r = -0.24$ $p = 0.15$	$r = -0.17$ $p = 0.32$	$r = -0.12$ $p = 0.46$
	FM (kg)	$r = -0.09$ $p = 0.6$	$r = -0.04$ $p = 0.82$	$r = 0.08$ $p = 0.61$	$r = -0.08$ $p = 0.65$	$r = -0.20$ $p = 0.22$	$r = -0.08$ $p = 0.63$	$r = -0.06$ $p = 0.70$
Transition period	Body mass (kg)	**$r = -0.47$** **$p = 0.00$*****	$r = -0.26$ $p = 0.11$	$r = -0.09$ $p = 0.60$	$r = -0.08$ $p = 0.63$	$r = 0.12$ $p = 0.46$	$r = 0.21$ $p = 0.22$	$r = -0.16$ $p = 0.32$
	BMI (kg/m^2)	**$r = -0.48$** **$p = 0.00$*****	$r = -0.28$ $p = 0.09$	$r = -0.10$ $p = 0.56$	$r = -0.08$ $p = 0.65$	$r = 0.13$ $p = 0.45$	$r = 0.19$ $p = 0.25$	$r = -0.17$ $p = 0.29$
	LBM (kg)	$r = -0.17$ $p = 0.31$	$r = 0.07$ $p = 0.67$	$r = 0.30$ $p = 0.07$	$r = 0.04$ $p = 0.80$	$r = 0.00$ $p = 1.00$	$r = 0.15$ $p = 0.38$	$r = 0.17$ $p = 0.29$

(Continued)

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TABLE 2 (Continued)

Variable	Nutrition knowledge sub-section	Weight management 0–12 points	Macronutrients 0–30 points	Micronutrients 0–13 points	Sport nutrition 0–12 points	Supplements 0–12 points	Alcohol 0–8 points	Total 0–87 points
SSM (kg)		$r = -0.17$	$\mathbf{r = -0.33}$	$r = -0.12$	$r = -0.10$	$r = -0.02$	$r = 0.09$	$r = -0.19$
		$p = 0.32$	$\mathbf{p = 0.05}$*	$p = 0.46$	$p = 0.57$	$p = 0.89$	$p = 0.59$	$p = 0.25$
%BF		$r = 0.03$	$r = 0.06$	$r = 0.14$	$r = 0.17$	$r = 0.21$	$\mathbf{r = -0.48}$	$r = 0.29$
		$p = 0.86$	$p = 0.71$	$p = 0.4$	$p = 0.30$	$p = 0.21$	$\mathbf{p = 0.00}$***	$p = 0.08$
FM (kg)		$r = -0.10$	$r = -0.07$	$r = 0.15$	$r = 0.06$	$r = 0.09$	$r = 0.23$	$r = 0.11$
		$p = 0.56$	$p = 0.66$	$p = 0.36$	$p = 0.72$	$p = 0.61$	$p = 0.10$	$p = 0.52$

LBM, lean body mass; SMM, skeletal muscle mass; %BF, percentage of body fat; FM, fat mass.
* = $p < 0.05$; *** = $p < 0.001$; r = Spearman's R' correlation. The bolding is to emphasize statistical significance.

composition during the competitive period in current research showed a decrease in lean body mass from 71.68 ± 5.85 kg to 71.14 ± 5.93 kg and an increase in body fat from 9.76 ± 2.57% to 10.32 ± 2.77%.

In the available literature, researchers have obtained inconclusive results regarding changes in body composition during the competitive period. In a study by Carling et al., lean body mass increased during this period, while fat content decreased (46). In the study by Kultu et al. (47) a reduction in lean body mass and a reduction in body fat were verified. Devlin et al. obtained results consistent with their own, lean body mass was reduced while body fat increased during the competitive period (48). During the preparatory period, training programmes include general training and high-intensity conditioning training, while a significant number of training sessions during the competitive period are related to game tactics, ball possession, and set pieces, which are characterized by less strain. These results suggest that match effort alone is insufficient as an incentive to maintain a consistent body weight of a player (48).

The transition period is characterized by complete cessation or significant reduction of training. Increased body mass resulting from an inappropriate diet affects the performance and efficiency of an athlete. Reduced muscle mass due to lack of training stimulus may result in decreased strength and endurance and thus an increased risk of injury when numerous intensive training units are reintroduced during the preparatory period (14). During the transition period in current research, the number of athletes with a BMI interpreted as overweight increased at the end of the transition period. 26.3% ($N = 10$) of the players were overweight, according to the interpretation of BMI. When analyzing body fat percentage, it was found that most athletes ($N = 21$) had normative body fat at the end of the transition period amongst all measurements analyzed. The transition period is the only period in the analyzed macrocycle when differences in body fat percentage are reflected by the BMI value. It is related to the limitation of physical activity and changes in the composition of the body weight typical of people with low physical activity. This study showed an increase in body weight in the transition period from an average of 79.27 ± 7.44 kg to 80.13 ± 7.47 kg. Similar results apply to the BMI values, which increased from an average of 23.74 ± 1.27 to 24 ± 1.26. Body composition analysis showed a reduction in lean body mass during the transition period and an increase in body fat percentage and body fat mass between the 4th and 6th measurements, i.e., from the second half of the athletic period to the end of the transition period. A study by Reinke et al. showed a reduction in body weight, lean body mass and an increase in body fat in the football players participating in the study (49). Ostojic et al. (50) results are in line with our own.

The authors' study also assessed the nutrition knowledge of professional football athletes; the NSKQ questionnaire was used for the assessment. This study showed that the use of this questionnaire in team sports athletes can be an important tool for screening athletes, requiring additional educational support (34). The athletes in the current study obtained an average of 51.49% of correct answers, and their nutritional awareness was rated as average. The athletes' nutrition knowledge of micronutrients and supplementation was at a poor level, while their nutrition knowledge of weight control, macronutrients, sports nutrition, and alcohol was at an average level.

The NSKQ questionnaire has been used to assess athletes' nutritional awareness by other researchers. A study by Jenner et al. (51) assessed the nutrition knowledge of 46 Australian professional football players. On average, the players obtained 46% of correct answers, and the overall nutrition knowledge of the study group was poor (51). O'Brien et al. analyzed the nutritional awareness of Gaelic football players. On average, the athletes obtained 47.6% of correct answers, and their knowledge was rated as poor (52). A study by Jagim et al. (53) analyzed body composition, nutrition knowledge, and ability to estimate macronutrient and energy requirements of 67 university athletes, including football players. The athletes answered 47.9% of the questions correctly on average, and the knowledge of the study group was rated as poor. In addition, the athletes did not correctly estimate energy and carbohydrate requirements according to their needs. In the above-mentioned study, it was established that the athletes with higher knowledge of the subsection of sports nutrition had lower body fat percentage and body fat mass. Accordingly, athletes with higher levels of sports nutrition knowledge may be better able to adjust macronutrient and energy requirements, thus maintaining desired body composition (53).

In the final stage of the author's study, the contribution of athletes' nutrition knowledge to the maintenance of constant body composition parameters during the preparatory, competitive, and transition periods was determined. The relationship between nutrition knowledge and body composition stability was demonstrated during the competitive period. Higher knowledge of the subsection of sports nutrition corresponded to lower dispersion of lean body mass content ($p = 0.004$), and skeletal muscle mass ($p = 0.03$). The above correlations suggest that athletes with higher nutritional awareness regarding sports nutrition during the competitive period are characterized by less muscle mass loss. During the competitive period, the body composition of the players is disintegrated, the muscle mass is reduced, while the fat tissue content increases, which is a highly unfavorable phenomenon due to the exercise capacity and sport form of athletes. Higher nutritional awareness in individual categories of nutrition knowledge results in the reduction of negative changes in the composition of the body weight during the competitive period. Correlations between nutrition knowledge and body composition stability were demonstrated during the transition period. Higher nutritional awareness of athletes from the weight control subsection corresponded to lower dispersion of body mass ($p = 0.00$) and BMI values ($p = 0.00$). Higher nutrition knowledge on macronutrients was associated with lower dispersion of skeletal muscle mass content ($p = 0.05$), while higher alcohol awareness was associated with lower dispersion of body fat percentage ($p = 0.00$). The described relationships prove that players with higher nutritional awareness of weight control are characterized by a lower increase in body weight and BMI value during the transition period. Athletes who are more knowledgeable about macronutrients in their diets are characterized by lower reductions in muscle mass and footballers with higher awareness of alcohol consumption—with a lower increase in body fat percentage.

The results of this study suggest that football players with higher nutritional knowledge are able to better manage body composition. During the preparatory period, they have better results regarding increasing lean body mass and reducing body fat, which has a positive effect on exercise capacity. During the competitive period, they are characterized by a smaller reduction in lean body mass, which can consequently contribute to a reduced risk of injury. In the transition period, they are characterized by a smaller increase in body mass and better maintain a constant body composition, which affects a quicker return to top athletic form after a period of reduced training intensity. The above results indicate the relevance of the level of nutritional knowledge in football players. Improving the nutrition knowledge of professional football players may provide benefits in terms of better management and maintenance of body composition during particular periods of the macrocycle. No studies were found in the available literature on this issue, so it may be of comparable value.

Strengths and limitations

The strengths of the study include the fact that despite the popularity of football, there are still few scientific studies analyzing changes in football players' body weight throughout the entire training macrocycle. Typically, body weight composition is assessed over the preparatory, competitive, or transition period, as well as individual measurements, are analyzed, as evidenced by the discussion of the results. Furthermore, in many reports, the study group consists of amateur or semi-professional football players whose training intensity and frequency, match requirements, daily physical activity, as well as salary are different from those of professional players. This study analyzed the results obtained from professional football players of the highest football league in Poland. Assessing the nutrition knowledge of football players is essential to orient education based on the athletes' knowledge insufficiency. Moreover, it can also be useful for screening assessments to identify players who require additional educational support. In the current research, a standardized questionnaire was used for this purpose, which, due to its structure, allows the targeting of educational activities. The results described can provide comparative value with professional athletes practicing other sports. The bibliometric analysis of GoogleScholar indicates that this is the first study analyzing the correlation between nutrition knowledge and changes in body composition in football players (as of 14 May 2022).

One of the limitations of the conducted research is the size of the study group. However, it should be emphasized that many exclusion criteria were defined, one of which was the 14-day exclusion from training related to quarantine or isolation due to COVID-19, which was common in the 2020/2021 football season. The study included players from two football clubs, and the limitation of the study is the lack of representativeness of players from other clubs and other sports disciplines. It is worth emphasizing, however, that each discipline is characterized by a different training macrocycle and a different intensity of matches, which may pose a problem in comparing them reliably. However, it is worth considering such an attempt and such studies are planned by the authors. A control group of non-football players was not included in the study because the type of questions asked would not allow comparisons to be made between such groups.

However, it should be highlighted that prediction equations based on BIA are instrument-dependent, and instrument sensitivities are variable. Therefore, comparisons cannot be made between studies that measure bioimpedance using different technologies (e.g., foot-to-hand or direct segmental measurement in the standing position) or sampling rates. However, it should be pointed out that the authors made every effort to minimize the systematic error in the study (54, 55).

Conclusion

Based on the results obtained, changes in the composition of the body weight of players were found, regardless of their function on the field in the preparatory, competitive, and transition periods of the PKO BP Ekstraklasa 2020/2021 spring round and their nutrition knowledge was average, according to the adopted classification. BMI did not allow the identification of changes in the composition of the mass (lean and adipose tissue). The applied BIA method more accurately reflected the changes in the body composition of the players in the analyzed periods. The body composition of the athletes, taking into account the spring season periods, underwent significant modifications. During the preparatory period, the mass of skeletal muscles increased, but the mass of fat tissue was stable. During the competitive period, the lean body mass decreased, but the content of fat tissue increased. During the transition period, the content of fat tissue increased in the study group. Correlations were found between elements of nutrition knowledge of athletes and stability of their body composition during all analyzed periods: preparatory, competitive, and transition periods.

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 human participants were reviewed and approved by Bioethics Committee of the Silesian Medical University in Katowice. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization and writing—original draft preparation: WS and EG-N. Methodology: WS, EG-N, and AB-D. Software: MG. Validation: GZ, MK, and SJ-S. Formal analysis: GZ and WS. Investigation: MK. Resources and supervision: OK. Data curation: GZ. Writing—review and editing: AB-D. Visualization: SJ-S. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

References

1. Dvorak J, Junge A, Graf-Baumann T, Peterson L. Football is the most popular sport worldwide. *Am J Sports Med.* (2004) 32:3S—4S. doi: 10.1177/0363546503262283

2. Portes LA, Canhadas IL, Silva RLP, de Oliveira NC. Anthropometry and fitness of young elite soccer players by field position. *Sport Sci Health.* (2015) 11:321–8. doi: 10.1007/s11332-015-0243-z

3. Atakan MM, Unver E, Demirci N, Cinemre A, Bulut S, Turnagol HH. Effect of body composition on fitness performance in young male football players. *Turk J Sport Exer.* (2017) 19:54–9.

4. Leão C, Silva AF, Badicu G, Clemente FM, Carvutto R, Greco G, et al. Body composition interactions with physical fitness: a cross-sectional study in youth soccer players. *Int J Environ Res Public Health.* (2022) 19:3598. doi: 10.3390/ijerph19063598

5. Mukherjee S, Chia M. Within-season variation in the body composition of Asian youth professional soccer players. *Sport Sci.* (2010) 3:15–22.

6. Bunc V, Hráský P, Skalska M. Changes in body composition, during the season, in highly trained soccer players. *Open Sports Sci J.* (2015) 8:21–4. doi: 10.2174/1875399X01508010018

7. Shinkaruk OA. The concept of forming a system of training, selection of athletes and their improvement. *Pedagog Psychol Med Biol Probl Phys Train Sports.* (2012) 12:144–8.

8. Bloomfield J, Polman R, O'Donoghue P. Physical demands of different positions in FA premier league soccer. *J Sport Sci Med.* (2007) 6:63–70.

9. Buchheit M, Racinais S, Bilsborough JC, Bourdon PC, Voss SC, Hocking J, et al. Monitoring fitness, fatigue and running performance during a pre-season training camp in elite football players. *J Sci Med Sport.* (2013) 16:550–5. doi: 10.1016/j.jsams.2012.12.003

10. Jeong TS, Reilly T, Morton J, Bae SW, Drust B. Quantification of the physiological loading of one week of "pre-season" and one week of "in-season" training in professional soccer players. *J Sports Sci.* (2011) 29:1161–6. doi: 10.1080/02640414.2011.583671

11. Clemente FM, Seerden G, Linden CM. Quantifying the physical loading of five weeks of pre-season training in professional soccer teams from Dutch and Portuguese leagues. *Physiol Behav.* (2019) 209:112588. doi: 10.1016/j.physbeh.2019.112588

12. Michaelides MA, Parpa KM, Zacharia AI. Effects of an 8-week pre-seasonal training on the aerobic fitness of professional soccer players. *J Strength Cond Res.* (2021) 35:2783–9.

13. Anderson L, Orme P, Di Michele R, Close GL, Morgans R, Drust B, et al. Quantification of training load during one-, two- and three-game week schedules in professional soccer players from the English premier league: implications for carbohydrate periodization. *J Sports Sci.* (2016) 34:1250–9. doi: 10.1080/02640414.2015.1106574

14. Silva JR, Brito J, Akenhead R, Nassis GP. The transition period in soccer: a window of opportunity. *Sports Med.* (2016) 46:305–13. doi: 10.1007/s40279-015-0419-3

15. Reilly T, Ekblom B. The use of recovery methods post-exercise. *J Sports Sci.* (2005) 23:619–27. doi: 10.1080/02640410400021302

16. Kraemer WJ, French DN, Paxton NJ, Häkkinen K, Volek JS, Sebastianelli WJ, et al. Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. *J Strength Cond Res.* (2004) 18:121–8. doi: 10.1519/00124278-200402000-00018

17. Melchiorri G, Ronconi M, Triossi T, Viero V, Sanctis DD, Tanceredi V, et al. Detraining in young soccer players. *J Sports Med Phys Fitness.* (2014) 54:27–33.

18. Tessitore A, Meeusen R, Cortis C, Capranica L. Effects of different recovery interventions on anaerobic performances following pre-season soccer training. *J Strength Cond Res.* (2007) 21:745–50. doi: 10.1519/00124278-200708000-00015

19. Bangsbo J, Iaia FM, Krustrup P. Metabolic response and fatigue in soccer. *Int J Sports Physiol Perform.* (2007) 2:111–27. doi: 10.1123/ijspp.2.2.111

20. Owen AL, Lago-Peñas C, Dunlop G, Mehdi R, Chtara M, Dellal A. Seasonal body composition variation amongst elite European professional soccer players: an approach of talent identification. *J Hum Kinet.* (2018) 62:177–84. doi: 10.1515/hukin-2017-0132

21. Mascherini G, Gatterer H, Lukaski H, Burtscher M, Galanti G. Changes in hydration, body-cell mass and endurance performance of professional soccer players through a competitive season. *J Sports Med Phys Fitness.* (2015) 55:749–55.

22. Mukherjee S, Chia M. Within-season variation in the body composition of asian youth professional soccer players. *Sport Sci.* (2010) 3:15–22.

23. Devlin BL, Leveritt MD, Kingsley M, Belski R. Dietary intake, body composition, and nutrition knowledge of australian football and soccer players: implications for sports nutrition professionals in practice. *Int J Sport Nutr Exerc Metab.* (2017) 27:130–8. doi: 10.1123/ijsnem.2016-0191

24. Lohman R, Carr A, Condo D. Nutritional intake in australian football players: sports nutrition knowledge and macro-nutrient and micronutrient intake. *Int J Sport Nutr Exerc Metab.* (2019) 29:289–96. doi: 10.1123/ijsnem.2018-0031

25. Francescato MP, Venuto I, Buoite Stella, Stel G, Mallardi F, Cauci S. Sex differences in hydration status among adolescent elite soccer players. *J Hum Sport Exerc.* (2019) 14:265–280. doi: 10.14198/jhse.2019.142.02

26. Denna I, Elmabsout A, Eltuhami A, Alagory S, Alfirjani T, Barakat F, et al. Evaluation of nutrition knowledge of professional football players. *Ibnosina J Med Biomed Sci.* (2018) 10:21–4. doi: 10.4103/ijmbs.ijmbs_34_17

27. World Health Organization Regional Office for Europe. Body mass index – BMI. Available online at: https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi (accessed June 07, 2022).

28. Kotler DP, Burastero S, Wang J, Pierson RN Jr. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *Am J Clin Nutr.* (1996) 64:489S—97S. doi: 10.1093/ajcn/64.3.489S

29. InBody Poland. InBody 770. Available online at: https://inbodypoland.pl/produkty/inbody-770/ (accessed June 07, 2022).

30. Yi Y, Baek JY, Lee E, Jung HW, Jang IY. A comparative study of high-frequency bioelectrical impedance analysis and dual-energy X-ray absorptiometry for estimating body composition. *Life.* (2022) 12:994. doi: 10.3390/life12070994

31. Blue M, Hirsch K, Brewer G, Cabre H, Gould L, Tinsley G, et al. The validation of contemporary body composition methods in various races and ethnicities. *Br J Nutri.* (2022) 3:1–11. doi: 10.1017/S0007114522000368

32. Antonio J, Kenyon M, Ellerbroek A, Carson C, Burgess V, Tyler-Palmer D, et al. Comparison of dual-energy X-ray absorptiometry (DXA) vs. a multi-frequency bioelectrical impedance (InBody 770) device for body composition assessment after a 4-week hypoenergetic diet. *J Funct Morphol Kinesiol.* (2019) 4:23. doi: 10.3390/jfmk4020023

33. McLester CN, Nickerson BS, Kliszczewicz BM, McLester JR. Reliability and agreement of various in body composition analysers as compared to dual-energy X-ray absorptiometry in healthy men and women. *J Clin Densitom.* (2020) 23:443–50. doi: 10.1016/j.jocd.2018.10.008

34. Trakman GL, Forsyth A, Hoye R, Belski R. Modifications to the nutrition for sport knowledge questionnaire (NSQK) and abridged nutrition for sport knowledge questionnaire (ANSKQ). *J Int Sci Sports Nutr.* (2019) 16:1–3. doi: 10.1186/s12970-019-0293-8

35. Trakman GL, Forsyth A, Hoye R, Belski R. The nutrition for sport knowledge questionnaire (NSKQ): development and validation using classical test theory and Rasch analysis. *J Int Sci Sports Nutr.* (2017) 14:1–11. doi: 10.1186/s12970-017-0182-y

36. Ling CH, Craen de. Slagboom PE, Gunn DA, Stokkel MPM, Westendrop RGJ, et al. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin Nutr.* (2011) 30:610–5. doi: 10.1016/j.clnu.2011.04.001

37. Kopeć A, Nowacka E, Klaja A, Leszczyńska T. Assessment of selected food frequency intake in football players. *Probl Hig Epidemiol.* (2013) 94:151–7.

38. Petri C, Mascherini G, Pengue L, Galanti G. Dietary habits in elite soccer players. *Sport Sci Health.* (2016) 12:113–9. doi: 10.1007/s11332-016-0264-2

39. Spehnjak M, Gušić M, Molnar S, Baić M, Andrašić S, Selimi M. Body composition in elite soccer players from youth to senior squad. *Int J Environ Res Public Health.* (2021) 18:4982. doi: 10.3390/ijerph18094982

40. Sylejmani B, Maliqi A, Gontarev S, Haziri S, Morina B, Durmishaj E, Bajrami A. Anthropometric characteristics and physical performance of young elite Kosovo soccer players. *Int J Morphol.* (2019) 37:1429–36. doi: 10.4067/S0717-95022019000401429

41. Sutton L, Scott M, Wallace J, Reilly T. Body composition of English premier league soccer players: influence of playing position, international status, and ethnicity. *J Sports Sci.* (2009) 27:1019–26. doi: 10.1080/02640410903030305

42. Boone J, Vaeyens R, Steyaert A, Vanden Bossche L, Bourgois J. Physical fitness of elite Belgian soccer players by player position. *J Strength Cond Res.* (2012) 26:2051–7. doi: 10.1519/JSC.0b013e318239f84f

43. Abbott W, Brickley G, Smeeton NJ. Physical demands of playing position within English premier league academy soccer. *J Hum Sport Exerc*. (2018) 13:285–95. doi: 10.14198/jhse.2018.132.04

44. McEwan GP, Drobnic F, Lizarraga A, Gómez Díazd A, Pons E, Dello Iacon A, et al. Changes in markers of body composition of professional male soccer players during pre-season. *Sports Med Health Sci.* (2020) 2:166–71. doi: 10.1016/j.smhs.2020.08.004

45. Caldwell BP, Peters DM. Seasonal variation in physiological fitness of a semiprofessional soccer team. *J Strength Cond Res.* (2009) 23:1370–7. doi: 10.1519/JSC.0b013e3181a4e82f

46. Carling C, Orhant E. Variation in body composition in professional soccer players: interseasonal and intraseasonal changes and the effects of exposure time and player position. *J Strenght Cond Res.* (2010) 24:1332–9. doi: 10.1519/JSC.0b013e3181cc6154

47. Kultu M, Sofi N, Bozkus T. Changes in body compositions of elite level amateur and professional soccer players during the competitive season. *Sport Hekimligi Dergisi.* (2007) 42:21–5.

48. Devlin BL, Kingsley M, Leveritt MD, Belski R. Seasonal changes in Soccer players' body composition and dietary intake practices. *J Strength Cond Res*. (2017) 31:3319–26. doi: 10.1519/JSC.0000000000001751

49. Reinke S, Karhausen T, Doehner W, Taylor W, Hottenrott K, Duda GN, et al. The influence of recovery and training phases on body composition, peripheral vascular function and immune system of professional soccer players. *PLoS ONE.* (2009) 4:e4910. doi: 10.1371/journal.pone.0004910

50. Ostoji SM. Seasonal alterations in body composition and sprint performance of elite soccer players. *J Am Soc Exerc Physiol.* (2003) 6:24–7.

51. Jenner SL, Trakman G, Coutts A, Kempton T, Ryan S, Forsyth A, et al. Dietary intake of professional Australian football athletes surrounding body composition assessment. *J Int Soc Sports Nutr.* (2018) 15:43. doi: 10.1186/s12970-018-0248-5

52. O'Brien L, Collins K, Amirabdollhian F. Exploring sports nutrition knowledge in elite gaelic footballers. *Nutrients.* (2021) 13:1081. doi: 10.3390/nu13041081

53. Jagim AR, Fields JB, Magee M, Kerksick C, Luedke J, Erickson J, et al. The influence of sport nutrition knowledge on body composition and perceptions of dietary requirements in collegiate athletes. *Nutrients.* (2021) 13:2239. doi: 10.3390/nu13072239

54. Campa F, Bongiovanni T, Trecroci A, Rossi A, Greco G, Pasta G, et al. Effects of the COVID-19 lockdown on body composition and bioelectrical phase angle in Serie A soccer players: a comparison of two consecutive seasons. *Biology.* (2021) 10:1175. doi: 10.3390/biology10111175

55. Campa F, Toselli S, Mazzilli M, Gobbo LA, Coratella G. Assessment of body composition in athletes: a narrative review of available methods with special reference to quantitative and qualitative bioimpedance analysis. *Nutrients*. (2021) 13:1620. doi: 10.3390/nu13051620

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Turmeric supplementation improves markers of recovery in elite male footballers: a pilot study

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Football match-play causes muscle damage and provokes an inflammatory response. Rapid recovery is paramount to optimising subsequent performance and reducing injury risk. Turmeric contains high concentrations of curcumin, a polyphenol that has been shown to reduce muscle damage and soreness post-exercise in recreational exercisers. However, it is unknown whether a curcumin-containing supplement can support elite footballers recovery between matches. This applied study explored whether a turmeric supplement could improve performance, subjective and physiological markers of recovery, in elite male footballers. Twenty-four elite male footballers divided into a turmeric group, who consumed 60mL of a turmeric drink twice per day, or a control group who did not. After 96 h of rest, baseline measurements of subjective soreness (leg and whole-body), plasma creatine kinase ([CK]), plasma C-reactive protein ([CRP]), isometric mid-thigh pull (IMTP) and counter movement jump (CMJ), were collected. Following eight competitive matches, subjective leg and whole-body soreness and plasma concentrations of inflammation markers ([CK] and [CRP]) were assessed immediately (0h), 40 and 64h post-match. Performance markers (IMTP and CMJ) were also assessed at 40 and 64h post-match. Percentage change from baseline showed a main effect of group (p=0.035, p=0.005) and time (p=0.002, p=0.002) for both leg and whole-body soreness, respectively. There was a group by time interaction effect (p=0.049) for [CRP]. There were no effects of turmeric on [CK], CMJ or IMTP. This applied study is the first in elite footballers to show that a curcumin-containing supplementation may attenuate a biomarker of inflammation [CRP] and soreness post-match play.

KEYWORDS
curcumin, polyphenols, elite athlete, recovery, football (soccer), inflamation

Introduction

Prolonged, high-intensity exercise causes muscle damage and produces reactive oxygen species, provoking an inflammatory response and altering cell function (1). Whilst inflammation is a necessary part of the tissue repair process (1), this often leads to muscular discomfort and a subsequent reduction in the ability to generate force (2). Following muscle damaging exercise, limitations of the endogenous antioxidant system to remove free-radicals delays recovery and reduces subsequent exercise performance in a time-course manner often referred to as delayed onset of muscle soreness (DOMS) (2).

Professional footballers in the UK are subject to a high number of competitive matches (>50 per season) and often short intervals between matches (48–72 h), which curtails recovery time and increases the risk of injury or sub-optimal performance (3). Football match-play is known to cause a significant increase in muscle damage and soreness up to 72 h post-match (4, 5), likely due to the high magnitude eccentric muscle loading (6). Professional footballers often use non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate DOMS and restore muscle function (7). However, concerns about the long-term use of NSAIDs and reported side effects, such as reduced muscle regeneration (7), and gastrointestinal problems (8) necessitates the need for alternative solutions (7).

Supplementation of certain dietary compounds may aid recovery by reducing inflammation, alleviating DOMS and restoring muscle function (9). Curcumin, a natural polyphenol found in high concentrations in turmeric, has anti-inflammatory, antioxidant, and analgesic properties, making it a candidate to accelerate post-exercise recovery. Laboratory-based studies of cycling, running and eccentric loading protocols (e.g., downhill running, eccentric resistance exercises), have shown that curcumin supplementation before and after an exercise period can reduce subjective soreness (10, 11), attenuate haematological inflammatory markers (11), and improve subsequent exercise performance (12, 13). However, there is an absence of applied studies in elite cohorts, likely due, in part, to challenges associated with conducting research in elite cohorts. One study, in elite rugby players, found that curcumin attenuated muscle damage and limited loss of muscle function after a muscle damaging protocol (12), while one other study in youth team male footballers found that curcumin attenuated DOMS and loss of muscle function after match-play (14). However, no study has explored whether a curcumin supplement can accelerate recovery from match-play in elite-level footballers. Elite sport is unique in terms of the playing intensity and specific contextual factors such as athlete skill level, psychological pressure and playing environment (crowd, score line, etc.), which make it hard to replicate in laboratory-based studies and/or with non-elite participants (15).

Whilst early findings are encouraging (15), whether curcumin can accelerate recovery and more rapidly restore muscle function in elite athletes is not well known. The aim of this study was to assesses if regular turmeric supplementation could accelerate markers of recovery, including, haematological inflammatory markers, subjective soreness, and performance, in elite male footballers following match-play. Participants consumed a commercially available turmeric supplement, formulated to contain 35 g of raw turmeric root with an estimated curcumin content of 1,400 mg (16), twice a day throughout the course of the study.

Methods

Participants

Twenty-four elite [Tier 4; (17)] male professional footballers (with 7.5 ± 3.7y experience as a professional footballer and undertaking 3–4 training sessions per week), competing in the English third tier during the 2020/21 season, volunteered to participate in the study. To qualify for enrolment, participants were required to be outfield players with no history of cardiovascular or gastrointestinal complaint and were not regularly consuming any supplements containing turmeric or curcumin. All participants provided written consent after they were informed verbally and in writing of the nature and requirements of the study. The study was approved by the Nottingham Trent University Human Invasive Ethics Committee (REF: 716).

Study design and measures

The study adopted a between-groups design. Baseline measures of subjective soreness (whole-body and leg-specific), performance [counter movement jump (CMJ) and isometric mid-thigh pull (IMTP)] and concentration of haematological markers of inflammation [C-reactive protein ([CRP]) and creatine kinase ([CK]) were collected after 96 h of rest. Following this, participants self-selected to a supplementation or control group. The supplementation group (n = 16; Age = 26 ± 3 y; Height = 1.83 ± 0.06 m; Mass = 80.2 ± 5.0 kg) consumed two 60 mL drinks per day (Raw Turmeric Original Shot, The Turmeric Co., UK)], each containing 35 g of raw turmeric root (estimated to contain 1,400 mg curcumin) and 200 mg of black pepper (estimated to contain 10 mg of piperine), throughout the course of the study. Compliance with the supplementation was verbally confirmed by participants prior to each match. The control group (n = 8, Age = 25 ± 4 y; Height = 1.82 ± 0.07 m; Mass = 79.5 ± 6.3 kg) were not provided with the supplement and were asked to avoid turmeric-containing supplements during the study. Participants were monitored during eight competitive matches taking place between October 2021 and April 2022 using Global Positing System (Vector, Catapult, Australia) for commonly used physical performance measures (total distance, high speed distance, accelerations, deceleration). There were no differences in GPS derived physical performance measures between the turmeric group and the control group (all p values <0.29; for data see Supplementary File 1). Participants were assessed immediately after each match for measures of subjective soreness and inflammation, and these were assessed again, along with performance measures, 40 h and 64 h following each match.

Procedures

Capillary blood samples (300 µL) were collected into EDTA collection tubes (Microvette, Sarstedt, Germany). Post-match samples were collected where the match took place, with follow-up samples collected on arrival at the training facility. Samples were stored on ice after collection (maximum of 30 min) before being centrifuged (13,000 g, 10 min, 4°C), and the resulting plasma being stored at −80°C. Plasma samples were analysed for [CK] and [CRP] using an ABX Pentra 400 (Horiba Medical, Kyoto, Japan; CV: 3.2%).

Subjective whole-body and leg-specific soreness were assessed on 100 mm visual analogue scales, anchored at 0 mm with ‘no pain’ and 100 mm with ‘as much pain as it could be’ (4, 18). Participants were asked to apply a mark on the line to indicate their level of soreness, which was then quantified with a ruler.

CMJ was assessed by participants standing in an upright position with their hands on their hips, squatting to their preferred depth, before jumping as high as possible whilst standing on a force plate (Hawkin Dynamics, ME, United States). Jump height was calculated

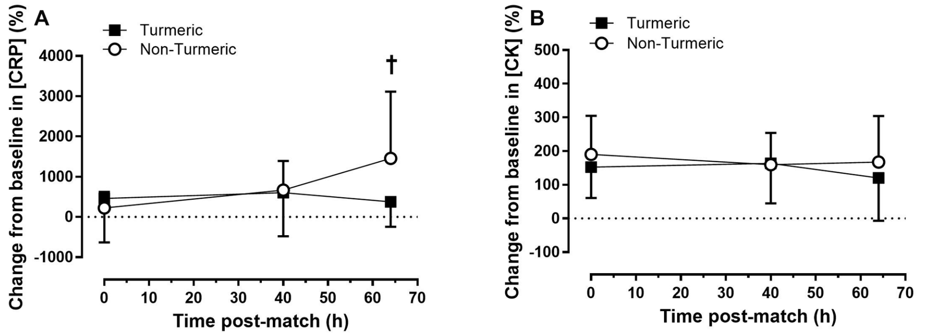

FIGURE 1
Plasma concentrations of c-reactive protein **(A)** and creatine kinase **(B)** immediately (0h), 40h and 64h post-match, in the turmeric (solid square) and non-turmeric (open circle) groups. † indicates a difference between groups at this time-point (p<0.05); Data are presented as percent change from baseline. Values are means with error bars representing standard error.

using the following equation: Jump Height = Take Off Velocity^2/2 x Gravity (Moir 2008). CMJs have been shown to be reliable [ICCs 0.96–0.97; (19)]. Participants prepared with an individualised warm-up followed by three jumps, with the highest jump recorded and used in subsequent analysis. Verbal encouragement was provided by the investigators for each jump.

The IMTP test was performed on two force platforms sampling at 1000 HZ (Hawkin Dynamics, ME, United States) and a test rack. The IMTP has been shown to be a reliable test [ICCs 0.89–0.98 (20)]. Participants used a standardised overhand grip while secured to the barbell with lifting straps to eliminate the influence of grip strength. Warm-up trials were performed prior to completing three maximal lifts of 3 s per lift. Verbal encouragement was provided by the investigators for each lift. Peak force and percentage change in peak force were used for the statistical analysis.

GPS

The physical demands for match-play were monitored using a 10 Hz GPS (Vector, Catapult, Australia). This system has been valid and reliable to assess athlete movements (21). Each player wore a bespoke garment containing a GPS unit positioned between the shoulder blades. Post-session, each GPS unit was downloaded and analysed using commercially available software (Viper, STATSports, Ireland). The physical performance variables assessed included: total distance covered (m), high speed (>5.5 m/s) distance covered (m), very high speed (>7.0 m/s) distance covered (m), number of accelerations above 0.5 m/s^2 for >0.5 s, and number of decelerations below −0.5 m/s^2 for >0.5 s (Supplementary Table 1).

Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) were conducted on each recovery marker with percentage change from baseline serving as the dependent variable and time and supplementation group as the independent variables. Where appropriate, significant main effects were followed-up with Bonferroni-corrected post-hoc tests. Statistical significance was accepted at the 95% confidence level ($p < 0.05$). Mean and standard error were used to describe the average and variability of data, unless stated otherwise. Partial eta squared statistics were used to indicate effect sizes and were interpreted as small (0.01–0.05), medium (0.06–0.13), and large (≥0.14) (22). Analyses were conducted using IBM SPSS Statistics (v.28).

Results

Markers of inflammation

There was no main effect of time (p = 0.08, $\eta^2_p = 0.034$) or group ($p = 0.13$, $\eta^2_p = 0.015$) for percentage change in plasma [CRP] but there was a significant group by time interaction ($p = 0.049$, $\eta^2_p = 0.040$; see Figure 1A). At 64 h post-match, [CRP] was 1,082 ± 447% lower in the turmeric group compared to the control group ($p = 0.017$). There was no main effect of time ($p = 0.64$, $\eta^2_p = 0.005$) or group ($p = 0.23$, $\eta^2_p = 0.008$), and no group by time interaction ($p = 0.56$, $\eta^2_p = 0.006$) for percentage change in plasma [CK] (see Figure 1B).

Subjective markers of soreness

The percentage change in leg soreness was 77 ± 36% lower overall in the turmeric group compared to the control group ($p = 0.035$, $\eta^2_p = 0.022$). There was also a main effect of time ($p = 0.002$, $\eta^2_p = 0.06$), for percentage change in leg soreness where post-hoc analysis revealed that leg soreness was 165 ± 46% lower in both groups at 64 h compared to 0 h post-match ($p < 0.001$), but there was no difference between 0 and 40 h ($p = 0.15$) or 40 and 64 h ($p = 0.21$) post-match. There was no group by time interaction effect for leg soreness ($p = 0.66$, $\eta^2_p = 0.004$; see Figure 2A).

Whole-body soreness was 106 ± 37% lower overall in the turmeric group compared to the control group ($p = 0.005$, $\eta^2_p = 0.038$). There was also a main effect of time for whole-body soreness ($p = 0.002$, $\eta^2_p = 0.061$) where post-hoc analysis revealed that soreness was lower in both groups at 64 h compared to 0 h post-match ($p = 0.001$; Figure 2B), but there were no differences between 0 and 40 h ($p = 0.14$)

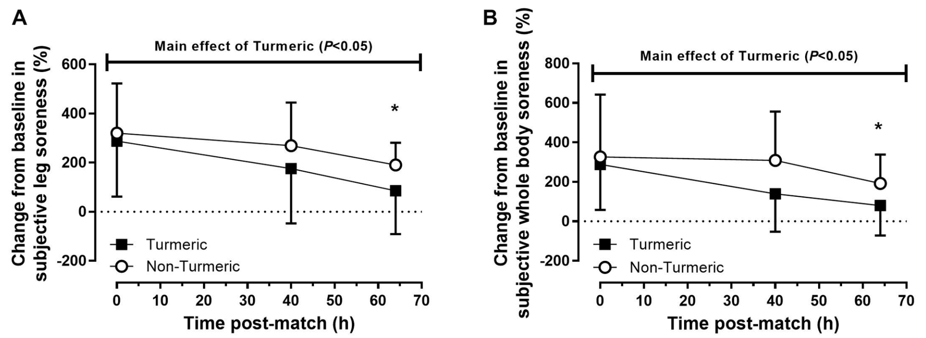

FIGURE 2
Subjective ratings of leg **(A)** and whole-body **(B)** soreness immediately (0h), 40h and 64h post-match, in the turmeric (solid square) and non-turmeric (open circle) groups. * indicates a difference compared to 0h in both groups (p<0.05). Data are presented as percent change from baseline. Values are means with error bars representing standard error.

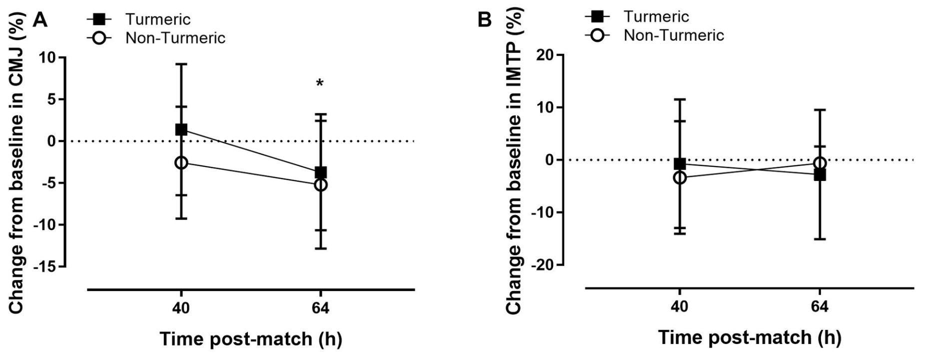

FIGURE 3
Countermovement jump height **(A)** and isometric mid-thigh pull **(B)** performance 40h and 64h post-match, in the turmeric (solid square) and non-turmeric (open circle) groups. * indicates a difference compared to 40h in both groups (p<0.05). Data are presented as percent change from baseline. Values are means with error bars representing standard error.

or 40 and 64 h ($p=0.19$) post-match. There was no group by time interaction effect for whole-body soreness ($p=0.29$, $\eta^2_p=0.012$).

Performance markers

CMJ performance was 3.9 ± 1.9% lower at 64 h compared to 40 h post-match ($p=0.049$, $\eta^2_p=0.052$). There was no main group ($p=0.16$, $\eta^2_p=0.026$) or group by time interaction ($p=0.53$, $\eta^2_p=0.006$) effects for percentage change in CMJ (see Figure 3A). There were no main effects of time ($p=0.93$, $\eta^2_p<0.001$), group ($p=0.95$, $\eta^2_p<0.001$), nor group by time interaction ($p=0.54$, $\eta^2_p<0.006$) for percentage change in IMTP (see Figure 3B).

Discussion

The aim of this study was to determine if twice daily consumption of a commercially available turmeric supplement, estimated to contain 1,400 mg of curcumin, could enhance recovery in a group of elite male footballers. We found that turmeric supplementation attenuated subjective markers of muscle soreness and reduced plasma [CRP] – a haematological marker of inflammation – at 64 h post-match, compared to a control group who did not consume the supplement. The results of this pilot study provide evidence that a commercially available turmeric supplement may be an effective and convenient method of accelerating recovery in elite male footballers.

Increased plasma [CRP] was observed in the control group 64 h post-match, but was attenuated in the turmeric supplementation group, indicating significant blunting of the systemic inflammatory response in participants consuming the turmeric supplement. CRP is a major acute phase protein synthesised by the liver in response to elevations in IL-6 and correlates with systemic inflammation (23). Turmeric contains high levels of the polyphenol curcumin, which has antioxidant and anti-inflammatory properties (24). Previous studies demonstrate that curcumin downregulates various inflammatory regulators, including nuclear factor kappa beta (NF-κB) activation and production of the enzyme cyclooxygenase-2 (COX-2), which are central components of the inflammatory cascade (11). Curcumin also increases blood antioxidant capacity, reducing the inflammation caused by free radicals (25). Therefore, the reduction in [CRP] at 64 h post-match likely reflects a reduction in systematic inflammation, indicating that turmeric supplementation has attenuated exercise-induced inflammation.

In contrast to [CRP], plasma [CK] did not differ between groups. CK is a protein found in the mitochondria of the muscle and can be used as a biomarker for muscle damage (26). The results of the present study are similar to another supplementation study, which found after an acute bout of eccentric bicep exercise, that [CRP] could be reduced by an antioxidant-docohexanoic supplement, but [CK] remained unchanged (27). Differences in [CK] and [CRP] response in the present study may be related to the training status of the population studied. Previous research has shown that plasma [CK] increases less in individuals accustomed to high volumes of exercise (28, 29) and can be influenced by muscle fibre type (30). Differences may also be due to the limited amount of control we were able to apply, due to the professional-status and elite nature of the population studied. For example, acute dietary intake of certain nutrients [such as vitamin C; (31)], which we were unable to measure or control in the present study, can influence plasma [CK]. Despite no club directed training taking place, it is also possible that the 5-days of rest prior to baseline measurements were insufficient for [CK] to reach nadir, which, coupled with these other factors, may explain why we did not observe a change in [CK] in response to turmeric supplementation.

Subjective muscle soreness was reduced with turmeric supplementation across all post-match time points. Previous studies have also similarly found that curcumin supplementation has reduced DOMS after exercise (10, 11), however, this finding is not consistent amongst all curcumin supplementation studies (12, 13, 32). Previous studies have ceased supplementation the day before (32) or 12 h after (13) exercise, which may explain the null findings. Tanabe et al. (33) found that curcumin supplementation for 4-days after exercise reduced muscle soreness, whereas supplementation for 7-days preceding exercise did not. These findings align with the current study and suggest that the analgesic effects of curcumin supplementation may be enhanced by continuing supplementation throughout the recovery period. This is likely explained by the pharmacokinetics of polyphenols, which typically peak a few hours after consumption before being metabolised and excreted (34).

Despite turmeric supplementation reducing CRP and subjective markers of soreness, this study found no effect of supplementation on CMJ and IMTP performance up to 48 h post-match. Previous studies have reported inconsistent findings regarding the effects of curcumin supplementation on performance, following a bout of exercise induced muscle damage. For example, curcumin supplementation can expedite recovery of some performance outcomes, such as MVC torque (13), range of motion (33) and 6 s sprint performance (12). However, other performance metrics, such at total work, mean peak torque (13) and MVC torque (33) were not different between when supplementing with curcumin. Abbott et al. (14) reported that curcumin supplementation offset the attenuation in CMJ performance observed after exercise-induced muscle damage from football match-play. These findings are directly comparable, in terms of exercise mode and performance metric, to the current study, yet present conflicting results. This may be due to the contextual differences in academy (14) compared to elite (current study) football match-play, and/or the degree of control that could be elicited around performance testing. For example, Abbott et al. (14) collected all measurements in the fasted state, whereas we were unable to control players dietary intake prior to measurements.

The supplement administered in this study was a 60 mL shot, which was well-tolerated and easy to incorporate into the nutrition plan of elite footballers. The supplement also contained piperine, an adjuvant that can greatly improve bioavailability of curcumin. High doses of curcumin (up to 12 g per day) are pharmacologically safe and well tolerated in humans (34). However, unformulated curcumin exhibits very poor bioavailability, with very low concentrations detected in serum and extracellular tissues after oral consumption, likely due to poor absorption, followed by rapid metabolism and elimination (24). Piperine slows the metabolism of curcumin by inhibiting hepatic and intestinal glucuronidation (24). Previous studies have shown that administering curcumin with piperine can increase serum concentrations of curcumin by up to 2000% (35), indicating that glucuronidation inhibition may be the major mechanism of increasing curcumin bioavailability (24). As such, the formulation of the supplement administered in the current study, which contained 35 g of raw turmeric root, (estimated to contain 1,400 mg of curcumin) and 10 mg of piperine, may have helped to increase serum concentrations of curcumin such that it could exert its biological action, whilst the mode of administration may have encouraged greater adherence. These are major strengths of this study, as these factors increase the likelihood of professional athletes utilising this supplement to expedite recovery.

Another strength of this study is the cohort of elite footballers studied, which is rare for this type of research. Whilst research in sub-elite populations can be informative, the contextual factors surrounding elite football are unique, and therefore, findings from sub-elite cannot always be readily extrapolated to elite athletes. Moreover, elite football match-play elicits a high degree of muscle damage and soreness (5), which results in many players using NSAIDs to manage pain and maintain performance amidst an intensive training and match-play schedule (36). However, chronic NSAID use has been associated with various adverse cardiovascular issues (37) and regular use is not recommended (38). As such, findings of the present study in this highly relevant population, provide evidence that a commercially available and easy to administer turmeric shot could reduce muscle soreness and expedite recovery after match-play.

This study is not without limitations. Firstly, despite the supplement being formulated to enhance bioavailability, this has not been empirically tested. Future studies should aim to measure the appearance of curcumin in blood, and the time course, to establish the optimal dosing strategy. Secondly, due to the novelty of the supplement provided and the population, we were unable to blind participants to the treatment they received. Previous research has showed that when participants perceive that they have ingested an active supplement this can distort perceptions of pain (39) and influence other outcomes, such as exercise performance (40). It's important that future studies incorporate a suitable placebo condition to mitigate this risk. Thirdly, due to the novelty of the population studied, sample size for this study was inherently small, and turmeric and non-turmeric users could not be matched, as we could not control the match-day playing team. This resulted in a larger number of sample points for the supplementation group (60) compared to control (16).

In conclusion, this applied pilot study found that twice-daily consumption of a turmeric supplement attenuated a blood marker of inflammation and subjective muscle soreness, in elite male footballers following match-play. These findings suggest that a commercially available and easy to consume turmeric supplement may accelerate post-match recovery and this warrants further investigation.

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 human participants were reviewed and approved by Nottingham Trent University Human Invasive Ethics Committee (REF: 716). The patients/participants provided their written informed consent to participate in this study.

Author contributions

DC, RB, and IV: conceptualisation. DC, RB, PH, RJ, and IV: methodology. DC, RB, PH, RJ, CS, SG, RM, and IV: formal analysis. DC, RB, PH, RJ, CS, AC, RS, SG, RM, and IV: data curation, writing—review and editing, and project administration. DC, PH, RJ, CS, and IV: writing—original draft preparation. All authors contributed to the article and approved the submitted version.

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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/fnut.2023.1175622/full#supplementary-material

References

1. Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. *Sports Med*. (2006) 36:327–58. doi: 10.2165/00007256-200636040-00004

2. Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. *Sports Med*. (2004) 34:49–69. doi: 10.2165/00007256-200434010-00005

3. Morgans R, Bezuglov E, Orme P, Burns K, Rhodes D, Babraj J, et al. The physical demands of match-play in academy and senior soccer players from the Scottish premiership. *Sports*. (2022) 10:150. doi: 10.3390/sports10100150

4. Varley I, Lewin R, Needham R, Thorpe RT, Burbeary R. Association between match activity variables, measures of fatigue and neuromuscular performance capacity following elite competitive soccer matches. *J Hum Kinet*. (2017) 60:93–9. doi: 10.1515/hukin-2017-0093

5. Ascensão A, Rebelo A, Oliveira E, Marques F, Pereira L, Magalhães J. Biochemical impact of a soccer match — analysis of oxidative stress and muscle damage markers throughout recovery. *Clin Biochem*. (2008) 41:841–51. doi: 10.1016/j.clinbiochem.2008.04.008

6. Mohr M, Draganidis D, Chatzinikolaou A, Barbero-Álvarez JC, Castagna C, Douroudos I, et al. Muscle damage, inflammatory, immune and performance responses to three football games in 1 week in competitive male players. *Eur J Appl Physiol*. (2016) 116:179–93. doi: 10.1007/s00421-015-3245-2

7. Schoenfeld BJ. The use of nonsteroidal anti-inflammatory drugs for exercise-induced muscle damage: implications for skeletal muscle development. *Sports Med*. (2012) 42:1017–28. doi: 10.1007/BF03262309

8. Sostres C, Gargallo CJ, Lanas A. Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. *Arthritis Res Ther*. (2013) 15:S3. doi: 10.1186/ar4175

9. Maughan RJ, Burke LM, Dvorak J, Larson-Meyer DE, Peeling P, Phillips SM, et al. IOC consensus statement: dietary supplements and the high-performance athlete. *Int J Sport Nutr Exerc Metab*. (2018) 28:104–25. doi: 10.1123/ijsnem.2018-0020

10. Kawanishi N, Kato K, Takahashi M, Mizokami T, Otsuka Y, Imaizumi A, et al. Curcumin attenuates oxidative stress following downhill running-induced muscle damage. *Biochem Biophys Res Commun*. (2013) 441:573–8. doi: 10.1016/j.bbrc.2013.10.119

11. Davis JM, Murphy EA, Carmichael MD, Zielinski MR, Groschwitz CM, Brown AS, et al. Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage. *Am J Physiol Regul Integr Comp Physiol*. (2007) 292:R2168–73. doi: 10.1152/ajpregu.00858.2006

12. Delecroix B, Abaïdia AE, Leduc C, Dawson B, Dupont G. Curcumin and Piperine supplementation and recovery following exercise induced muscle damage: a randomized controlled trial. *J Sports Sci Med*. (2017) 16:147–53.

13. Tanabe Y, Maeda S, Akazawa N, Zempo-Miyaki A, Choi Y, Ra SG, et al. Attenuation of indirect markers of eccentric exercise-induced muscle damage by curcumin. *Eur J Appl Physiol*. (2015) 115:1949–57. doi: 10.1007/s00421-015-3170-4

14. Abbott W, Hansell EJ, Brett A, Škarabot J, James LJ, Clifford T. Curcumin attenuates delayed-onset muscle soreness and muscle function deficits following a soccer match in male professional players. *Int J Sports Physiol Perform*. (2023) 18:347–53. doi: 10.1123/ijspp.2022-0283

15. Campbell MS, Carlini NA, Fleenor BS. Influence of curcumin on performance and post-exercise recovery. *Crit Rev Food Sci Nutr*. (2021) 61:1152–62. doi: 10.1080/10408398.2020.1754754

16. Singh S, Joshi RK, Nayak S. Identification of elite genotypes of turmeric through agroclimatic zone based evaluation of important drug yielding traits. *Ind Crop Prod*. (2013) 43:165–71. doi: 10.1016/j.indcrop.2012.07.006

17. McKay AKA, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL, et al. Defining training and performance caliber: a participant classification framework. *Int J Sports Physiol Perform*. (2022) 17:317–31. doi: 10.1123/ijspp.2021-0451

18. Kabongo K, Emeran A, Bosch A. Do upper leg compression garments aid performance and reduce exercise-induced muscle damage in recreational marathon runners? *South Afr J Sports Med [Internet]*. (2022) 34:v34i1a14169. doi: 10.17159/2078-516X/2022/v34i1a14169

19. Heishman AD, Daub BD, Miller RM, Freitas EDS, Frantz BA, Bemben MG. Countermovement jump reliability performed with and without an arm swing in NCAA division 1 intercollegiate basketball players. *J Strength Cond Res*. (2020) 34:546–58. doi: 10.1519/JSC.0000000000002812

20. De Witt JK, English KL, Crowell JB, Kalogera KL, Guilliams ME, Nieschwitz BE, et al. Isometric Midthigh pull reliability and relationship to deadlift one repetition maximum. *J Strength Cond Res*. (2018) 32:528–33. doi: 10.1519/JSC.0000000000001605

21. Johnston RJ, Watsford ML, Kelly SJ, Pine MJ, Spurrs RW. Validity and interunit reliability of 10 Hz and 15 Hz GPS units for assessing athlete movement demands. *J Strength Cond Res*. (2014) 28:1649–55. doi: 10.1519/JSC.0000000000000323

22. Sink CA, Mvududu NH. Statistical power, sampling, and effect sizes: three keys to research relevancy. *Couns Outcome Res Eval*. (2010) 1:1–18. doi: 10.1177/2150137810373613

23. Michigan A, Johnson TV, Master VA. Review of the relationship between C-reactive protein and exercise. *Mol Diagn Ther*. (2011) 15:265–75. doi: 10.1007/BF03256418

24. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm*. (2007) 4:807–18. doi: 10.1021/mp700113r

25. Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I. Curcumin induces glutathione biosynthesis and inhibits NF-κB activation and Interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. *Antioxid Redox Signal*. (2005) 7:32–41. doi: 10.1089/ars.2005.7.32

26. Baird MF, Graham SM, Baker JS, Bickerstaff GF. Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *J Nutr Metab*. (2012) 2012:1–13. doi: 10.1155/2012/960363

27. Phillips T, Childs AC, Dreon DM, Phinney S, Leeuwenburgh C. A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males. *Med Sci Sports Exerc*. (2003) 35:2032–7. doi: 10.1249/01.MSS.0000099112.32342.10

28. Hoffman JR, Kang J, Ratamess NA, Faigenbaum AD. Biochemical and hormonal responses during an intercollegiate football season. *Med Sci Sports Exerc*. (2005) 37:1237–41. doi: 10.1249/01.mss.0000170068.97498.26

29. Vincent H, Vincent K. The effect of training status on the serum Creatine kinase response, soreness and muscle function following resistance exercise. *Int J Sports Med*. (1997) 28:431–7. doi: 10.1055/s-2007-972660

30. Isaacs AW, Macaluso F, Smith C, Myburgh KH. C-reactive protein is elevated only in high Creatine kinase responders to muscle damaging exercise. *Front Physiol*. (2019) 10:86. doi: 10.3389/fphys.2019.00086

31. Childs A, Jacobs C, Kaminski T, Halliwell B, Leeuwenburgh C. Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. *Free Radic Biol Med*. (2001) 31:745–53. doi: 10.1016/S0891-5849(01)00640-2

32. Jäger R, Purpura M, Kerksick CM. Eight weeks of a high dose of curcumin supplementation may attenuate performance decrements following muscle-damaging exercise. *Nutrients*. (2019) 11:1692. doi: 10.3390/nu11071692

33. Tanabe Y, Chino K, Sagayama H, Lee HJ, Ozawa H, Maeda S, et al. Effective timing of curcumin ingestion to attenuate eccentric exercise-induced muscle soreness in men. *J Nutr Sci Vitaminol (Tokyo)*. (2019) 65:82–9. doi: 10.3177/jnsv.65.82

34. Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med*. (2006) 6:10. doi: 10.1186/1472-6882-6-10

35. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas P. Influence of Piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med*. (1998) 64:353–6. doi: 10.1055/s-2006-957450

36. Rosenbloom C, Okholm Kryger K, Carmody S, Broman D. Non-steroidal anti-inflammatory drugs in football – a "keeping SCORE" approach to judicious use. *Sci Med Footb*. (2022):1–5. doi: 10.1080/24733938.2022.2140189

37. Pope JE. A Meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. *Arch Intern Med*. (1993) 153:477. doi: 10.1001/archinte.1993.00410040045007

38. Antman EM, Bennett JS, Daugherty A, Furberg C, Roberts H, Taubert KA. Use of nonsteroidal Antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association. *Circulation*. (2007) 115:1634–42. doi: 10.1161/CIRCULATIONAHA.106.181424

39. Trojian TH, Beedie CJ. Placebo effect and athletes. *Curr Sports Med Rep*. (2008) 7:214–7. doi: 10.1249/JSR.0b013e31817ed050

40. Saunders B, de Oliveira LF, da Silva RP, de Salles PV, Gonçalves LS, Yamaguchi G, et al. Placebo in sports nutrition: a proof-of-principle study involving caffeine supplementation. *Scand J Med Sci Sports*. (2017) 27:1240–7. doi: 10.1111/sms.12793

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The impact of nutrition on visual cognitive performance in the nutrition, vision, and cognition in sport study

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Introduction: The purpose of this study was to examine the influence of nutritional intake on visual perceptual-cognitive performance (VCP) in young healthy adults.

Methods: Ninety-eight healthy men (n=38) and women (n=60) aged 18–33 years participated and maintained their usual dietary intake throughout the study. VCP was measured using the NeuroTracker™ CORE (NT) 3-Dimensional (3-D) software program (15 training sessions) over a 15-day period. Food logs and extensive lifestyle measures including body composition, cardiovascular health, sleep and exercise patterns, and general readiness to perform were collected. Mean intake from 10 food logs collected over the 15 days were analyzed using Nutribase software. Statistical analyses were performed in SPSS using repeated measures ANOVA including significant covariates when appropriate.

Results: Males consumed significantly more calories, macronutrients, cholesterol, choline, and zinc and performed significantly better on VCP than the females. Participants who consumed more than 40% of kcals from carbohydrates (p=0.038), less than 24% of kcals from protein (p=0.009), more than 2,000 µg/ day lutein/zeaxanthin or more than 1.8 mg/ day vitamin B2 performed significantly better on VCP than those who consumed less than those amounts, respectively.

Discussion: VCP is an important dimension of cognitive function and in the present study is influenced by higher carbohydrate, lutein/ zeaxanthin, and vitamin B2 dietary intake while high protein consumption and the female sex negatively impacted VCP.

KEYWORDS

carbohydrates, protein, micronutrients, lutein, zeaxanthin, cognition, cognitive performance, vision

1. Introduction

Traditionally, athletes spend a lot of time physically training skeletal muscle and cardiovascular systems to enhance sports performance. However, an increasing prevalence of studies suggest that vision and perceptual-cognitive processing are also critical factors that enhance or limit overall sports performance (1–4). While both visual "hardware" (eye health) and "software" (visual processing) domains have been evaluated in relation to visual cognitive ability, the relative importance of each of these appears dependent on the requirements of the specific sport (3–7). Athletes ranging from hockey players to baseball players have unique visual processing requirements that impact their overall athletic performance (1, 3–7).

Computer technology in sport-specific vision training programs may be used to enhance perceptual cognitive ability and testing has been associated with overall sport success (1, 2, 8–10). The 3-dimensional (3D) multi-object-tracking (MOT) is a robust computerized training intervention that requires tracking multiple objects in 3D that are moving among distractors in a broad field using a speed threshold to measure performance (8–10). This technology sensitively differentiates low and high visual-cognitive performance, requires peripheral vision, individualizes speed and difficulty levels, and consistently challenges trainees to progress (10). The NeuroTracker™ CORE (NT) 3D software program from CogniSens Inc. is a single-task integrative perceptual cognitive training system that is equivalent to the 3D-MOT system. It evaluates the individual's perceptual cognitive ability, provides training, and has been shown to be effective in improving cognitive performance after a specified number of training sessions (8, 9).

Previous investigations using the NT 3D-MOT system utilized maximal speed thresholds and adaptive training responses to differentiate between professional, elite amateurs, and non-athletes. Professional athletes demonstrated significantly greater perceptual cognitive processing with a much steeper learning curve in a dynamic environment when compared to elite amateur athletes. Similarly, elite amateur athletes performed significantly better and had a significantly steeper learning curve than non-athletes (8). College age non-athletes, who completed 10 NT training sessions, experienced significant improvements in cognitive performance, attention span, memory, and reaction time, in addition to quantitative changes in brain function when comparing baseline and final sessions (10).

While the importance of visual processing speed in sport performance is increasingly recognized, the understanding of the variability between individuals is poor. Individual intake of macronutrients including the dietary ratio of carbohydrates, protein, and fat appear to impact overall cognitive ability. Some studies report mixed effects of high carbohydrate diets that are dependent on the type of carbohydrate consumed. Simple carbohydrates have been shown to reduce cognitive function as well as lead to pathological cognitive decline through deleterious effects on the brain. Complex carbohydrates with high fiber content are reported to be beneficial for cognition through improved blood glucose control and thus reduce metabolic dysregulation. Studies also support a balanced protein intake due to evidence that inadequate or overconsumption of protein can negatively impact cognition while adequate intake improves certain cognitive tests. Additionally, research supports higher intakes in healthy fats. For example, polyunsaturated fats are linked to improved cognitive function while diets high in saturated fatty acids and cholesterol are shown to have a negative correlation with global cognitive function (11–18).

Micronutrient intake can also greatly influence nervous and sensory function including choline, B vitamins, calcium, magnesium, zinc, copper, beta-carotene, vitamins C and E, ω-3 fatty acids, lutein/zeaxanthin. These nutrients have been studied most often for their role in visual cognitive function, performance and/or eye health (19–26). Lutein/zeaxanthin also positively influenced visual cognitive performance by serving as antioxidant and anti-inflammatory agents to reduce oxidative stress and inflammation, filtering short-wave blue light that damage the retina and diminish visual sharpness and supporting retinal light processing through synaptic membrane regulation (2, 20, 21, 26). The follow-up to the Age-Related Eye Disease Study 2 (AREDS2) study reported lutein/zeaxanthin contributed to eye health by positively impacting those with late age-related macular degeneration (AMD) (26).

The purpose of the IONSport study was to examine the influence of free-living nutritional intake on visual perceptual-cognitive performance in young healthy adults. The focus was to examine macronutrients and micronutrients in the current literature that reportedly impact eye health and/or cognitive performance. We employed a cross-sectional design to characterize cognitive performance and dietary intake. Participants kept food logs on each day of cognitive testing to evaluate the effect of variability in a free-living diet on cognitive performance; detailed 10-day nutrient averages were used to represent individual long term nutrient intake. The NT 3D-MOT software program was used to identify individual perceptual cognitive ability. We are unaware of any studies that have evaluated the relationship of nutrient intake to visual cognitive testing using the NT software.

2. Materials and methods

2.1. Subjects

Men and women aged 18–33 years were recruited for the IONSport study using a convenience sampling strategy. Email, posted fliers, and direct contact with student groups on the Texas A&M University campus were used to recruit participants. Individuals with a body mass index (BMI) less than 18, had a pacemaker, experienced vertigo, or had difficulty with 3D viewing were excluded. Persons who self-reported their inability to distinguish between yellow and orange due to colorblindness on a pre-screening form were also excluded since the training program required the ability to distinguish these colors. All participants signed an informed consent, and the Texas A&M University Human Subject's Institutional Review Board (IRB) approved the study protocol.

2.2. Procedures

Prior to the start of the study, baseline data including blood pressure (BP); heart rate (HR) (Omron Healthcare, Inc. Bannockburn, IL, United States); visual acuity using the Snellen Chart; and body composition using the Biometric Beurer BF 520 BIA technology (Beurer, Germany) were collected (27). Subjects completed the Pittsburgh Sleep Quality Index, medical history, and the Modifiable Activity Questionnaire to provide detailed sleep, health, and physical activity background (28, 29). Participants came to the lab 10 times over a 15-day period to complete cognitive testing. Daily data, anthropometrics, and food logs were collected at each visit.

2.3. Nutrition education and monitoring

Subjects were instructed to continue their usual eating behaviors and physical activity throughout the study. A registered dietitian nutritionist (RDN) prepared and emailed five, one-to-two-minute, online instructional videos and provided participants direct guidance on how to accurately assess and document food and beverages consumed. Subjects were asked to log all food and beverages consumed on each of the 10 cognitive training days. Each food log was reviewed daily by an RDN and, if necessary, additional details were collected to ensure logs were complete and accurate. NutriBase 19 Pro Edition, v. 19.2 software (NB) (CyberSoft, Inc., Houston, TX) was used for dietary analyses.

2.4. Visual cognitive performance testing

Participants completed 15 cognitive training sessions using the NT 3D software program over 10 training days that included alternating single and double training sessions with 4–5 interspersed days of no training (15 total days of the study). During the first training session, the cognitive testing procedures were explained to participants. Participants were seated in a chair aligned 4½' from a 50" 3D television in a dark, quiet testing room with the seat adjusted so that their eyes were positioned at the center of the screen. They wore active 3D glasses that directly interacted with 3D software and noise-canceling headphones to minimize distractions. Participants who relied on glasses for corrected vision wore them under the 3D glasses when training.

Each training session included tracking the spatial location of four pre-identified target spheres that were initially a distinct color from the other four spheres. Once identified, these spheres became identical in color to the four other spheres. All eight identical spheres moved among each other at a given speed within a 3D virtual space, passing in front of or behind each other, colliding with each other or the edges of the screen and changing directions. After 6 s of movement, the spheres stopped, and the participant picked out the four pre-identified spheres. If the subject selected all four of the correct spheres, the speed of sphere movement increased for the next 6 s trial. If one or more spheres was missed, the speed of sphere movement decreased for the next trial in a 0.05 log up 0.05 log down pattern (30, 31) subjects performed 20 trials within a single training session obtaining a "speed threshold," the level at which the participant correctly tracked and selected the correct objects 50% of the time. The final speed threshold for each training session and the progression over 15 sessions were the primary determinants of cognitive performance.

Each of the 10 cognitive training days included collection of recent physical activity, fluid intake, most recent urine color (validated urine color scale), blood pressure, heart rate, readiness to perform, body composition, level of sleepiness (Stanford Sleepiness questionnaire) and actual hours of sleep the previous night (32, 33).

2.5. Statistical analyses

IBM SPSS 27.0 software (SPSS Inc., Chicago, IL) was used for all the analyses. NB was used to calculate the 10-day food log mean of individual nutrients which was used in cognitive performance analyses. Lutein/zeaxanthin were analyzed together since NB reported these nutrients as one. Visual cognitive performance was assessed using the 15-day NT speed threshold. Speed threshold analysis included: (1) evaluating mean and maximal performance and (2) comparing baseline sessions 1–3 to the final 3 sessions. The relationship of nutritional intake and cognitive performance was evaluated based on sex and comparison of top and poor performers on the NT cognitive tests. Data are presented as estimated mean ± SD, and $p < 0.05$ was considered statistically significant.

A repeated measures analysis of variance (ANOVA) was performed to compare the effect of nutrient intake on visual cognitive performance. Nutrients which showed suggestive positive or negative associations to cognitive performance parameters were further analyzed by categorizing intakes according to recommended levels and natural groupings within the data. Stepwise linear regression was used to determine the independent effect of cognitive performance using variables that continued to show a consistent effect on cognitive performance parameters.

3. Results

3.1. Subjects

The IONSport Study enrolled 109 participants, and 98 (38 males, 60 females) had complete data sets for analyses (at least 9 daily food records, 15 NT sessions, and at least 9 site visits in 15 days). Table 1 shows the characteristics of all male and female participants. There were significant differences among male and female participants in age, height, weight, lean mass, BMI, and percent body fat. Regarding blood pressure, there was a significant difference in the systolic but not diastolic blood pressure. There was also a significant difference in hydration status as indicated by urine color. There was no significance between the hours of sleep or responses on the Stanford Sleepiness scale. Most participants were Caucasian (49%).

TABLE 1 Participant characteristics.

	Male (*n*=38)		Female (*n*=60)		
	Mean	SD	Mean	SD	P (sex)
Age (years)	22.7	3.0	21.1	3.6	0.032
Height (m)	1.78	0.09	1.64	0.06	<0.001
Weight (kg)	80.5	10.4	60.9	9.3	<0.001
Lean mass (kg)	69.8	7.9	46.0	4.8	<0.001
BMI (kg•m^{-2})	25.3	2.7	22.5	3.2	<0.001
Systolic BP (mmHg)	122.8	10.4	108.4	8.9	<0.001
Diastolic BP (mmHg)	73.4	4.9	71.3	7.4	0.159
Fat (%)	12.9	3.9	23.8	5.5	<0.001
Urine color (AU)	2.6	0.9	2.1	0.6	0.002
Sleep (hours)	6.8	0.9	6.8	0.9	0.871
Stanford sleepiness	2.3	0.9	2.3	0.6	0.859
Race (% white)	40.5		55.1		

3.2. Average dietary intake and cognitive performance

There was variance between men and women in both average dietary intake and cognitive performance outcomes as displayed in Table 2. Males consumed significantly more calories (per day) carbohydrates (grams/day), protein (g/day and % calories), and fat (g/day). Additionally, cholesterol (mg/day), choline (mg/day) and zinc (mg/day) consumption was higher in males than females. There were no significant differences in the consumption of B vitamins, calcium, magnesium, copper, beta-carotene, vitamins C and E, ω-3 fatty acids, fiber, or lutein/zeaxanthin. VCP was significantly better for males than the females and males performed significantly better on the three baseline and three final cognitive performance sessions. However, the change from baseline to the final sessions was similar between males and females.

A repeated measures ANOVA compared the effect of the percent of calories from carbohydrate and protein consumption on visual cognitive performance in Figures 1, 2, respectively. There was a statistically significant difference in cognitive performance and participants who consumed more than 40% carbohydrates ($p = 0.038$) compared to those who consumed less than that amount. A significant breakpoint in the data was identified in those who consumed less than 24% of total calories from protein and those who consumed 26% of total calories from protein. Participants who consumed less than 24% protein performed significantly better on the visual cognitive performance task ($p = 0.009$) than those who consumed 26% calories from protein.

3.3. Top performers and cognitive performance

There were also differences in average dietary intake and cognitive performance when comparing the top performers to poor performers. Top performers were defined as the 7 males and 7 females who

TABLE 2 Participants average dietary intake and cognitive performance.

		Male (n=38)		Female (n=60)		
		Mean	SD	Mean	SD	P (sex)
Macronutrients	Calories (kcal•day^{-1})	2214	492	1727	402	<0.001
	Calories (kcal•kg^{-1})	28.1	7.0	29.0	7.4	0.554
	Carbs (g•day^{-1})	249.5	75.6	211.5	63.7	0.008
	Carbs (g•kg^{-1}•day^{-1})	3.2	1.2	3.6	1.1	0.135
	Carbs (% Calories)	42.3	9.2	45.8	8.4	0.056
	Sugars (g•day^{-1})	77.8	37.7	73.3	29.3	0.507
	Fiber	21.2	8.91	21.4	10.4	0.902
	Protein (g•day^{-1})	122.2	49.1	79.8	27.2	<0.001
	Protein (g•kg^{-1}•day^{-1})	1.5	0.6	1.3	0.4	0.036
	Protein (g•kg lean^{-1}•day^{-1})	1.8	0.6	1.7	0.6	0.782
	Protein (% Calories)	23.4	8.4	19.3	4.4	0.002
	Fat (g•day^{-1})	81.4	24.0	65.4	22.9	0.001
	Fat (% Calories)	33.5	6.0	34.3	6.6	0.564
Micronutrients	Omega-3 (g•day^{-1})	0.4	0.6	0.3	0.4	0.176
	Cholesterol (mg•day^{-1})	431.0	313.5	232.7	168.8	<0.001
	Choline (mg•day^{-1})	267.7	258.9	142.0	121.4	0.002
	Betaine (mg•day^{-1})	45.9	152.1	20.8	28.2	0.216
	Vitamin B2 (μg •day^{-1})	3.6	10.0	1.4	1.6	0.105
	Vitamin B5 (μg •day^{-1})	5.8	6.8	3.8	5.8	0.136
	Vitamin C (mg•day^{-1})	91.5	85.3	95.6	87.5	0.818
	Vitamin E (mg•day^{-1})	13.1	21.2	11.7	26	0.766
	Copper (mg•day^{-1})	0.9	0.7	0.8	0.6	0.662
	Zinc (mg•day^{-1})	9.3	6.4	5.9	3.1	0.001
	Lut/Zea (μg •day^{-1})	2383	5930	2481	4285	0.925
Cognitive performance	Mean of 15	1.75	0.34	1.52	0.33	0.001
	Maximum of 15	2.38	0.45	2.08	0.43	0.001
	Baseline (3)	1.60	0.40	1.34	0.38	0.001
	Final (3)	1.88	0.35	1.67	0.38	0.007
	Change	0.26	0.30	0.33	0.26	0.185

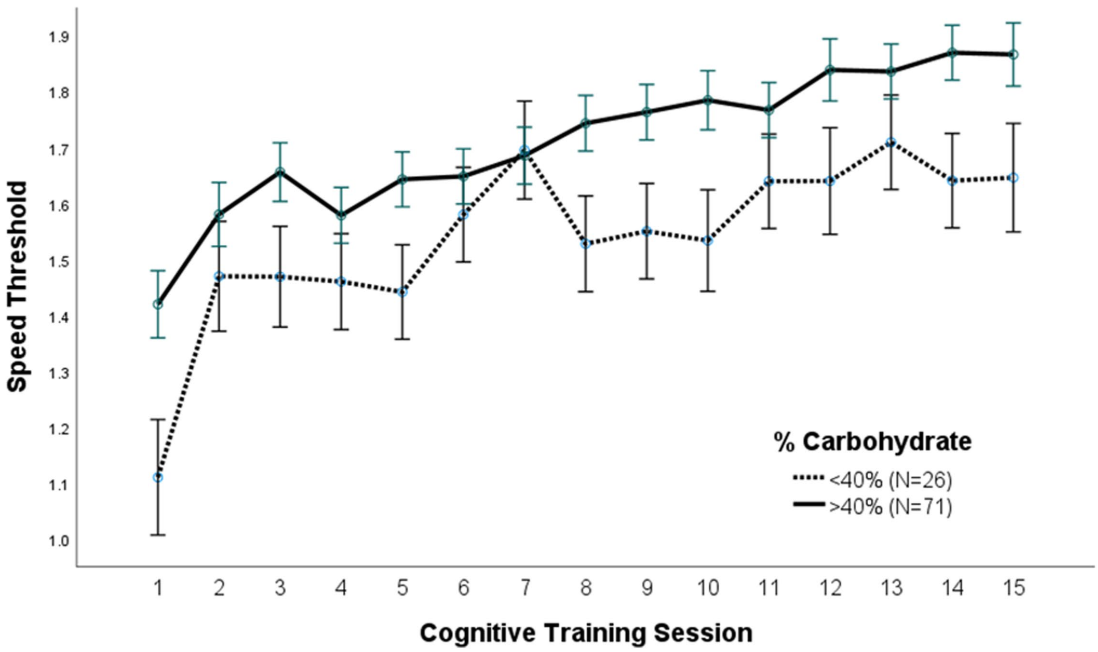

FIGURE 1
Carbohydrate (% calories) intake. Covariates appearing in the model are evaluated at the following values: Gender=1.6146, LUTZEA2000=1.2708; Error bars: +/− 1 SE. Repeated measures ANOVA $p=0.038$.

performed best on 15 cognitive training sessions, while the poor performers were the 8 males and 8 females who performed the worst on the same number of sessions. Mean ($p<0.001$) and maximal ($p<0.001$) cognitive performance was significantly higher in top performers when compared to poor performers (Table 3). Top performers also consumed significantly more carbohydrates (% calories) and significantly less protein (g/kg/day and g/kg lean/day) than poor performers (Table 3). There was no significant difference in the consumption of fat, choline, B vitamins, calcium, magnesium, copper, zinc, beta-carotene, vitamins C and E, ω-3 fatty acids, or lutein/zeaxanthin (Table 3).

3.4. Lutein/zeaxanthin intake

A repeated measures ANOVA compared the effect of lutein/zeaxanthin consumption on visual cognitive performance (Figure 3). Dietary intake of 2,000 μg/day lutein/zeaxanthin was used to distinguish participants who consumed high vs. low lutein/zeaxanthin levels since this is the upper average intake by American adults (34, 35). Mean ($p=0.004$) and maximal ($p=0.031$) cognitive performance was significantly higher in those that consumed >2000 μg/day lutein/zeaxanthin. However, both groups improved at the same rate.

3.5. Vitamin B2 (riboflavin)

A repeated measures ANOVA compared the effect of vitamin B2 (riboflavin) consumption on visual cognitive performance (Figure 4). Dietary intake of 1.8 mg /day was used as a cutoff for high vs. low B2 (riboflavin) consumption based on the average dietary intake for women (36). There was a significantly higher mean ($p=0.017$) and maximal ($p=0.029$) cognitive performance in those consuming >1.8 mg/day B2. While the recommended dietary intake for men and women ages 19–51 is 1.3 mg and 1.1 mg, respectively, these differences in cognitive performance were not affected by controlling for sex (EMM ± SE, 0.27 ± 0.03 vs. 0.41 ± 0.06; $p=0.032$).

3.6. Regression

All independent variables that appeared to have an influence on the NT mean and/or maximum speed threshold were considered in the linear regression analysis, only significant/consistent predictors were reported in the linear regression models. There were significant independent effects of sex, protein intake (% kcal), vitamin B2, lutein/zeaxanthin and age on the mean (Table 4) and maximal (Table 5) NT speed threshold; however, lutein/zeaxanthin was not a significant predictor in the model for mean or maximal NT speed thresholds. Although carbohydrate intake positively influenced cognitive performance in the repeated measures ANOVA model, it was not retained in the linear regression models High protein intake was a significant negative predictor of both the mean (Table 4) and the maximal (Table 5) NT speed threshold. Similarly, there was a significant negative relationship between age and the maximal (Table 5) NT speed threshold. Younger subjects had significantly better maximal NT speed threshold than older participants; however, there was not a significant relationship between age and the mean NT speed threshold. Vitamin B2 (riboflavin) had a significantly positive relationship with both the mean (Table 4) and the maximal (Table 5) NT speed threshold.

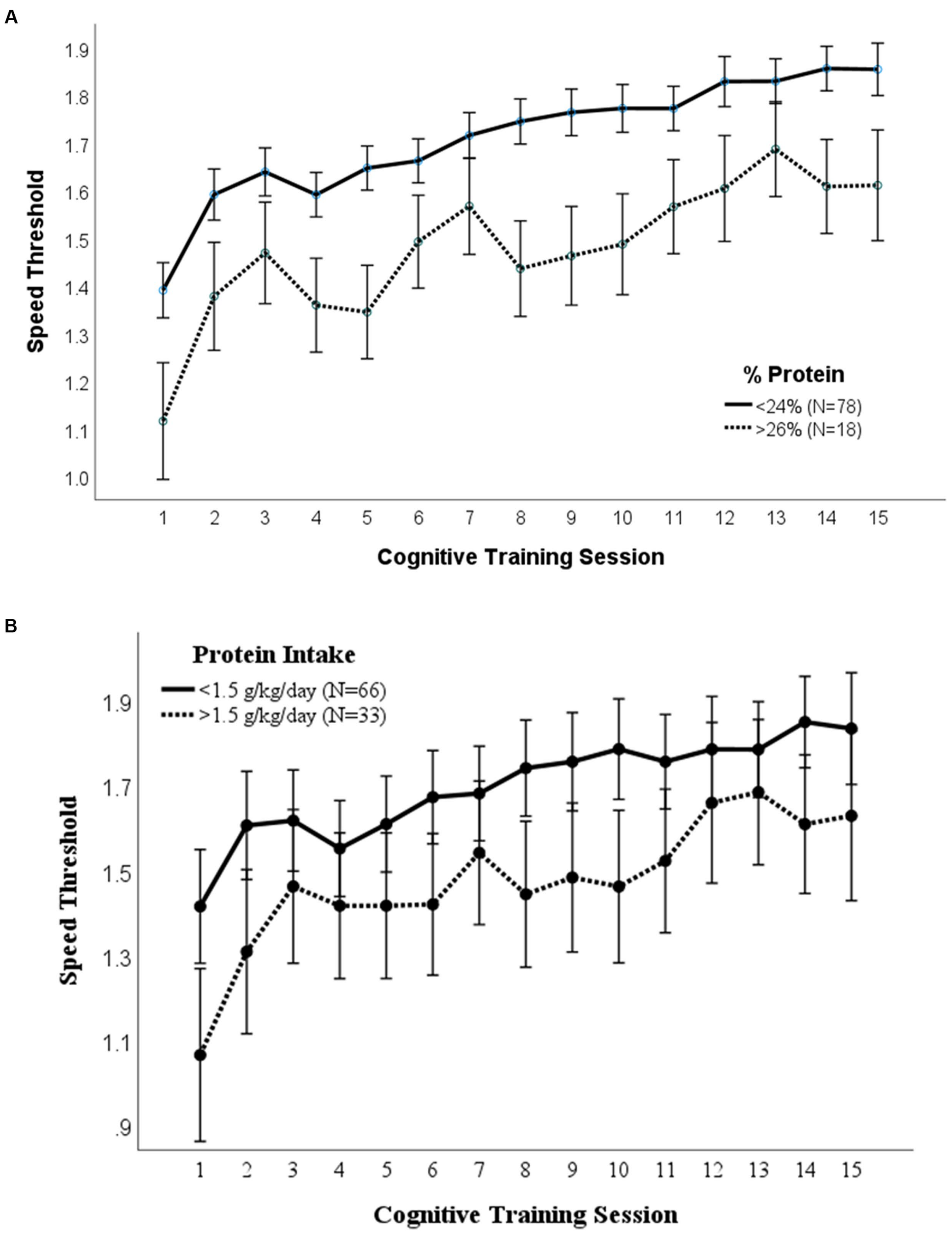

FIGURE 2
(A) Protein (% calories) intake. Covariates appearing in the model are evaluated at the following values: Gender=1.6146, LUTZEA2000=1.2708; Error bars: +/− 1 SE. Repeated measures ANOVA p=0.009. **(B)** Protein (g/kg/day) intake. Covariates appearing in the model are evaluated at the following values: Gender=1.6146, LUTZEA2000=1.2708; Error bars: +/− 1 SE. Repeated measures ANOVA p=0.035.

4. Discussion

The results of this study demonstrated a significant association of macronutrients on visual cognitive performance in a group of healthy male and females aged 18–33 who recorded food intake following their normal dietary patterns and completed visual cognitive performance testing on 10 days within a 15-day period. Results of this study demonstrated a significantly positive relationship between individuals who consumed >40% of their macronutrient intake from carbohydrates and visual cognitive performance.

Previous investigations have found that carbohydrates contribute to athletic performance and endurance, but there has been limited emphasis on the relationship between carbohydrate intake and cognitive ability (13). While carbohydrate recommendations for endurance athletes vary based on their intensity and length of performance, recent studies demonstrated a positive relationship

TABLE 3 Top performers compared to poor performers nutrient intake and cognitive performance.

		Top performers (7M, &7F)		Poor performers (8M, 8F)		Covary sex
		Mean	SD	Mean	SD	P (group)
Macronutrients	Calories (kcal•day^{-1})	2087	390	2014	670	0.765
	Calories (kcal•kg^{-1})	30.9	6.3	31.3	8.4	0.896
	Carbs (g•day^{-1})	272	49	231	98	0.174
	Carbs (g•kg^{-1}•day^{-1})	4.1	1.1	3.6	1.4	0.337
	Carbs (% Calories)	49.6	6.0	43.0	10.5	0.047*
	Sugars (g•day^{-1})	85.9	30.8	81.3	43.1	0.763
	Protein (g•day^{-1})	88.7	29.6	109	51.8	0.139
	Protein (g•kg^{-1}•day^{-1})	1.25	0.33	1.65	0.60	0.035*
	Protein (g•kg lean^{-1}•day^{-1})	1.53	0.39	1.99	0.71	0.048*
	Protein (% Calories)	17.8	3.4	23.1	10.7	0.07
	Fat (g•day^{-1})	73.4	17.1	75.6	33.3	0.785
	Fat (% Calories)	32.3	4.2	33.1	7.9	0.762
Micronutrients	Omega-3 (g•day^{-1})	0.30	0.26	0.46	0.74	0.443
	Cholesterol (mg•day^{-1})	240.9	133.7	357.2	322.3	0.158
	Choline (mg •day^{-1})	163	118	208	245	0.493
	Betaine (mg •day^{-1})	85	254	18	17	0.334
	Vitamin B2 (µg •day^{-1})	1.5	1.1	1.6	1.0	0.878
	Vitamin B5 (µg •day^{-1})	3.5	2.3	4.0	2.5	0.547
	Vitamin C (mg •day^{-1})	106.9	124.0	80.7	60.9	0.461
	Vitamin E (mg •day^{-1})	7.2	5.2	9.4	10.1	0.452
	Copper (mg •day^{-1})	0.9	0.7	0.8	0.5	0.401
	Zinc (mg •day^{-1})	7.5	4.5	7.9	5.6	0.779
	Lut/Zea (µg •day^{-1})	2240	2441	1469	1814	0.274
Cognitive performance	Mean of 15	2.22	0.12	1.20	0.17	<0.001*
	Maximum of 15	2.97	0.24	1.72	0.23	<0.001*
	Baseline (3)	2.06	0.28	0.99	0.27	<0.001*
	Final (3)	2.36	0.24	1.31	0.23	<0.001*
	Change	0.30	0.36	0.35	0.13	0.602

*$p < 0.05$. Top performers were the males and females who performed the best on cognitive testing while poor performers were the males and females who performed the worst on cognitive testing.

between carbohydrate mouth rinses and improved accuracy and precision in cognitive processing (14, 15). However, there is limited research evaluating the relationship of carbohydrate consumption and cognitive performance (15, 16). A small study that evaluated the relationship between three different mouth rinses containing 1.6 g/25 mL carbohydrate, 0.4 g/25 mL guarana complex or 67 mg/25 mL caffeine used at the beginning and twice during a 40-min exercise period reported that all three enhanced temporal activity. The carbohydrate rinse also decreased the rating of perceived exertion (RPE) when compared to the other rinses (16). Another small study that evaluated the impact of maltodextrin mouth rinses also reported that fatigued fencing athletes displayed enhanced accuracy in this skill-based sport after using a maltodextrin mouth rinse (17). Brain imaging of participants using 1.6 g/25 mL maltodextrin mouth rinse displayed enhanced activity in the orbitofrontal cortex of the brain but no influence on reaction rate (18). However, more research on the relationship between habitual carbohydrate consumption and cognitive performance is needed.

Our research showed a significantly negative linear relation between total protein intake and the mean and maximal speed threshold in cognitive testing. Previous investigations reporting a positive relationship between dietary protein consumption and cognitive response were conducted in aging persons, which often consume less protein than the needed protein for maintaining their health (37, 38). However, our population was younger, and many consumed above the RDA for protein, which may have influenced the conflict with the present result. In a study that examined the impact of dietary protein on cognitive performance compared the impact of carbohydrates alone to a protein and carbohydrate mixture, no significant differences in cognitive ability were demonstrated after a

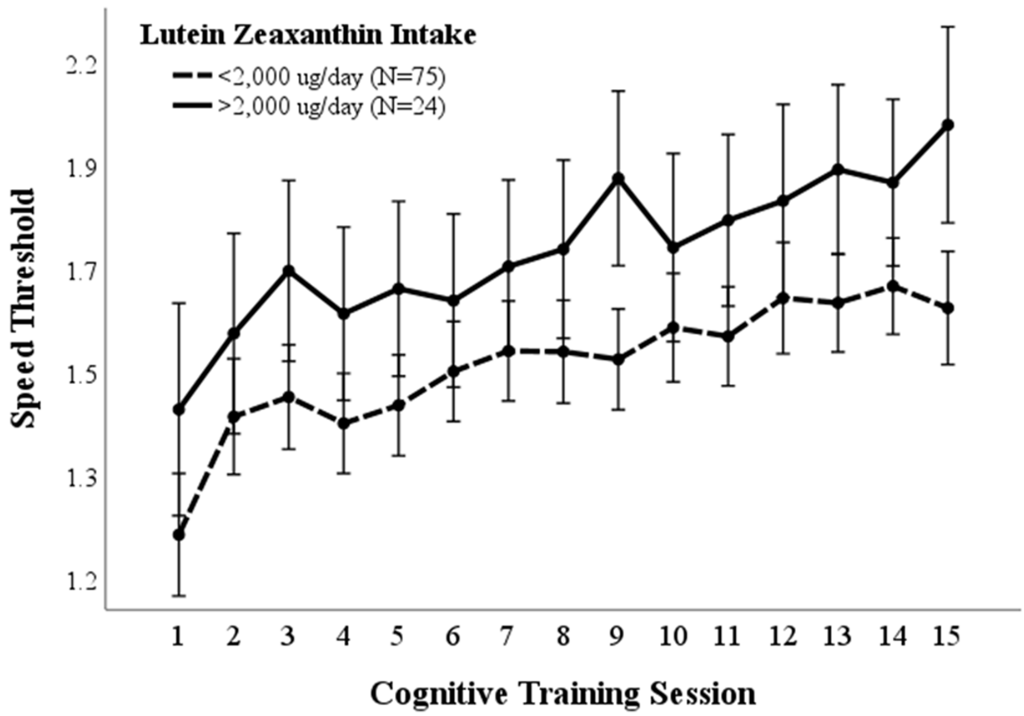

FIGURE 3
Lutein/Zeaxanthin intake. Covariates appearing in the model are evaluated at the following values: Gender=1.6146 Error bars: +/– 2 SE. Repeated measures ANOVA *p*=0.004.

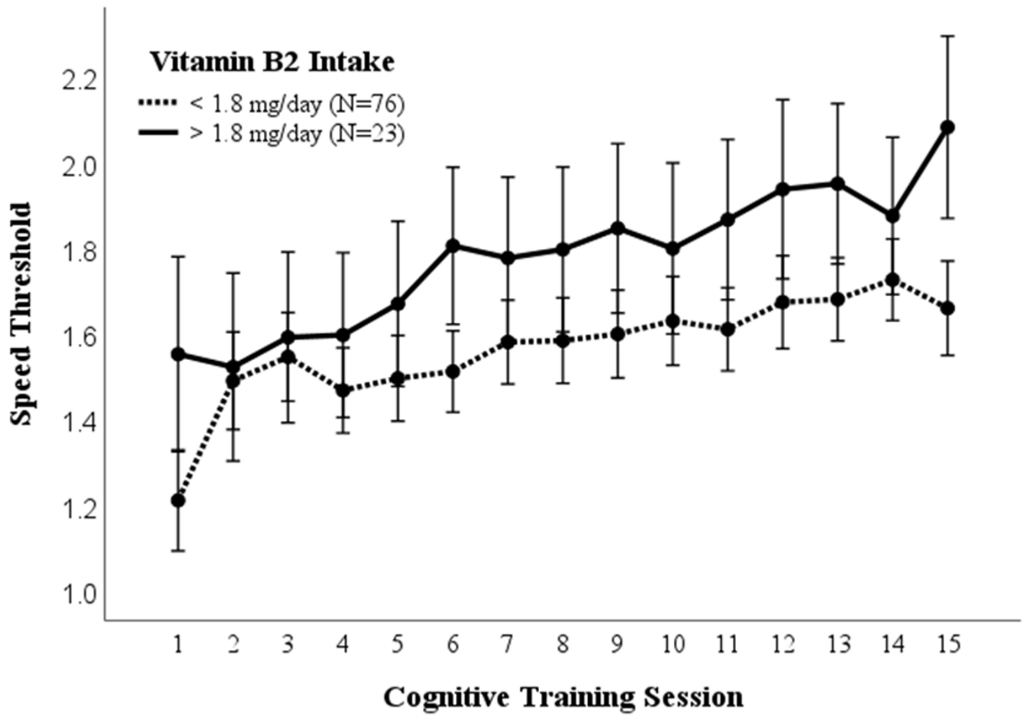

FIGURE 4
Vitamin B2 intake. Covariates appearing in the model are evaluated at the following values: Gender=1.6146, PROTEIN=97.1975 Error bars: +/– 2 SE. Repeated measures ANOVA *p*=0.014.

TABLE 4 Linear regression analysis with neurotracker (NT) mean speed threshold.

Model		Unstandardized coefficients		Standardized coefficients	*t*	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.417	0.326		7.413	<0.001
	Sex (1 = M, 2 = F)	−0.301	0.074	−0.407	−4.073	<0.001
	Protein (% kcal)	−0.019	0.005	−0.346	−3.457	0.001
	Vitamin B2 (1 = low, 2 = high)	0.217	0.089	0.253	2.451	0.017
	Lut/Zea (1 = low, 2 = high)	0.162	0.083	0.189	1.961	0.054
	Age (years)	−0.018	0.011	−0.162	−1.652	0.103

[a]Dependent variable: NT mean.

TABLE 5 Linear regression analysis with neurotracker (NT) maximum speed threshold.

Model		Unstandardized coefficients		Standardized coefficients	*t*	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.523	0.427		8.251	<0.001
	Sex (1 = M, 2 = F)	−0.393	0.097	−0.408	−4.062	<0.001
	Protein (% kcal)	−0.026	0.007	−0.362	−3.600	0.001
	Vitamin B2 (1 = low, 2 = high)	0.317	0.116	0.283	2.728	0.008
	Lut/Zea (1 = low, 2 = high)	0.117	0.108	0.105	1.080	0.284
	Age (years)	−0.030	0.014	−0.215	−2.177	0.033

Dependent variable: NT max.

30-min period of exercise (39). Studies evaluating the impact of protein on cognitive performance most often focus on branched chain amino acids (40) whereas we evaluated total protein consumption.

The present results demonstrating better cognitive performance when intake of carbohydrate was >40% and protein <24% is similar to the acceptable macronutrient distribution ranges (AMDR) recommended by the Institute of Medicine including 45–65% carbohydrates, 10–35% protein, and 20–35% fat (40). These percentages also align with other research studies that support the positive relationship of complex carbohydrates with cognitive performance (11, 12). Many diets promote extreme ratios of macronutrients for distinct reasons including, but not limited to, weight loss and athletic performance (14). However, these diets have not evaluated the impact of the varying macronutrient ratios on cognitive performance suggesting additional investigations of macronutrient ratios and cognitive performance is warranted.

Additionally, the results of this study align with other research studies that show a positive relation between lutein/zeaxanthin and enhanced visual cognitive performance (2, 21, 35, 41). For example, a double-blind placebo-controlled study using a lutein and zeaxanthin supplement showed that this daily supplementation for 1 year improved spatial memory, reasoning ability, and complex attention (42). Additionally, a study investigating just 4 months of lutein and zeaxanthin supplementation showed that, compared to placebo, those receiving the intervention significantly increased visual processing speed in healthy young adults (20). Because lutein/zeaxanthin are not endogenously produced (35), individuals must modify their dietary patterns to include lutein/zeaxanthin to receive the benefits of enhanced visual cognitive performance that these nutrients offer. This, in part, explains why much of the literature surrounding cognitive performance and lutein/zeaxanthin utilize supplementation.

Our data suggest a positive relationship between vitamin B2 (riboflavin) and visual cognitive performance. Prior work has demonstrated riboflavin's role in neurotransmitter synthesis (19). Additionally, dietary riboflavin has been shown to serve as a protective factor for global cognitive ability (43). The relationship between vitamin B2 and cognitive performance resulted in a significant positive result on cognitive performance in those consuming >1.8 mg/day B2, which is greater than the recommended dietary intake for men and women ages 19–51 years. Vitamin B2 is essential for macronutrient metabolism, energy generation, and many other physiological functions including but not limited to antioxidant and anti-inflammatory roles (44).

Limitations of this study include the subject population and the length of the study. The young, college-enrolled population limits the generalization of the results to other age and demographic groups. While we used 10 days of food logs in analyzing dietary intake, reliance on dietary recall to assess nutrient status may result in potential errors due to underreporting which is common in self-reported food records. However, we provided baseline participant education on the level of detail required for reporting and handouts with portion size estimates were given to participants. Food logs were also checked upon submission for detail. Additionally, 10 food records greatly exceed the standard 3 food records for dietary assessment and may capture more of the variability and reflect longer term intake better. Furthermore, this study is limited by the lack of biochemical assessment indicators such as serum analysis and macular pigment optical density for objective evaluation (42, 45–47).

This study provided evidence that several nutrients, particularly lutein/zeaxanthin, vitamin B2, protein, and carbohydrates, contribute to enhanced visual cognitive performance. Athletes who often manipulate macronutrient ratios for muscular performance gains may consider optimizing this balance of protein for muscular gains and carbohydrate for both high energy metabolism and visual cognitive performance. Other micronutrients including lutein/zeaxanthin and B2 offer additional opportunities to enhance/optimize performance of cognitive function outside the traditional skeletal muscle and cardiovascular nutritional interventions and may be highly relevant in aging populations that are impacted by decline in visual cognitive performance.

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 human participants were reviewed and approved by the Texas A&M University Human Subject's Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

NG conducted the research. CS and KB coordinated and supervised the research. KB drafted the manuscript. JG and AR edited the manuscript. SR conception and design of the research, analyzed the data, and interpreted the results of experiments. KB and SR edited and approved final version of manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

References

1. Appelbaum GL, Erickson G. Sports vision training: a review of the state-of-the-art in digital training technique. *Int Rev Sport Exerc Psychol.* (2016) 11:160–89. doi: 10.1080/1750984x.2016.1266376

2. Erickson GB. Optimizing visual performance for sport. *Adv Ophthalmol Optom.* (2018) 3:1–19. doi: 10.1016/j.yaoo.2018.05.001

3. Poltavaki D, Biberdorf D. The role of visual perception measures used in sports vision programmes in predicting actual game performance in division I collegiate hockey players. *J Sports Sci.* (2014) 33:597–608. doi: 10.1080/02640414.2014.951952

4. Mann DT, Williams AM, Ward P, Janelle CM. Perceptual-cognitive expertise in sport: a meta-analysis. *J Sport Exerc Psychol.* (2007) 29:457–78. doi: 10.1123/jsep.29.4.457

5. Elmurr P. The relationship of vision and skilled movement – a general review using cricket batting. *Eye Contact Lens.* (2011) 37:164–6. doi: 10.1097/ICL.0b013e318217cc09

6. Laby DM, Kirschen DG, Pantall P. The visual function of olympic-level athletes – an initial report. *Eye Contact Lens.* (2011) 37:116–22. doi: 10.1097/ICL.0b013e31820c5002

7. Laby DM, Davidson JL, Rosenbaum LJ, Strasser C, Mellman MF, Rosenbaum AL, et al. The visual function of professional baseball players. *Am J Ophthalmol.* (1996) 122:476–85. doi: 10.1016/s0002-9394(14)72106-3

8. Faubert J. Professional athletes have extraordinary skills for rapidly learning complex neutral dynamic visual scenes. *Sci Rep.* (2013) 3:1154. doi: 10.1038/srep01154

9. Moen F, Hrozanova M, Stiles T. The effects of perceptual-cognitive training with neurotracker on executive brain functions among elite athletes. *Cogent Psychol.* (2018) 5:1544105. doi: 10.1080/23311908.2018.1544105

10. Parsons B, Magill T, Boucher A, Zhang M, Zogbo K, Bérubé S, et al. Enhancing cognitive function using perceptual-cognitive training. *Clin EEG Neurosci.* (2014) 47:37–47. doi: 10.1177/1550059414563746

11. Muth AK, Park SQ. The impact of dietary macronutrient intake on cognitive function and the brain. *Clinical Nutr.* (2021) 40:3999–4010. doi: 10.1016/j.clnu.2021.04.043

12. Ding B, Xiao R, Ma W, Zhao L, Bi Y, Zhang Y. The association between macronutrient intake and cognition in individuals aged under 65 in China: a cross-sectional study. *BMJ Open.* (2018) 8:e018573. doi: 10.1136/bmjopen-2017-018573

13. Cermak NM, van Loon LJC. The use of carbohydrates during exercise as an ergogenic aid. *Sports Med.* (2013) 43:1139–55. doi: 10.1007/s40279-013-0079-0

14. Vitale K, Getzin A. Nutrition and supplement update for the endurance athlete: review and recommendations. *Nutrients.* (2019) 11:1289. doi: 10.3390/nu11061289

15. Pomportesa L, Brisswalter J. Carbohydrate mouth rinse effects on physical and cognitive performance: benefits and limitations in sports. *Sci Sports.* (2020) 35:200–6. doi: 10.1016/j.scispo.2020.06.001

16. Pomportes L, Brisswalter J, Casini L, Hays A, Davranche K. Cognitive performance enhancement induced by caffeine, carbohydrate, and guarana mouth rinsing during submaximal exercise. *Nutrients.* (2017) 9:589. doi: 10.3390/nu9060589

17. Rowlatt G, Bottoms L, Edmonds CJ, Buscombe R. The effect of carbohydrate mouth rinsing on fencing performance and cognitive function following fatigue-inducing fencing. *Eur J Sport Sci.* (2017) 17:433–40. doi: 10.1080/17461391.2016.1251497

18. de Pauw K, Roelands B, Knaepen K, Polfliet M, Stiens J, Meeusen R. Effects of caffeine and maltodextrin mouth rinsing on P300, brain imaging, and cognitive performance. *J Appl Physiol*. (2015) 118:776–82. doi: 10.1152/japplphysiol.01050.2014

19. Huskisson E, Maggini S, Ruf M. The influence of micronutrients on cognitive function and performance. *Int J Med Res*. (2007) 35:1–19. doi: 10.1177/147323000703500101

20. Bovier ER, Hammond BR. A randomized placebo-controlled study on the effects of lutein and zeaxanthin on visual processing speed in young healthy subjects. *Arch Biochem Biophys*. (2015) 572:54–7. doi: 10.1016/j.abb.2014.11.012

21. Lien EL, Hammond BR. Nutritional influences on visual development and function. *Prog Retin Eye Res*. (2011) 30:188–203. doi: 10.1016/j.preteyeres.2011.01.001

22. Murakami K, Murata N, Noda Y, Tahara S, Kaneko T, Kinoshita N, et al. SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid β protein oligomerization and memory loss in mouse model of Alzheimer disease. *J Biol Chem*. (2011) 286:44557–68. doi: 10.1074/jbc.M111.279208

23. Naber M, Hommel B, Colzato LS. Improved human visuomotor performance and pupil constriction after choline supplementation in a placebo-controlled double-blind study. *Sci Rep*. (2015) 5:13188. doi: 10.1038/srep13188

24. Bauer I, Crewther DP, Pipingas A, Rowsell R, Cockerell R, Crewther SG. Omega-3 fatty acids modify human cortical visual processing–a double-blind, crossover study. *PLoS One*. (2011) 6:e28214. doi: 10.1371/journal.pone.0028214

25. D'Ascoli TA, Mursu J, Voutilainen S, Kauhanen J, Tuomainen TP, Virtanen JK. Association between serum long-chain omega-3 polyunsaturated fatty acids and cognitive performance in elderly men and women: the Kuopio Ischaemic heart disease risk factor study. *Eur J Clin Nutr*. (2016) 70:970–5. doi: 10.1038/ejcn.2016.59

26. Chew EY, Clemons TE, Agrón E, Domalpally A, Keenan TDL, Vitale S, et al. Long-term outcomes of adding lutein/zeaxanthin and ω-3 fatty acids to the AREDS supplements on age-related macular degeneration progression: AREDS2 report 28. *JAMA Ophthalmol*. (2022) 140:692–8. doi: 10.1001/jamaophthalmol.2022.1640

27. Hetherington R. The Snellen chart as a test of visual acuity. *Psychol Forsch*. (1954) 24:349–57. doi: 10.1007/BF00422033

28. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh sleep quality index: a newinstrument for psychiatric practice and research. *Psychiatry Res*. (1989) 28:193–213. doi: 10.1016/0165-1781(89)90047-4

29. Momenan AA, Delshad M, Sarbazi N, Ghaleh NR, Ghanbarian A, Azizi F. Reliability and validity of the modifiable activity questionnaire (MAQ) in an Iranian urban adult population. *Arch Iran Med*. (2012) 15:279–82.

30. Levitt H. Transformed up-down methods in psychoacoustics. *J Acoust Soc Am*. (1971) 49:467–77. doi: 10.1121/1.1912375

31. Spaner CR, Musteata S, Kenny R, Gawryluk J, Hofer S, Christie BR. 3-dimensional multiple object tracking training can enhance selective attention, psychomotor speed, and cognitive flexibility in healthy older adults. *Ageing Sci Ment Health Stud*. (2019) 3:1–12.

32. Armstrong LE, Maresh CM, Castellani JW, Bergeron MF, Kenefick RW, LaGasse KE, et al. Urinary indices of hydration status. *Int J Sport Nutr Exerc Metab*. (1994) 4:265–79. doi: 10.1123/ijsn.4.3.265

33. Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness a new approach. *J Psychophysiol*. (1973) 10:431–6. doi: 10.1111/j.1469-8986.1973.tb00801.x

34. Ranard KM, Jeon S, Mohn ES, Griffiths JC, Johnson EJ, Erdman JW. Dietary guidance for lutein: consideration for intake recommendations is scientifically supported. *Eur J Nutr*. (2017) 56:37–42. doi: 10.1007/s00394-017-1580-2

35. Arunkumar R, Gorusupudi A, Bernstein PS. The macular carotenoids: a biochemical overview. *Biochim Biophys Acta Mol Cell Biol Lipids*. (2020) 1865:158617. doi: 10.1016/j.bbalip.2020.158617

36. National Institutes of Health Office of Dietary Supplements. (2022). Riboflavin fact sheet for health professionals. Available at: https://ods.od.nih.gov/factsheets/Riboflavin-HealthProfessional/#en2 (Accessed June 25, 2022).

37. Li Y, Li S, Wang W, Zhang D. Association between dietary protein intake and cognitive function in adults age 60 years and older. *J Nutr Health Aging*. (2020) 24:223–9. doi: 10.1007/s12603-020-1317-4

38. Glenn JM, Madero EN, Bott NT. Dietary protein, and amino acid intake: links to the maintenance of cognitive health. *Nutrients*. (2019) 11:1315. doi: 10.3390/nu11061315

39. Sun FH, Cooper SB, Gui Z. Effects of carbohydrate and protein co-ingestion during short-term moderate-intensity exercise on cognitive function. *J Sports Med Phys Fitness*. (2020) 60:656–63. doi: 10.23736/S0022-4707.20.10322-0

40. Lindsey B, Nuccio RP, Jeukendrup AE. Acute effects of dietary constituents on motor skill and cognitive performance in athletes. *Nutr Rev*. (2014) 72:790–802. doi: 10.1111/nure.12157

41. Renzi-Hammond LM, Bovier ER, Fletcher LM, Miller L, Mewborn C, Lindbergh C, et al. Effects of a lutein and zeaxanthin intervention on cognitive function: a randomized, double-masked, placebo-controlled trial of younger healthy adults. *Nutrients*. (2017) 9:1246. doi: 10.3390/nu9111246

42. Kijlstra A, Tian Y, Kelly ER, Berendschot TTJM. Lutein: more than just a filter for blue light. *Prog Retin Eye Res*. (2012) 31:303–15. doi: 10.1016/j.preteyeres.2012.03.002

43. Tao L, Liu K, Chen S, Yu H, An Y, Wang Y, et al. Dietary intake of riboflavin and unsaturated fatty acid can improve the multi-domain cognitive function in middle-aged and elderly populations: a 2-year prospective cohort study. *Front Aging Neurosci*. (2019) 11:226. doi: 10.3389/fnagi.2019.00226

44. Suwannasom N, Kao I, Pruß A, Georgieva R, Bäumler H. Riboflavin: the health benefits of a forgotten natural vitamin. *Int J Mol Sci*. (2020) 21:950. doi: 10.3390/ijms21030950

45. Abdel-Aal ESM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. *Nutrients*. (2013) 5:1169–85. doi: 10.3390/nu5041169

46. Lieblein-Boff JC, Johnson EJ, Kennedy AD, Lai CS, Kuchan MJ. Exploratory metabolomic analyses reveal compounds correlated with lutein concentration in frontal cortex, hippocampus, and occipital cortex of human infant brain. *PLoS One*. (2015) 10:1–19. doi: 10.1371/journal.pone.0136904

47. Vishwanathan R, Schalch W, Johnson EJ. Macular pigment carotenoids in the retina and occipital cortex are related in humans. *Nutr Neurosci*. (2016) 19:95–101. doi: 10.1179/1476830514Y.0000000141

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Perspectives and practices of nutritionists on dietary supplements for elite soccer teams: a cross-sectional survey study

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Introduction and objectives: Dietary supplements are part of the nutritional strategies frequently applied in sports performance support. With growing research on this subject and high demand from athletes, nutritionists need to keep up to date with the latest evidence and utility of dietary supplements, particularly in real-world contexts. As information about the use of dietary supplements among elite soccer players is still scarce, this work aimed to know how nutritionists working with elite soccer teams perceive and use these substances in their daily practice.
Methods: A questionnaire previously used to describe nutritionists' beliefs and attitudes regarding the use of dietary supplements in a clinical context was adapted for this study. The online questionnaire was addressed to nutritionists working with elite soccer teams from six European Leagues and Brazil, between November 2022 and February 2023.
Results: Overall, the participants considered themselves well-trained (76.9%), knowledgeable (95.4%), and interested in dietary supplements (95.4%). The majority (70.8%) of the participants agreed or strongly agreed to recommend dietary supplements to soccer players. Personal usage of dietary supplements was associated with recommending supplements ($p < 0.001$), but no relationships were found with years of experience and academic level.
Discussion: Nutritionists working with elite soccer players consider the use of dietary supplements for performance-enhancement purposes and not only to compensate for nutritional deficits, which might contribute to their higher interest, training and perceived knowledge about this topic. Participants recognize players' interest in dietary supplements, and are mindful of the safety and efficacy of these products. The present study suggests that nutritionists working with elite soccer teams are among the highest prescribers of dietary supplements, although personal usage is lower than that of nutritionists working in a clinical context.

KEYWORDS
football, questionnaire, ergogenic, recovery, nutrition knowledge

Highlights

- Knowledge about the perspectives and practices of nutritionists working with elite soccer teams are important to better understand the reasons for recommendation and use of dietary supplements.
- Most nutritionists in this survey recommend dietary supplements to elite soccer players, and are aware of the challenges regarding safety and effectiveness.
- Nutritionists working with elite soccer teams are interested in dietary supplements and also recognize the importance of training and research in this area.

1. Introduction

Nutrition care is well established as part of support strategies used to improve performance and recovery in soccer (1). Nevertheless, the way nutritional strategies are applied can change considerably depending on numerous factors, such as players' individual preferences or team competitive calendars, and has deserved the attention of researchers and practitioners in recent years. Energy and nutrient requirements (2, 3) and the effects of dietary supplements are some of the topics studied in the context of elite soccer. Available data suggest a high prevalence of the use of dietary supplements by elite soccer players (4, 5). Given the growing number of research enrolling dietary supplements and the high number and variety of products available on the market, it is relevant to understand better how these substances are being used in soccer.

Several definitions exist for dietary supplements, including those established by regulatory entities [such as EFSA in the European Union (6)]. However, for the purpose of the present study, the definition recently proposed in the International Olympic Committee (IOC) consensus statement ("a food, food component, nutrient, or non-food compound that is purposefully ingested in addition to the habitually-consumed diet with the aim of achieving a specific health and/or performance benefit") was considered the most suitable (7). This includes substances such as caffeine, creatine, or vitamins, and products like isotonic drinks or protein shakes, typically used among soccer players (8). Recent publications have described the evidence regarding the benefits of dietary supplements in soccer players' performance or recovery (1, 9). However, information on how nutritionists address the use of these products remains scarce.

To our knowledge, the first work addressing the practices and recommendations of sports nutritionists was conducted by Grandjean in 1993 (10). In this survey, it was found a positive correlation ($p < 0.008$) between personal usage of dietary supplements and the recommendation of these products to athletes. Nevertheless, most of the professionals worked with recreational athletes and were more concerned about encouraging a healthy diet than improving athletic performance. More recently, other surveys assessed nutrition knowledge, practices and perceptions of nutritionists regarding dietary supplements (11–14), but research was focused on a clinical context, and the use of dietary supplements to balance food intake or address particular health conditions. In the context of sports nutrition, Wardenaar and Hoogervorst (15), in a survey conducted among sports health professionals (18% of which were nutritionists) working with Olympic and non-Olympic athletes, noted that sports dietitians were ranked as the most knowledgeable professionals about nutritional supplements (74%). Not surprisingly, nutritionists have been considered among the most well-informed professionals and preferred source of information about dietary supplements in surveys conducted among athletes (16). Thus, it is important to better understand how nutritionists' perspectives on dietary supplements can influence their use by athletes, particularly at the elite level.

In elite soccer, studies assessing the use of dietary supplements among players are still scarce (4, 5, 8) and the available results pointed out health concerns as the main reason for using these products. Yet, accelerate recovery, improve performance, and prevent injury are also among the most frequently reported motivations for using dietary supplements. These data suggest that male and female elite players consider dietary supplements as part of the strategies for dealing with physical and physiological demands of elite soccer. Though, since the training and playing demands have increased (17), support teams, namely health and performance professionals, are more required to attend to the needs imposed by such demanding fixtures properly. The high number of matches per season [nowadays, some players from the most competitive teams can play over 60 games per season (18)] and the popularity of soccer also mean more visibility and scrutiny of the practices and procedures of players and teams. Additionally, as elite teams and players are considered the benchmark of good practices for all soccer players, nutritionists working in the context of elite sports must be able to ensure evidence-based nutritional strategies are adjusted to the very specific conditions and needs of the real world. Thus, the present study aimed to assess the perceptions and practices of nutritionists supporting elite soccer teams regarding dietary supplements.

2. Materials and methods

2.1. Participants and settings

The scope of this work was limited to nutritionists working with soccer players from elite clubs. A list of the nutritionists working with elite soccer teams from six European leagues (English Premier League, Spanish La Liga, Italian Serie A, German Bundesliga, French Ligue 1, and Portuguese Primeira Liga) and Brazil (Serie A) was defined, based on authorś personal knowledge, as well as information available from each club. After approval by the Ethical Committee of the Portugal Football School (PFS 16/2022), the nutritionists were contacted via e-mail and professional and personal networks. Invitations to participate were also addressed to all clubs via institutional e-mail.

Data collection took place between November 7th 2022 and February 17th 2023 using an anonymous questionnaire (available in **Supplementary Appendix S1**), adapted from a study used for a similar purpose (12) after obtaining the authors' permission. The questionnaire was provided in English and administered online using Microsoft Forms®.

2.2. Variables and instruments

The questionnaire used in the present study comprised 19 questions grouped into three sections: (a) participants' information; (b) perceptions about dietary supplements; and (c) practices regarding the use and recommendation of dietary supplements. Overall, questions from the original questionnaire used by Marx et al. (12) were kept, but since it was focused on nutritionists working within a clinical context, some minor adaptations were applied in order to fit the nutritional context of elite soccer. Generally, questions using the word "patients" were adapted to "soccer players" and questions about the perceptions and recommendation of dietary supplements were adapted to include answers regarding the conditions faced by sports nutritionists (such as, athletic performance enhancement or fatigue recovery).

2.2.1. Information from participants

Information from participants was collected via open-ended and multiple-choice questions. Data collected in this section included the following: age; contractual relationship with the club; experience, measured as years working with nutrition in a soccer club context; education, measured as completed degree; and workload with soccer players.

2.2.2. Perceptions about dietary supplements

In this section, several perceptions about dietary supplements were assessed using a list of 20 sentences, to which participants were asked to respond using a 5-point Likert scale (from 1, strongly disagree, to 5, strongly agree). Questions included statements regarding participants perception about their personal interest, training, and knowledge in dietary supplements, about nutritionists' role in research, education and prescription of dietary supplements, and also about dietary supplements use in soccer.

2.2.3. Practices regarding the use and recommendation of dietary supplements

Finally, the use and recommendation of dietary supplements was also assessed with multiple-choice questions. Participants were asked if they personally used dietary supplements in the past 6 months, and if they sell dietary supplements as part of their practice. Recommendation of dietary supplements, as well as barriers and enablers for recommending were also assessed in this section.

2.3. Statistical methods

Statistical analysis was performed using Jamovi 2.3.21.0 software. Normal distribution was tested using the Shapiro-Wilk test. Descriptive statistics were reported as percentages, as mean (standard deviation) for variables following a normal distribution (i.e., age), or median (Q_1; Q_3) for variables not following a normal distribution (i.e., participants' experience). The items of the 5-point Likert-type scales were grouped into three categories for the analysis (strongly disagree and disagree; neutral; agree and strongly agree). The Mann-Whitney test was used to compare the difference between the experience (continuous variable, not normally distributed) of participants selling and not selling dietary supplements (nominal variable, dichotomous). As Likert-type scales (e.g., recommending dietary supplements) can be regarded as both nominal and ordinal variables, the following analysis were conducted. The Kruskal-Wallis test was used to compare the difference between recommending dietary supplements (nominal variable, three independent groups) with regard to participants' experience (continuous variable, not normally distributed). Spearman's correlation was used to assess the relationship between recommending dietary supplements (ordinal variable) and the participants' education (ordinal variable) and participants' experience (continuous variable, not normally distributed). The Fisher's exact test was used to assess the association between selling (nominal variable, dichotomous) and taking dietary supplements (nominal variable, dichotomous). Finally, the Freeman-Halton extension of the Fisher's exact test was used to assess the association between recommending dietary supplements (nominal variable, three independent groups) and selling dietary supplements (nominal variable, dichotomous) and between recommending and taking dietary supplements (nominal variable, dichotomous). Values of $p < 0.05$ were considered statistically significant.

3. Results

One hundred thirty-eight invitations were sent out, with four recused or undelivered. From those, 69 responses were received (50.0% response rate); however, three belonged to nutritionists who refrained from participating due to their club's policy. Of the 66 questionnaires obtained, one was excluded due to incorrect fulfillment. Therefore, 65 participants were included in the statistical analysis presented (**Figure 1**).

A resume of participants' characteristics can be found in **Table 1**.

3.1. Perceptions regarding dietary supplements

Results from the questions assessing nutritionists' perceptions regarding dietary supplements using a 5-point Likert-type scale

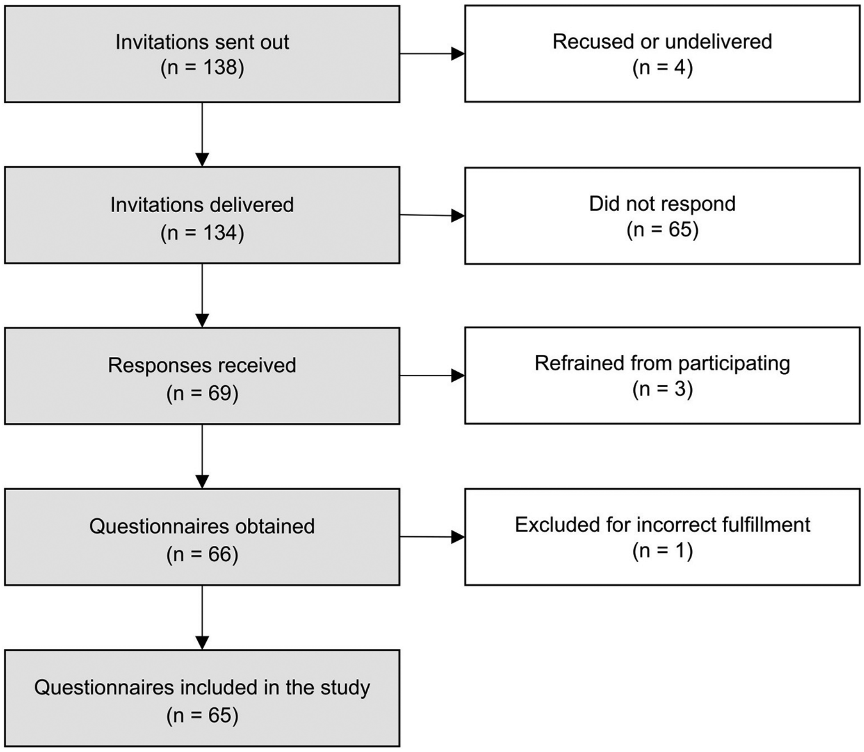

FIGURE 1
Flow diagram of participant recruitment.

TABLE 1 Participants' characteristics.

Age	34.7 (5.6) years
Years as a nutritionist in soccer	6.0 (4.0; 9.3) years
Education	
Diploma	9.2%
Bachelor	26.2%
Masters	50.8%
PhD	13.8%
Contractual relationship with soccer club	
Full-time	60.0%
Part-time	32.3%
Consultant/advisor	7.7%
Workload spent with soccer players	
Daily	72.3%
At least once a week	26.2%
Occasionally (every 2 weeks, once a month, beginning of season, upon request)	1.5%

Values are expressed as percentages, mean (standard deviation), or median (Q_1; Q_3). Data were available for all participants (n = 65) except for age (n = 63) and years as a nutritionist in soccer (n = 64).

are summarized in Figure 2. Almost all participants reported interest in dietary supplements (95.4% agreed or strongly agreed with this statement) and considered themselves knowledgeable about supplementation (95.4%). When asked if they were well trained in dietary supplements, 76.9% of participants agreed or strongly agreed, and 87.7% agreed or strongly agreed that they can access trustworthy information about dietary supplements.

Concerning participants' perceptions about their professional colleagues, most believe that nutritionists/dietitians are knowledgeable about dietary supplements (67.7% agreed or strongly agreed with this statement) and that nutritionists/dietitians should be considered an authority on dietary supplements (93.8%). Additionally, most participants agreed or strongly agreed that nutritionists/dietitians should play a more significant role in the prescription of dietary supplements (83.1%), education regarding dietary supplements (98.5%), and research on the use of dietary supplements (96.9%).

Regarding participants' perception of the efficacy and safety of dietary supplements, approximately half believe dietary supplements are effective (53.8% agreed or strongly agreed with this statement); however, only 23.1% agreed or strongly agreed that dietary supplements are safe.

When asked who the primary source of information on dietary supplements should be, almost all participants pointed out nutritionists/dietitians (98.5%), followed by medical doctors (55.4%), pharmacists (24.6%), and sports scientists (24.6%). Conversely, when participants were asked who the primary source of information was, the most frequent responses were nutritionists/dietitians (73.8%), coach/fitness coaches (67.7%), and friends and family (53.8%).

Sports performance (physical and physiological) (93.8%), fatigue recovery (81.5%), and sports performance (mental and

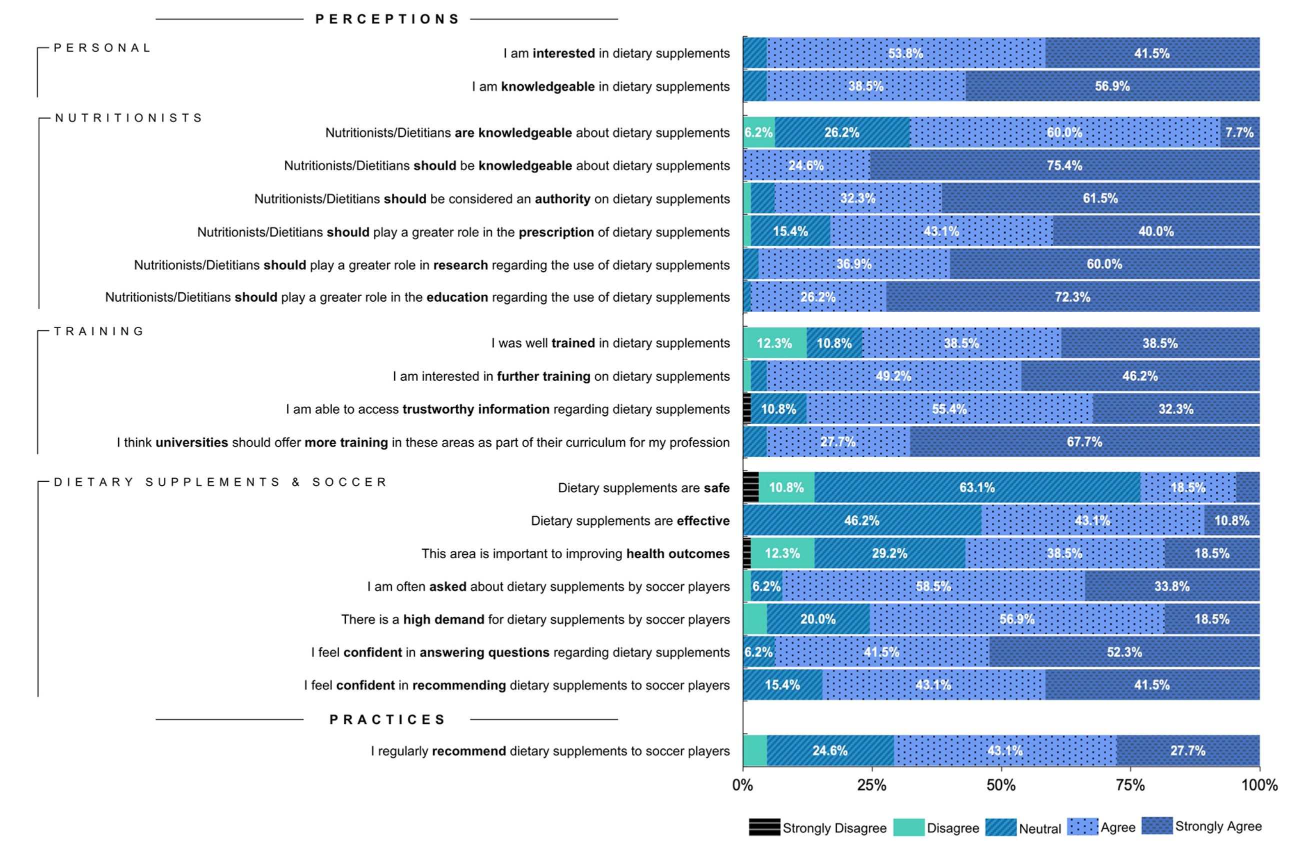

FIGURE 2
Nutritionists' perceptions regarding dietary supplements.

cognitive) (76.9%) were mentioned as the areas where dietary supplements are most effective.

Most participants agreed that they are often asked about dietary supplements (92.3% agreed or strongly agreed with this statement) and recognized a high demand for dietary supplements by soccer players (75.4% agreed or strongly agreed with this statement).

Finally, participants considered that the topics they would like to learn more about were the usage of dietary supplements for sports performance (69.2%), specific dietary supplements (53.8%), and reliable sources of information (30.8%).

3.2. Practices regarding dietary supplements

Of all participants, 15.4% admitted selling dietary supplements, with all of them considering there is no conflict of interest in such practice. When asked about their personal use of dietary supplements, 58.8% reported taking at least one in the last 6 months.

Participants reported getting information regarding dietary supplements mainly from evidence databases and academic journals (93.8%), followed by conferences (58.5%), and guidelines by their professional bodies (43.1%). Regarding the minimum level of evidence required before feeling confident in utilizing or recommending specific dietary supplements to soccer players, most participants reported systematic reviews (44.6%), randomized controlled trials (36.9%), and meta-analysis (7.7%).

Although 26.2% of participants reported not perceiving barriers to recommending dietary supplements to soccer players, concerns about the regulation of dietary supplements (50.8%), potential adverse effects of dietary supplements (26.2%), and perceived lack of efficacy and quality of dietary supplements (both with 24.6% of responses), were the most frequent answers. On the other hand, enablers to recommending the use of dietary supplements to soccer players included sufficient training (78.5%), sufficient autonomy to recommend dietary supplements (63.1%), and sufficient research to show the efficacy, as well as high-quality dietary supplements available on the market (both options with 47.7% of responses).

Regarding the factors that might relate to the recommendation of dietary supplements, relationships between individual factors (i.e., personal use, selling, experience, and education) and advice on dietary supplements were investigated. No differences were found between the median years of experience of participants who sell and those who do not sell dietary supplements ($p = 0.656$). In addition, the median years of experience did not differ with regard to recommending dietary supplements ($p = 0.225$). An association between personal usage of dietary supplements and recommending dietary supplements to soccer players was found ($p < 0.001$). However, no association was found between selling dietary supplements and recommending their use (p = 0.123), nor between selling supplements and use them personally (p = 0.175). Finally, no relationships between nutritionists' experience (p = 0.131) or education (diploma, bachelor, master, or Ph.D.) (p = 0.861) and recommending dietary supplements were found.

4. Discussion

In general, nutritionists working with Europe and Brazil's top soccer teams demonstrate a high interest in dietary supplements and consider themselves knowledgeable about them. Most participants on this survey regularly recommend supplements to elite soccer players, and most also personally use them.

To the best of our knowledge, this is the first time that the practices and perceptions of nutritionists working in the context of elite soccer teams regarding dietary supplements have been explored. In 1993, Grandjean (10) assessed sports nutritionists' practices, including recommendations regarding dietary supplements, among others. More recently, similar works have assessed the beliefs and attitudes towards dietary supplements of nutritionists working in a clinical context (11–13). Notably, differences in some of the objectives and methodology between studies make it difficult to compare results. Even so, topics such as interest, perceived knowledge, and recommendation of dietary supplements, as well as personal usage by nutritionists, were assessed in these studies.

In our work, 95.4% of the nutritionists agreed or strongly agreed that they are interested in dietary supplements, a higher percentage than that found in previous studies: in 2006, Hetherwick et al. (11) found that 87% of registered dietitians in the USA were interested in dietary supplements, and, in 2016, Marx et al. (12) reported that 68% of dietitians in Australia agreed or strongly agreed that they are interested in dietary supplements.

When comparing the perceived knowledge about dietary supplements, we can also observe differences between nutritionists working with elite soccer teams (95.4% agreed or strongly agreed that they are knowledgeable about dietary supplements, according to our findings) and those working in the clinical context [60% agreed or strongly agreed that they are knowledgeable about dietary supplements, in the survey by Marx et al. (12)]. More recently, a survey conducted among licensed dietitians working in a clinical context in Lebanon (13) assessed knowledge about dietary supplements, founding that 30% had a good knowledge score and 46% had a very good knowledge score.

Regarding their practices, 70.8% of nutritionists working with elite soccer teams agreed or strongly agreed that they regularly recommend dietary supplements. These results compare similarly with the ones found among registered dietitians in California (74% of participants declared to recommend dietary supplements) (13), but are higher than those reported by more recent works: only 27% of dietitians in Australia agreed or strongly agreed that they regularly recommend dietary supplements (12). And in 2021, 39.5% of licensed dietitians in Lebanon reported recommending dietary supplements to their patients (13).

Interestingly, in our study, the percentage of nutritionists declaring to use dietary supplements personally (58.8%) was lower when compared to the results from the surveys carried out with professionals working in a clinical context; 65% of dietitians in Australia (12), 69% of dietitians in California (11) and 73.7% of dietitians in Lebanon (13) declared to use dietary supplements. In the survey conducted by Grandjean (10), 55.0% of the sports nutritionists declared using dietary supplements. On the contrary, we found a higher percentage (15.4%) of nutritionists claiming to sell dietary supplements compared to 4% reported by Marx et al. (12) and 5.3% in the work of Nacouzi et al. (13).

The specific context of sports nutrition, where dietary supplements can be used for performance-enhancement purposes and not only to compensate for nutritional deficits or, allegedly, prevent diseases, might explain some of the differences between the results observed in our survey and those conducted among dietitians working in a clinical context. Dietary supplements such as creatine, caffeine, or protein powders currently gather broad consensus and evidence regarding the benefits for athletes' physical performance and body composition management (1, 7, 19–21), meaning that nutritionists working with elite soccer players may be more likely to recommend these substances. Also, the health and performance demands of elite soccer might also explain why nearly all nutritionists who participated in our survey considered themselves interested and knowledgeable about dietary supplements, more than their fellows working in a clinical context (11–13). These results should be interpreted with caution as these variations might also be related to differences in cultural habits, purchase power, and availability of dietary supplements in each country, as studies were conducted in the United States of America (specifically in California), Australia, Lebanon, and, in our study, in six different European countries and Brazil. It is also worth comparing the results from our survey with the available data from elite players' responses to questionnaires about the usage of dietary supplements. Although 70.8% of the nutritionists inquired agreed or strongly agreed to regularly recommended dietary supplements, reported use of these substances by elite players ranges between 82.0% (8) and 98.2% (5). This might suggest that players can be using dietary supplements by themselves or recommended by others than the nutritionists from their clubs. But it should be mentioned that the available studies regarding the usage of dietary supplements by elite players are from Turkey (5), Saudi Arabia (4) or female National Teams (8), whilst our survey was conducted with nutritionists from six elite European Leagues and Brazil.

Additionally, it would be worth understanding if the dietary supplements reportedly used by elite players coincide with those recommended by nutritionists and with established evidence regarding their benefits. Although the questionnaire used did not assess which dietary supplements participants recommend to players, sports drinks, vitamins (vitamin C, vitamin D, and multivitamins) and minerals (namely, magnesium) have been the most frequently reported by soccer players in previous studies (4, 5, 8). Participants in our survey recognize sports performance (athletic and cognitive) and fatigue recovery as the areas where dietary supplements can be most effective, which is supported by evidence from recent systematic reviews with professional and elite soccer players (22, 23). Even if it is not possible to directly compare results from available surveys conducted with players and nutritionists, it would be relevant to understand if there are differences between practitioners' recommendations and dietary supplements used by players, as this raises efficacy and safety questions. Therefore, future research should address these issues, either through validated tools for the specific context of elite soccer, or through the application of questionnaires to nutritionists and players supported by them. We believe this will allow a better understanding of how nutritionists' perceptions and practices impact elite soccer players' dietary supplement usage and, consequently, their overall performance.

4.1. Strengths and limitations

To our knowledge, this is the first work about the perceptions and practices of nutritionists on dietary supplements for elite soccer players. Research with elite athletes is typically more scarce, either because these athletes are fewer, or because their availability to participate in studies is also more limited. Given the role of nutritionists working in the context of elite soccer in the definition and application of nutritional strategies, this survey allows a first portrait of the opinion of these professionals and their recommendations. The results of this work clearly show that nutritionists working with major leagues clubs are interested and dedicated to knowing more about dietary supplements and their applications in the specific context of elite soccer. Additionally, the application of instruments already used in previous surveys, contributes to a better understanding of the perspectives of these nutritionists, when compared with colleagues who work in a clinical context.

Even so, the present survey has some limitations to consider in future research. Given that the questionnaire used in this work was adapted from one designed and validated for a clinical context, it should be considered the development of a specific questionnaire for professionals working in sports nutrition. This questionnaire may include not only the perceptions, but also directly assess some nutritional knowledge. Finally, it will also be relevant that the tools to be developed for this purpose include questions about which supplements are recommended by nutritionists and reasons for doing so.

5. Conclusions

Current evidence about the benefits of dietary supplements on athletic performance and recovery, paired with the great interest shown by players, are important reasons for knowing perceptions and practices of nutritionists working with elite soccer teams. For the first time, these practitioners' perspectives were explored, resulting in better knowledge on their opinion about dietary supplements and their recommendation to elite soccer players. Next steps in the research of this topic should contribute to understand how the nutritionists' recommendations are followed by players.

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.

Ethics Statement

Approval was obtained by the Ethical Committee of the Portugal Football School (PFS 16/2022).

Author contributions

Conceptualization and methodology: RA, JB, VT; Distribution of questionnaires and data collection: RA; Data analysis: RA, CO; writing—original draft preparation: RA, CO; writing—review and editing: JB, VT. All authors contributed to the article and approved the submitted version.

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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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspor.2023.1230969/full#supplementary-material

References

1. Collins J, Maughan RJ, Gleeson M, Bilsborough J, Jeukendrup A, Morton JP, et al. UEFA expert group statement on nutrition in elite football. Current evidence to inform practical recommendations and guide future research. *Br J Sports Med*. (2021) 55(8):416. doi: 10.1136/bjsports-2019-101961

2. Hulton AT, Malone JJ, Clarke JD, MacLaren DPM. Energy requirements and nutritional strategies for male soccer players: a review and suggestions for practice. *Nutrients*. (2022) 14(3). doi: 10.3390/nu14030657

3. Danielik K, Książek A, Zagrodna A, Słowińska-Lisowska M. How do male football players meet dietary recommendations? A systematic literature review. *Int J Environ Res Public Health*. (2022) 19(15). doi: 10.3390/ijerph19159561

4. Aljaloud SO, Ibrahim SA. Use of dietary supplements among professional athletes in Saudi Arabia. *J Nutr Metab*. (2013) 2013:245349. doi: 10.1155/2013/245349

5. Günalan E, Çavak EY, Turhan S, Cebioğlu K, Domínguez R, Sánchez-Oliver AJ. Dietary supplement use of Turkish footballers: differences by sex and competition level. *Nutrients*. (2022) 14(18). doi: 10.3390/nu14183863

6. EFSA. Directive 2002/46/EC of the European parliament and of the council of 10 June 2002 on the approximation of the laws of the member states relating to food supplements. In: T.E.P.A.T.C.O.T.E. UNION, editor. 2002/46/EC. (2002). p. 6. Official Journal.

7. Maughan RJ, Burke LM, Dvorak J, Larson-Meyer DE, Peeling P, Phillips SM, et al. IOC consensus statement: dietary supplements and the high-performance athlete. *Int J Sport Nutr Exerc Metab*. (2018) 28(2):104–25. doi: 10.1123/ijsnem.2018-0020

8. Oliveira CB, Sousa M, Abreu R, Ferreira Â, Figueiredo P, Rago V, et al. Dietary supplements usage by elite female football players: an exploration of current practices. *Scand J Med Sci Sports*. (2022) 32:73–80. doi: 10.1111/sms.14001

9. Abreu R, Figueiredo P, Beckert P, Marques JP, Amorim S, Caetano C, et al. Portuguese football federation consensus statement 2020: nutrition and performance in football. *BMJ Open Sport Exerc Med*. (2021) 7(3). doi: 10.1136/bmjsem-2021-001082

10. Grandjean AC. Practices and recommendations of sports nutritionists. *Int J Sport Nutr*. (1993) 3(2):232–42. doi: 10.1123/ijsn.3.2.232

11. Hetherwick C, Morris MN, Silliman K. Perceived knowledge, attitudes, and practices of California registered dietitians regarding dietary supplements. *J Am Diet Assoc*. (2006) 106(3):438–42. doi: 10.1016/j.jada.2005.12.005

12. Marx W, Kiss N, McKavanagh D, Isenring E. Attitudes, beliefs and behaviours of Australia dietitians regarding dietary supplements: a cross-sectional survey. *Complement Ther Clin Pract*. (2016) 25:87–91. doi: 10.1016/j.ctcp.2016.09.002

13. Nacouzi C, Tarazi V, Kabalane L, Hosry M, Hleyhel M. Knowledge, use and practices of licensed dietitians related to dietary supplements in Lebanon. *Public Health Nutr*. (2021) 25(7):1–11. doi: 10.1017/s136898002100495x

14. Dickinson A, Bonci L, Boyon N, Franco JC. Dietitians use and recommend dietary supplements: report of a survey. *Nutr J*. (2012) 11:14. doi: 10.1186/1475-2891-11-14

15. Wardenaar FC, Hoogervorst D. How sports health professionals perceive and prescribe nutritional supplements to olympic and non-olympic athletes. *Int J Environ Res Public Health*. (2022) 19(19). doi: 10.3390/ijerph191912477

16. Daher J, Mallick M, El Khoury D. Prevalence of dietary supplement use among athletes worldwide: a scoping review. *Nutrients*. (2022) 14(19). doi: 10.3390/nu14194109

17. Bush M, Barnes C, Archer DT, Hogg B, Bradley PS. Evolution of match performance parameters for various playing positions in the English premier league. *Hum Mov Sci*. (2015) 39:1–11. doi: 10.1016/j.humov.2014.10.003

18. Carling C, Le Gall F, Dupont G. Are physical performance and injury risk in a professional soccer team in match-play affected over a prolonged period of fixture congestion? *Int J Sports Med*. (2012) 33(1):36–42. doi: 10.1055/s-0031-1283190

19. Jäger R, Kerksick CM, Campbell BI, Cribb PJ, Wells SD, Skwiat TM, et al. International society of sports nutrition position stand: protein and exercise. *J Int Soc Sports Nutr*. (2017) 14:20. doi: 10.1186/s12970-017-0177-8

20. Kreider RB, Kalman DS, Antonio J, Ziegenfuss TN, Wildman R, Collins R, et al. International society of sports nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. *J Int Soc Sports Nutr*. (2017) 14:18. doi: 10.1186/s12970-017-0173-z

21. Guest NS, Van Dusseldorp TA, Nelson MT, Grgic J, Schoenfeld BJ, Jenkins NMD, et al. International society of sports nutrition position stand: caffeine and exercise performance. *J Int Soc Sports Nutr*. (2021) 18(1):1. doi: 10.1186/s12970-020-00383-4

22. Aguinaga-Ontoso I, Guillen-Aguinaga S, Guillen-Aguinaga L, Alas-Brun R, Guillen-Grima F. Effects of nutrition interventions on athletic performance in soccer players: a systematic review. *Life (Basel)*. (2023) 13(6). doi: 10.3390/life13061271

23. Abreu R, Oliveira CB, Costa JA, Brito J, Teixeira VH. Effects of dietary supplements on athletic performance in elite soccer players: a systematic review. *J Int Soc Sports Nutr*. (2023) 20(1):2236060. doi: 10.1080/15502783.2023.2236060

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Marine-derived protein: peptide bioresources for the development of nutraceuticals for improved athletic performance

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1. Introduction

Marine ecosystems, a prominent reservoir of biodiversity, have been globally acknowledged for their vast potential as sources of food and bioactive compounds. In recent years, an increasing number of studies have reported the discovery of novel proteins and peptides from marine organisms, thereby highlighting the untapped potential of these resources (1). These marine-derived proteins and peptides, owing to their unique amino acid composition, bioavailability, and bioactive properties, are being explored as a promising source of nutraceuticals and functional food ingredients (2) (**Table 1**).

Functional foods, or nutraceuticals, are dietary elements with added ingredients that offer health benefits beyond basic nutrition. They serve to enhance overall health, boost the immune system, reduce the risk of illness, and manage health conditions. Among the array of potential ingredients for functional foods, peptides have garnered significant attention. Peptides are short chains of amino acids that can be designed to have specific physiological benefits based on their structure and composition. They have been observed to possess various bioactive properties, such as antioxidant, antimicrobial, and anti-inflammatory activities, which potentially contribute to human health and wellbeing (3, 4).

The rapidly growing interest in functional foods is mirrored in the field of sports nutrition, where diet strategies aimed at optimizing athletic performance and recovery have become progressively more nuanced and specialized. Athletes continuously seek

TABLE 1 Marine-derived protein observed in several studies.

	Products/samples	Value/bioactivities	Reference
1	Antioxidant peptide Leu-Trp-His-Thr-His (LWHTH) from *Styela clava* (marine tunicate) (48)	ACE-Inhibitor	Kang et al. (2020)
2	Novel NCWPFQGVPLGFQAPP peptide (NCW peptide) from *Marphysa sanguinea* (marine polychaeta) (49)	antioxidant and anti-inflammatory	Park et al. (2020)
3	PFAOP peptide from *Pinctada fucata* (marine bivalve) (50)	Antioxidant	Ma et al. (2021)
4	HVGGCG peptide from *Oratosquilla woodmasoni* (marine squilla) (51)	ACE-Inhibitor and antioxidant	Joshi et al. (2021)
5	Gln-Trp-Arg Peptide from *Gadus chalcogrammus* (marine fish) (52)	Enhance glucose uptake to the muscle and lower blood glucose level	Ayabe et al. (2015)
6	Phe-Gly-Met-Pro-Leu-Asp-Arg (FGMPLDR; MW 834.41 Da) and Met-Glu-Leu-Val-Leu-Arg (MELVLR; MW 759.43 Da) peptide from *Ulva intestinalis* (microalgae) (53)	ACE-Inhibitor	Sun et al. (2019)
7	SFYYGK, RLVPVPY, and YIGNNPAKG peptide from *Gracilariopsis lemaneiformis* (marine red algae) (54)	ACE-Inhibitor	Su et al. (2022)
8	two phycobiliproteins (PBP): C-phycocyanin (C-PC) and allophycocyanin (APC) from *A. plantensis* (microalgae) (8)	Improve glucose metabolism	Karunarathne et al. (2020)
9	Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe (chlorella-11) from *C. vulgaris* and Leu-Asn-Gly-Asp-Val-Trp from *C. ellpsiodea* (microalgae) (55)	anti-inflamatory, blood glucose regulator	Ramos-Romero et al. (2021)
10	Skipjack Enzymatic Peptide (SEP) from *Katsuwonus pelamis* (marine fish) (56)	Anti-inflammatory	Wang et al. (2019)
11	DPP-IV inhibitor peptide from *Phaeodactylum tricornutum* and *Porphyridium purpureum* (microalgae) (57)	Antioxidant and antidiabetic	Stack et al. (2018)
12	DPPH-Scavenging peptides from *Dunaliella salina* (microalgae) (58)	Provitamin A, antioxidant, and food suplement for athlete diet	Çelebi et al. (2021)
13	Astaxanthin, carotenoids, protein, lutein, and fatty acid from *Haematococcus pluvialis* (microalgae) (59)	Anti-inflammatory, antioxidant, heal muscle soreness	Oslan et al. (2021)
14	GIISHR peptide from *Mustelus griseus* (Marine fish) (60)	Antioxidant	Ahmadi-Vavsari F et al. (2019)
15.	*I. galbana* peptide from *Isochrysis galbana* (microalgae) (61)	Anti-inflammatory	Bonfanti et al. (2018)
16	Marine peptide hydrolysate from salmon fish (62)	Metabolic influences during endurance cycling	Siegler et al. (2013)
17	Sardine scale peptide (63)	Improvement of the speed and strength indicators of the athletes from the test group and acceleration of recovery of the athletes after physical training	Mezenova et al. (2021)

innovative dietary strategies that can safely improve performance, enhance recovery, and maintain overall health. The development of functional foods targeted at athletes, thus, represents a significant area of potential growth and research. Current trends in this field include the use of natural and sustainable sources of proteins and peptides, personalized nutrition strategies, and a focus on enhancing both physical and mental aspects of performance (5).

Despite the promising potential of marine-derived proteins and peptides in the development of nutraceuticals, there are still significant gaps in our understanding. The bioactivity of these compounds is influenced by several factors including their source, extraction methods, and the individual's physiological response, all of which need to be comprehensively understood to maximize their benefits. Moreover, the specific applications of these marine-derived proteins and peptides in sports nutrition are relatively unexplored. Athletes have historically harnessed the nutritional advantages of various marine products to enhance their performance and recovery. Among these, fish-based products have been particularly popular. Fish oil supplements, rich in omega-3 fatty acids, have been widely used to reduce inflammation, improve joint health, and support cardiovascular function in athletes. Additionally, marine protein supplements, often derived from sources like fish and shellfish, offer a concentrated source of essential amino acids, aiding in muscle repair and growth. Marine collagen supplements, obtained from fish scales and skin, have gained traction for their potential to enhance joint and connective tissue health, crucial for athletes engaging in high-impact activities. Furthermore, certain marine algae, such as spirulina and chlorella, have gained attention for their nutrient density, including protein content, making them suitable additions to athletes' diets for improved energy and recovery. These marine-derived products exemplify the diverse range of options available to athletes seeking to optimize their nutritional intake and gain a competitive edge (6).

The aim of this article is to review the potential of marine-derived proteins and peptides as a novel source of nutraceuticals, with a specific focus on their application in enhancing athletic performance. We strive to elucidate the current knowledge regarding their bioactive properties, discuss the challenges in their extraction and utilization, and explore the potential pathways for their incorporation into functional foods aimed at athletes. Ultimately, we hope to contribute to the broader understanding of marine bioresources and their role in the future of sports nutrition.

2. Marine natural product as bioresources in foods term

The marine environment is a reservoir of various natural products that have been widely used for medicine and beauty

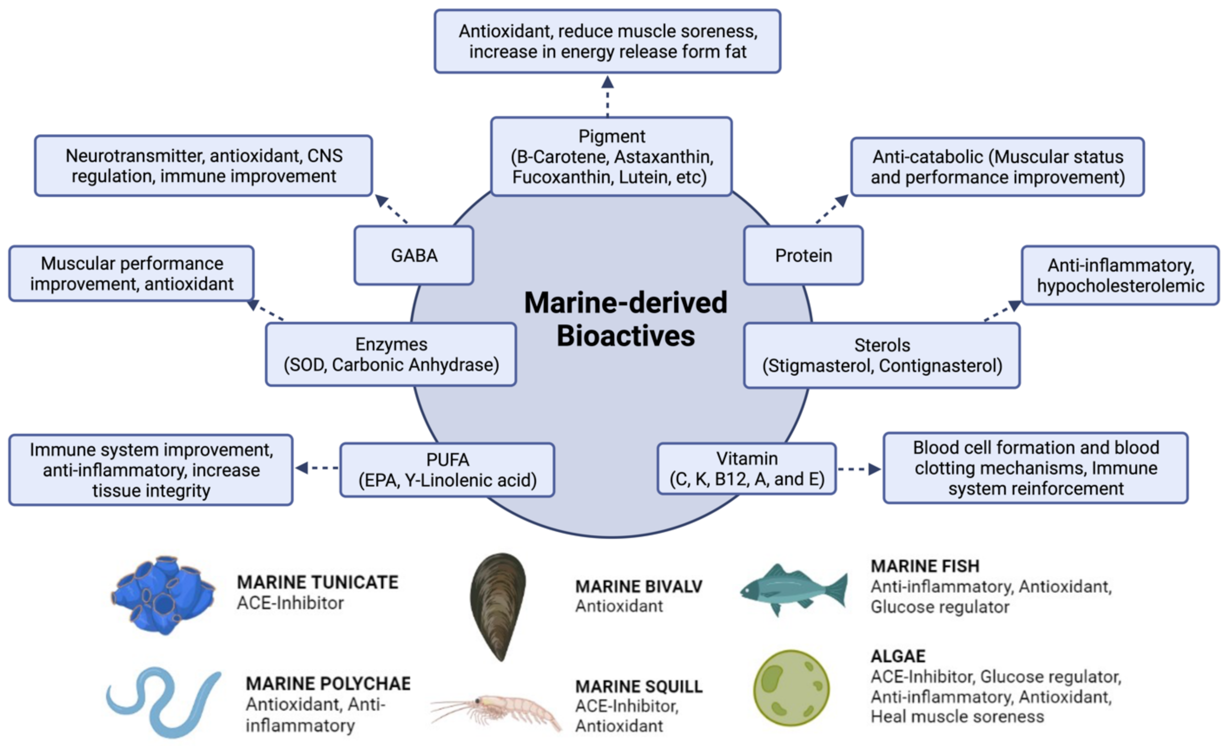

FIGURE 1
Marine organisms and their bioactive properties.

supplements, and have become a source of creation of new functional foods and nutritional supplements (1, 7). Carbohydrates, polyphenols, peptides, proteins, pigments, and essential fatty acids are examples of bioactive compounds obtained from various types of marine organisms, such as prokaryotes, algae, crustaceans, and other invertebrates, as well as various vertebrates (8, 9). Marine organisms have developed a wide range of bioactive chemicals that are not found in other organisms due to the diversity of their complex living conditions that give them a unique way of survival to grow and reproduce (8, 10). One of the widely used bioactive compounds is marine-derived peptides. Marine organisms that are well known for their peptide benefits are tunicates, fishes, seaweed, and various microorganisms (1). Here are some marine natural products as bioresources in food terms (**Figure 1**).

3. Marine-derived molecules and their nutritional values

In the past few years, functional and bioactive compounds from marine organisms such as sponges, bacteria, mollusks, and algae have been shown to have beneficial effects on health and could potentially be applied in medical activities (11). Unique bioactive compounds found in marine organisms, for example, peptides, polysaccharides, and fatty acids (12). Peptides from marine organisms are involved in the fundamental mechanisms that allow organisms to sustain life, including their reproduction, growth, and defense (13). The method for producing marine bioactive peptides is by solvent extraction or microbial protein fermentation which produces fragments with 3–20 amino acid residues (11). Marine-based purified peptide was found to exhibit potent ACE Inhibitor activity (12). Peptides derived from seaweed have shown potential to prevent cardiovascular disease and diabetes (14). Bioactive peptides sourced from fish are proposed to have an impact on the pathways that play a role in controlling blood pressure, as well as in regulating lipid and glucose metabolism and body composition (13). Peptides are also a promising alternative to antibiotics, such as peptide extracted from *Mytilus coruscus* (15).

Furthermore, bioactive peptides from marine microorganisms are starting to be applied as part of athlete's diet (16). Bioactive peptides were found to have a positive effect on body composition, namely increasing lean body mass and decreasing fat mass (17). Other effects include increasing muscle strength (17, 18), enhancing glucose intake into muscles (19), helping to heal muscle soreness and recovery from heavy exercise (20), and increasing the amount of upregulated proteins (myosin proteins, actin-binding proteins and tropomyosins) associated with resistance exercise adaptations (21). In addition, bioactive peptides have also been found to increase the translocation of GLUT-4 and GLUT-1 glucose transporters from the cytoplasm to the plasma membrane (22) which can have an impact on the enhancement of muscle glycogen and provide anti-stress effect (23). The ACE-inhibitory effect of bioactive peptides has also been found to improve endothelial function which is potentially beneficial for endurance sports (24, 25). Moreover, plasma biomarkers for muscle damage and inflammation were found to be lower in the group with bioactive peptide supplementation which shows that bioactive peptides can accelerate musculoskeletal adaptation and recovery through the possibility of extracellular matrix remodeling (26, 27).

Branched-Chains Amino Acids (BCAAS) consisting of leucine, isoleucine, and valine as peptide forming products also have many benefits for muscles, such as stimulating the synthesis of muscle protein (28), increasing physical performance, muscle strength, and muscle mass (29), and limiting muscle damage resulting from exercise (30). BCAA supplementation has been proven to improve the performance of athletes. Cheng et al. found that the supplementation of the BCAA could enhance endurance performance in college runners (31). Meanwhile, Chen et al. found that the supplementation of BCAA could alleviate the exercise-induced central fatigue in taekwondo athletes (32). In addition, leucine as a dietary supplement was also found to have an important therapeutic role in stress condition like burn, trauma, and sepsis, and also useful in slowing the degradation of muscle tissue. Leucine was found very high in various type of fishes, such as *S. Waitei*, *R. Kanagurta*, *L. Rohita*, *C. Mrigala*, *C. Batrachus* and *H. Fossilis*. Isoleucine is found in *O. Mykiss* and *L. Rohita* (33). Other studies found that leucine, isoleucine, and valine were contained in various other marine products such as tunas, mackerels, emperor fish, silky shark, and crustaceans such as lobsters and crabs. A serving of fish is found to provide approximately around or above 100% of the daily amounts of other essential amino acids recommended by the FAO and WHO, and a serving of crustaceans from the Palinuridae (spiny lobster) and Raninidae (spanner crab) families can be found to cover 60- 67% of valine, leucine, and isoleucine (34).

Marine products have the potential to be a source of ergogenic aids. Fish dan algae contain abundant beta alanine, creatine, and hydroxymethylbutyrate (HMB) that can improve the performances of athletes (35, 36). Beta alanine was proven to Increase time to exhaustion in athletes and increasing power output during strength training (37, 38). Creatine supplement was found to be able to delay fatigue at the time of exercise (39). Supplementing with HMB in athletes offers several benefits, including a favorable decrease in body fat while increasing lean muscle mass, enhancing anaerobic peak power, average power, and reducing post-anaerobic exercise lactate levels. Additionally, it helps limit the elevation of stress hormone response, preventing overreaching (40, 41).

Marine-derived antioxidants could also improve athlete performance and immune function by inhibiting the formation of muscle oxidative stress (42). Attenuation of oxidative stress found in young soccer athletes with antioxidant supplementation which was characterized by an increase in markers of lipid peroxidation malondialdehyde and total lipid peroxidation as well as a decrease in the ratio of glutathione to oxidized glutathione (43). Athletes who train at high altitudes also benefit from antioxidant supplementation, namely by reducing deformation of red blood cells (44). Reduced recovery period and delay of fatigue were also found when administering antioxidants immediately before and during exercise (45).

ACE-inhibitor content found in marine products also provides benefits for athlete performance. When exercising, the heart rate will increase to circulate blood throughout the body. An increase in heart rate will cause an increase in blood pressure, called autoregulation. The components of marine peptides have the opportunity to act as ACE inhibitors which work by inhibiting the enzyme that converts angiotensinogen to angiotensin (12). This will help control blood pressure. This condition causes dilation of blood vessels and a decrease in blood pressure. Marine peptide compounds help the heart work more efficiently and with less effort. This can reduce the risk of overworking the heart when athletes do intensive exercise. During intense exercise, blood pressure can increase significantly due to the increased oxygen demand by the muscles. This is the body's normal response to the physical load exerted during exercise. In addition to helping the heart work more efficiently, the opportunity of ACE inhibitors on marine peptides can provide additional protection for the kidneys. This effect can help reduce pressure on the glomerulus and the athlete's kidneys do not do extra work.

After exercise, there is a gradual decrease in blood pressure to normal levels or even below. This is caused by the release of blood vessel relaxing hormones, such as nitric oxide, which helps blood vessels widen and allows better blood flow (46). Marine's peptide compound has the opportunity to become an alternative food for athletes. Although there is research that currently states that the use of ACE inhibitor drugs has a non-synergistic effect on athletes, the use of natural ingredients in the form of Marine peptide compounds has not been studied further (47).

4. Future implication and direction in nutraceutical application for athletes

Advanced nutritional interventions are one of the main subjects of elite sports performance globally. Moderate to high intensity sports require a high percentage of muscle mass with minimum body weight to generate the maximum power. Nutraceutical foods can be useful, to prevent and treat athletes' typical ailments, also improving their performance (64). Some negative physiological changes occur in long-lasting heavy training with immune system disturbance, inflammation, and stress oxidative could be deprived. Athletes and coaches ought to conduct thorough assessments tailored to each athlete's unique needs. To do so, they can delve into scientific information, focusing on essential aspects. For instance, in-depth analysis of human physiological fluids like blood, urine, and feces can provide valuable insights into dietary necessities and nutritional objectives. This information can then inform the selection of appropriate medical supplements and sports nutrition. These personalized dietary plans can be customized to cater to an athlete's requirements through various means, such as consolidating multiple nutrients into a single delivery method or integrating diverse delivery systems containing different nutrients (**Figure 2**) (65, 66).

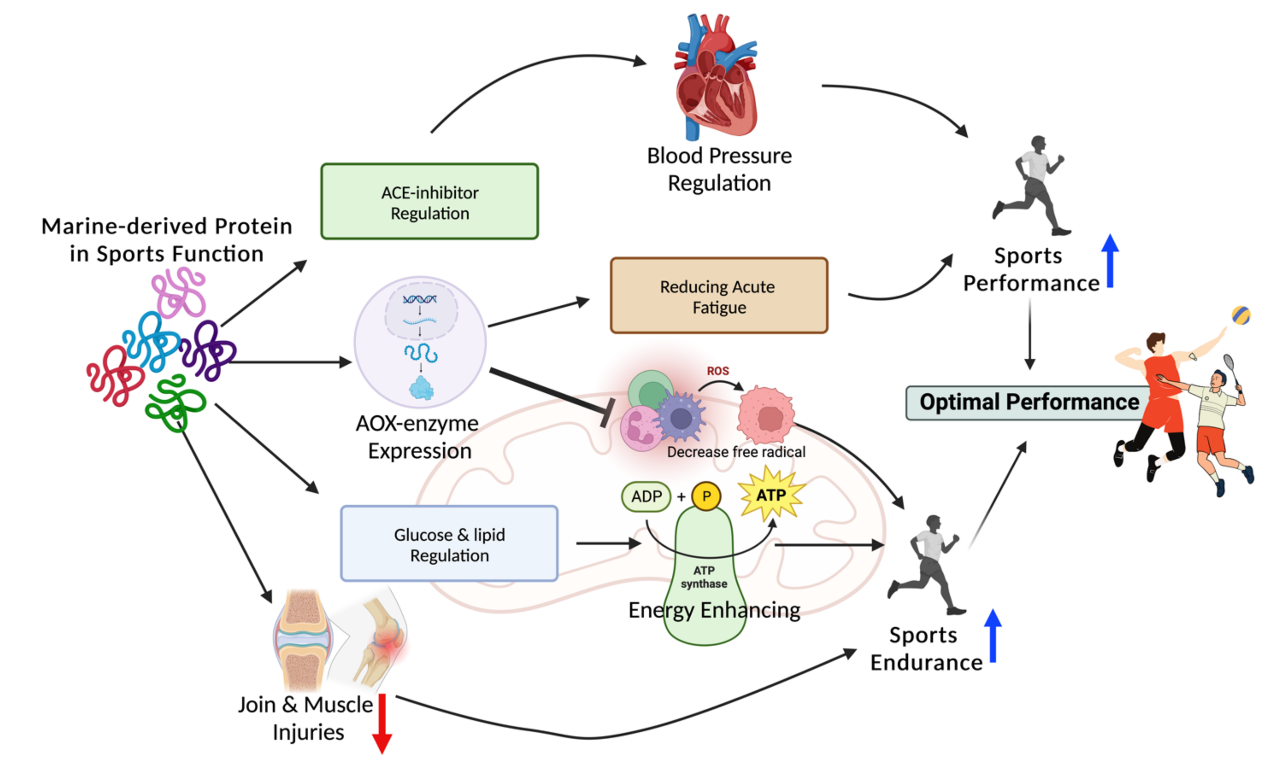

FIGURE 2
Marine-derived protein has beneficial effect to enhance sport performance and endurance by influence (1) ACE-inhibitor regulation affect to blood pressure regulation; (2) Antioxidant-enzyme expression affect to reducing acute fatigue and suppress reactive oxygen species (ROS) production; (3) glucose & lipid regulation affect to energy enhancing; and (4) decrease joint and muscle Marine-derived protein has beneficial effect to enhance sport performance and endurance by influence (1) ACE-inhibitor regulation affect to blood pressure regulation; (2) Antioxidant-enzyme expression affect to reducing acute fatigue and suppress reactive oxygen species (ROS) production; (3) glucose & lipid regulation affect to energy enhancing; and (4) decrease joint and muscle injuries.

In recent years, there has been a growing interest in the potential of marine-derived substances to combat obesity-related health issues, such as dyslipidemia, diabetes, oxidative stress, and inflammation. These bioactive compounds have shown promising effects in addressing these conditions and have thus become a focus of research and development. Marine-based products, known for their abundance of natural bioactive molecules like omega-3 fatty acids, proteins, biopeptides, carotenoids, glucosamine, and minerals, have the potential to be developed into a valuable source of nutritional food for athletes. These products offer a range of benefits, including enhanced performance, improved recovery, and overall support for the unique nutritional needs of individuals engaged in intense physical activity (64). Recent studies have shown that omega-3 fatty acids, found in marine-based products, can have a significant impact on the metabolic and functional responses of skeletal muscle during exercise training. These fatty acids not only offer potential anti-inflammatory and antioxidant benefits but also contribute to faster cell regeneration, aiding in the recovery process for athletes (64–67). Another example is positive impact on muscular performance and reduced muscle damage found after administration of bioactive peptides (68). This shows that as a nutritional source that is environmentally friendly and has a diverse product offering, as well as possessing many unique nutrients that are not often found in traditional sports supplements, marine-derived products could be a sustainable source of supplements for athletes and able to compete effectively with established commercial athletic products. Currently, there is a lack of knowledge regarding the suitable type and concentration of various bioactive components for specific individuals. As a result, it is anticipated that in the coming years, new formulations will be developed, considering potential benefits over traditional ones and advancements in oral bioavailability. These advancements may involve the use of innovative techniques to enhance the delivery and effectiveness of marine-based bioactive compounds. Further studies were needed focusing on marine-derived protein development and functional food manufacturing for high-performance athletes. Practical forms in a combination of marine-derived protein with daily dietary intake or as a dietary supplementation were expected. This article calls on researchers to promote marine-derived bioactivities, especially in the athlete population.

Author contributions

MP: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. GP: Data curation, Writing – original draft, Writing – review & editing. CR: Data curation, Writing – original draft, Writing – review & editing. AS: Data curation, Writing – original draft, Writing – review & editing. AP: Formal Analysis, Supervision, Writing – review & editing. RA: Formal Analysis, Supervision, Writing – review & editing. WG: Formal Analysis, Supervision, Writing – review & editing. DS: Formal Analysis, Supervision, Writing – review & editing. RK: Formal Analysis, Supervision, Writing – review & editing. NT: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. HH: Formal Analysis, Supervision, Writing – review & editing. FN: Conceptualization, Data curation, Formal Analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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References

1. Ghosh S, Sarkar T, Pati S, Kari ZA, Edinur HA, Chakraborty R. Novel bioactive compounds from marine sources as a tool for functional food development. *Front Mar Sci*. (2022) 9:76. doi: 10.3389/fmars.2022.832957

2. Najafian L. A review of bioactive peptides as functional food ingredients: mechanisms of action and their applications in active packaging and food quality improvement. *Food Funct*. (2023) 14(13):5835–57. doi: 10.1039/d3fo00362k

3. Salgado PR, Molina Ortiz SE, Petruccelli S, Mauri AN. Functional food ingredients based on sunflower protein concentrates naturally enriched with antioxidant phenolic compounds. *J Am Oil Chem Soc*. (2012) 89:825–36. doi: 10.1007/s11746-011-1982-x

4. Helkar PB, Sahoo AK, Patil NJ. Review: food industry by-products used as a functional food ingredients. *Int J Waste Resour*. (2016) 6(3):1–6. doi: 10.4172/2252-5211.1000248

5. Manjarrez C, Birrer R. Nutrition and athletic performance. *Am Fam Physician*. (1983) 28(5):105–15.

6. Vitale K, Getzin A. Nutrition and supplement update for the endurance athlete: review and recommendations. *Nutrients*. (2019) 11(6):1289. doi: 10.3390/nu11061289

7. Collins JE, Vanagt T, Huys I, Vieira H. Marine bioresource development–stakeholder's challenges, implementable actions, and business models. *Front Mar Sci*. (2020) 7(62):1–14. doi: 10.3389/fmars.2020.00062

8. Karunarathne S, Mendis E, Kim SK. The potential of developing additives from marine bioresources for the food industry. *Encyclop Mar Biotechnol*. (2020) 5:3085–107. doi: 10.1002/9781119143802.ch137

9. Karthikeyan A, Joseph A, Nair BG. Promising bioactive compounds from the marine environment and their potential effects on various diseases. *J Genet Eng Biotechnol*. (2022) 20(1):1–38. doi: 10.1186/s43141-021-00290-4

10. Giordano D. Bioactive molecules from extreme environments. *Mar Drugs*. (2020) 18(12):640. doi: 10.3390/md18120640

11. Šimat V, Elabed N, Kulawik P, Ceylan Z, Jamroz E, Yazgan H, et al. Recent advances in marine-based nutraceuticals and their health benefits. *Mar Drugs*. (2020) 18(12):627. doi: 10.3390/md18120627

12. Pujiastuti DY, Ghoyatul Amin MN, Alamsjah MA, Hsu JL. Marine organisms as potential sources of bioactive peptides that inhibit the activity of angiotensin i-converting enzyme: a review. *Molecules*. (2019) 24(14):2541. doi: 10.3390/molecules24142541

13. Ovchinnikova TV. Structure, function, and therapeutic potential of marine bioactive peptides. *Mar Drugs*. (2019) 17(9):505. doi: 10.3390/md17090505

14. Admassu H, Gasmalla MA, Yang R, Zhao W. Bioactive peptides derived from seaweed protein and their health benefits: antihypertensive, antioxidant, and antidiabetic properties. *J Food Sci*. (2018) 83(1):6–16. doi: 10.1111/1750-3841.14011

15. Oh R, Lee MJ, Kim YO, Nam BH, Kong HJ, Kim JW, et al. Myticusin-beta, antimicrobial peptide from the marine bivalve, Mytilus coruscus. *Fish Shellfish Immunol*. (2020) 99:342–52. doi: 10.1016/j.fsi.2020.02.020

16. Çelebi H, Bahadır T, Şimşek İ, Tulun Ş. *Use of dunaliella salina in environmental applications. Proceedings of 1st international electronic conference on biological diversity, ecology and evolution*; 2021. p. 9411.

17. Jendricke P, Centner C, Zdzieblik D, Gollhofer A, König D. Specific collagen peptides in combination with resistance training improve body composition and regional muscle strength in premenopausal women: a randomized controlled trial. *Nutrients*. (2019) 11(4):892. doi: 10.3390/nu11040892

18. Kirmse M, Oertzen-Hagemann V, de Marées M, Bloch W, Platen P. Prolonged collagen peptide supplementation and resistance exercise training affects body composition in recreationally active men. *Nutrients*. (2019) 11(5):1154. doi: 10.3390/nu11051154

19. Roblet C, Doyen A, Amiot J, Pilon G, Marette A, Bazinet L. Enhancement of glucose uptake in muscular cell by soybean charged peptides isolated by electrodialysis with ultrafiltration membranes (EDUF): activation of the AMPK pathway. *Food Chem*. (2014) 147:124–30. doi: 10.1016/j.foodchem.2013.09.108

20. Clifford T, Ventress M, Allerton DM, Stansfield S, Tang JC, Fraser WD, et al. The effects of collagen peptides on muscle damage, inflammation and bone turnover following exercise: a randomized, controlled trial. *Amino Acids*. (2019) 51:691–704. doi: 10.1007/s00726-019-02706-5

21. Oertzen-Hagemann V, Kirmse M, Eggers B, Pfeiffer K, Marcus K, de Marées M, et al. Effects of 12 weeks of hypertrophy resistance exercise training combined with collagen peptide supplementation on the skeletal muscle proteome in recreationally active men. *Nutrients*. (2019) 11(5):1072. doi: 10.3390/nu11051072

22. Morato PN, Lollo PC, Moura CS, Batista TM, Camargo RL, Carneiro EM, et al. Whey protein hydrolysate increases translocation of GLUT-4 to the plasma membrane independent of insulin in wistar rats. *PloS One*. (2013) 8(8):e71134. doi: 10.1371/journal.pone.0071134

23. Moura CS, Lollo PC, Morato PN, Risso EM, Amaya-Farfan J. Bioactivity of food peptides: biological response of rats to bovine milk whey peptides following acute exercise. *Food Nutr Res*. (2017) 61(1):1290740. doi: 10.1080/16546628.2017.1290740

24. Martin M, Hagemann D, Nguyen TT, Schwarz L, Khedr S, Moskopp ML, et al. Plasma concentrations and ACE-inhibitory effects of tryptophan-containing peptides from whey protein hydrolysate in healthy volunteers. *Eur J Nutr*. (2020) 59:1135–47. doi: 10.1007/s00394-019-01974-x

25. Toldra F, Wu J. *Biologically active peptides from basic science to applications for human health*. London: Academic Press (2021).

26. Lopez HL, Ziegenfuss TN, Park J. Evaluation of the effects of biocell collagen, a novel cartilage extract, on connective tissue support and functional recovery from exercise. *Integr Med*. (2015) 14(3):30.

27. Khatri M, Naughton RJ, Clifford T, Harper LD, Corr L. The effects of collagen peptide supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise: a systematic review. *Amino Acids*. (2021) 53(10):1493–506. doi: 10.1007/s00726-021-03072-x

28. Jackman SR, Witard OC, Philp A, Wallis GA, Baar K, Tipton KD. Branched-chain amino acid ingestion stimulates muscle myofibrillar protein synthesis following resistance exercise in humans. *Front Physiol*. (2017) 8:390. doi: 10.3389/fphys.2017.00390

29. Ko CH, Wu SJ, Wang ST, Chang YF, Chang CS, Kuan TS, et al. Effects of enriched branched-chain amino acid supplementation on sarcopenia. *Aging (Albany NY)*. (2020) 12(14):15091. doi: 10.18632/aging.103576

30. Fouré A, Bendahan D. Is branched-chain amino acids supplementation an efficient nutritional strategy to alleviate skeletal muscle damage? A systematic review. *Nutrients*. (2017) 9(10):1047. doi: 10.3390/nu9101047

31. Cheng IS, Wang YW, Chen IF, Hsu GS, Hsueh CF, Chang CK. The supplementation of branched-chain amino acids, arginine, and citrulline improves endurance exercise performance in two consecutive days. *J Sports Sci Med*. (2016) 15(3):509.

32. Chen IF, Wu HJ, Chen CY, Chou KM, Chang CK. Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated matches in taekwondo athletes: a randomized controlled trial. *J Int Soc Sports Nutr*. (2016) 13(1):28. doi: 10.1186/s12970-016-0140-0

33. Mohanty B, Mahanty A, Ganguly S, Sankar TV, Chakraborty K, Rangasamy A, et al. Amino acid compositions of 27 food fishes and their importance in clinical nutrition. *J Amino Acids*. (2014) 2014:1–7. doi: 10.1155/2014/269797

34. Jensen IJ, Bodin N, Govinden R, Elvevoll EO. Marine capture fisheries from western Indian ocean: an excellent source of proteins and essential amino acids. *Foods*. (2023) 12(5):1015. doi: 10.3390/foods12051015

35. Yanshin N, Kushnareva A, Lemesheva V, Birkemeyer C, Tarakhovskaya E. Chemical composition and potential practical application of 15 red algal species from the white sea coast (the Arctic ocean). *Molecules*. (2021) 26(9):2489. doi: 10.3390/molecules26092489

36. Oehlenschläger J. Seafood quality assessment. *Seafood Processing*. (2013) 2:359–86. doi: 10.1002/9781118346174.ch14

37. Hobson RM, Harris RC, Martin D, Smith P, Macklin B, Gualano B, et al. Effect of beta-alanine with and without sodium bicarbonate on 2,000-m rowing performance. *Int J Sport Nutr Exerc Metab*. (2013) 23(5):480–7. doi: 10.1123/ijsnem.23.5.480

38. Maté-Muñoz JL, Lougedo JH, Garnacho-Castaño MV, Veiga-Herreros P, Lozano-Estevan MD, García-Fernández P, et al. Effects of β-alanine supplementation during a 5-week strength training program: a randomized, controlled study. *J Int Soc Sports Nutr*. (2018) 15(1):19. doi: 10.1186/s12970-018-0224-0

39. Butts J, Jacobs B, Silvis M. Creatine use in sports. *Sports Health*. (2018) 10 (1):31–4. doi: 10.1177/1941738117737248

40. Durkalec-Michalski K, Jeszka J, Podgórski T. The effect of a 12-week beta-hydroxy-beta-methylbutyrate (HMB) supplementation on highly-trained combat sports athletes: a randomised, double-blind, placebo-controlled crossover study. *Nutrients*. (2017) 9(7):753. doi: 10.3390/nu9070753

41. Albert FJ, Morente-Sánchez J, Ortega FB, Castillo MJ, Gutiérrez Á. Usefulness of β-hydroxy-β-methylbutyrate (hmb) supplementation in different sports: an update and practical implications. *Nutr Hosp*. (2015) 32(1):20–33. doi: 10.3305/nh.2015.32.1.9101

42. Gammone MA, Gemello E, Riccioni G, D'Orazio N. Marine bioactives and potential application in sports. *Mar Drugs*. (2014) 12(5):2357–82. doi: 10.3390/md12052357

43. de Oliveira DC, Rosa FT, Simões-Ambrósio L, Jordao AA, Deminice R. Antioxidant vitamin supplementation prevents oxidative stress but does not enhance performance in young football athletes. *Nutrition*. (2019) 63:29–35. doi: 10.1016/j.nut.2019.01.007

44. Higgins MR, Izadi A, Kaviani M. Antioxidants and exercise performance: with a focus on vitamin E and C supplementation. *Int J Environ Res Public Health*. (2020) 17 (22):8452. doi: 10.3390/ijerph17228452

45. Canals-Garzón C, Guisado-Barrilao R, Martínez-García D, Chirosa-Ríos IJ, Jerez-Mayorga D, Guisado-Requena IM. Effect of antioxidant supplementation on markers of oxidative stress and muscle damage after strength exercise: a systematic review. *Int J Environ Res Public Health*. (2022) 19(3):1803. doi: 10.3390/ijerph19031803

46. Ashfaq A, Sara K, Zdravka D, Maleeha A, Li N, Li P-L, et al. Review: role of nitric oxide in the cardiovascular and renal systems. *Int J Mol Sci* (2018) 19:2605. doi: 10.3390/ijms19092605

47. Sjúrðarson T, Bejder J, Breenfeldt Andersen A, Bonne T, Kyhl K, Róin T, et al. Effect of angiotensin-converting enzyme inhibition on cardiovascular adaptation to exercise training. *Physiol Rep*. (2022) 10(13). doi: 10.14814/phy2.15382

48. Kang N, Ko SC, Kim HS, Yang HW, Ahn G, Lee SC, et al. Structural evidence for antihypertensive effect of an antioxidant peptide purified from the edible marine animal styela clava. *J Med Food*. (2020) 23(2):132–8. doi: 10.1089/jmf.2019.4415

49. Park YR, Park CI, Soh Y. Antioxidant and anti-inflammatory effects of NCW peptide from clam worm (marphysa sanguinea). *J Microbiol Biotechnol*. (2020) 30 (9):1387. doi: 10.4014/jmb.2003.03050

50. Ma Y, Huang K, Wu Y. In vivo/in vitro properties of novel antioxidant peptide from pinctada fucata. *J Microbiol Biotechnol*. (2021) 31(1):33. doi: 10.4014/jmb.2006.06002

51. Joshi I, Abdul NR. Angiotensin I-converting enzyme (ACE-I) inhibition and antioxidant peptide from a squilla Species. *Protein Pept Lett*. (2021) 28(11):1238–45. doi: 10.2174/0929866528666210616122835

52. Ayabe T, Mizushige T, Ota W, Kawabata F, Hayamizu K, Han L, et al. A novel Alaska pollack-derived peptide, which increases glucose uptake in skeletal muscle cells, lowers the blood glucose level in diabetic mice. *Food Funct*. (2015) 6(8):2749–57. doi: 10.1039/C5FO00401B

53. Sun S, Xu X, Sun X, Zhang X, Chen X, Xu N. Preparation and identification of ACE inhibitory peptides from the marine macroalga ulva intestinalis. *Mar Drugs*. (2019) 17(3):179. doi: 10.3390/md17030179

54. Su Y, Chen S, Shen J, Yi Z, Liu S, Cai S, et al. Screening and molecular mechanisms of novel ACE-inhibitory peptides from gracilariopsis lemaneiformis. *Int J Mol Sci*. (2022) 23(23):14850. doi: 10.3390/ijms232314850

55. Ramos-Romero S, Torrella JR, Pagès T, Viscor G, Torres JL. Edible microalgae and their bioactive compounds in the prevention and treatment of metabolic alterations. *Nutrients*. (2021) 13(2):563. doi: 10.3390/nu13020563

56. Wang ZG, Ying XG, Gao P, Wang CL, Wang YF, Yu XW, et al. Anti-inflammatory activity of a peptide from skipjack (katsuwonus pelamis). *Mar Drugs*. (2019) 17(10):582. doi: 10.3390/md17100582

57. Stack J, Le Gouic AV, Tobin PR, Guihéneuf F, Stengel DB, FitzGerald RJ. Protein extraction and bioactive hydrolysate generation from two microalgae, porphyridium purpureum and phaeodactylum tricornutum. *J. Food Bioact*. (2018) 1:153–65. doi: 10.31665/JFB.2018.1134

58. Çelebi H, Bahadır T, Şimşek İ, Tulun Ş. *Use of dunaliella salina in environmental applications*. In *Proceedings of 1st international electronic conference on biological diversity, ecology and evolution* 2021 Mar 12 (p. 9411).

59. Oslan SN, Tan JS, Oslan SN, Matanjun P, Mokhtar RA, Shapawi R, et al. Haematococcus pluvialis as a potential source of astaxanthin with diverse applications in industrial sectors: current research and future directions. *Molecules*. (2021) 26(21):6470. doi: 10.3390/molecules26216470

60. Ahmadi-Vavsari F, Farmani J, Dehestani A. Recombinant production of a bioactive peptide from spotless smooth-hound (mustelus griseus) muscle and characterization of its antioxidant activity. *Mol Biol Rep*. (2019) 46:2599–608. doi: 10.1007/s11033-018-4468-1

61. Bonfanti C, Cardoso C, Afonso C, Matos J, Garcia T, Tanni S, et al. Potential of microalga isochrysis galbana: bioactivity and bioaccessibility. *Algal Res*. (2018) 29:242–8. doi: 10.1016/j.algal.2017.11.035

62. Siegler JC, Page R, Turner M, Mitchell N, Midgely AW. The effect of carbohydrate and marine peptide hydrolysate co-ingestion on endurance exercise metabolism and performance. *J Int Soc Sports Nutr*. (2013) 10:1–7. doi: 10.1186/1550-2783-10-29

63. Mezenova NY, Verkhoturov VV. Study of the combined effect of a biologically active composition based on apiculture products and peptides extracted from fish scalein the training of athletes in speed and strength athletics disciplines. *IOP Conference Series*. (2021) 689(1):012045. doi: 10.1088/1755-1315/689/1/012045

64. Tirla A, Islam F, Islam MdR, Ioana Vicas S, Cavalu S. New insight and future perspectives on nutraceuticals for improving sports performance of combat players: focus on natural supplements, importance and advantages over synthetic ones. *Appl Sci*. (2022) 12(17):8611. doi: 10.3390/app12178611

65. Gupta C, Prakash D, Gupta S. Nutraceuticals for athletes. *Adv Food Technol Nutr Sci*. (2016) 2(2):73–92. doi: 10.17140/AFTNSOJ-2-132

66. Angelo S D', Tafuri D. Nutraceuticals: their role in improving sports performance. *Sport Sci*. (2020) 13(1):7–12.

67. Buonocore D, Negro M, Arcelli E, Marzatico F. Anti-inflammatory dietary interventions and supplements to improve performance during athletic training. *J Am Coll Nutr*. (2015) 34(1):62–7. doi: 10.1080/07315724.2015.1080548

68. König D, Kohl J, Jerger S, Centner C. Potential relevance of bioactive peptides in sports nutrition. *Nutrients*. (2021) 13(11):3997. doi: 10.3390/nu13113997

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Investigating pre-professional dancer health status and preventative health knowledge

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Introduction: Dance is a highly demanding physical pursuit coupled with pressure to conform to aesthetic ideals. Assessment of health status and preventative health knowledge of pre-professional dancers may help inform educational strategies promoting dancers' health and career longevity. The aim of this research was to establish a baseline understanding of dance students at a single pre-professional institution based on metrics focused on current health, nutrition, lifestyle, and wellbeing while also gauging knowledge of longer-term health implications.

Methods: Adopting a cross-sectional study design, the Dance-Specific Energy Availability Questionnaire was tailored for Australian participants and administered online.

Results: The response rate was 59.5% (69/116 eligible students) and the survey was completed in full by 63 students. Mean BMI was 20 kg/m^2, although among females, 47% had a BMI $<$ 20 (range 16 to 25 kg/m^2), and at their lowest reported weight BMI was 14 to 25 kg/m^2. Over a third had either experienced (31%) or were currently experiencing (3.4%) secondary amenorrhea (period absence $\geq$ 3 consecutive months). Most dancers did not exclude food groups, however, 24% had been advised to exclude particular foods in the past, mostly by dance teachers. A large percentage used nutritional supplements (68%) with 60% supplementing with iron and more than half (53%) taking two or more supplements. Only 25% had ever utilised a qualified dietitian, although 16% reported a history of eating disorders and 25% reported vegetarian or vegan eating patterns. REDs risk scores ranged from −16 to +16 points with negative scores indicating LEA and higher risk of REDs. The mean score for males was 5.2 (SD = 3.9) and 2.1 (SD = 5.9) for females, with 33.3% producing a negative score.

Conclusion: Results provide insight to health knowledge and particular issues pertinent for dancers and highlights the need for specific education strategies to promote a preventative health focus for those entering a pre-professional programme. This study also highlights the need for improved awareness of LEA and REDs among all practitioners working with dancers along with cultural and structural changes within the broader dance community to help protect and promote the wellbeing of dancers.

KEYWORDS
dance, low energy availability, REDs, nutrition, health, weight, menstrual disturbance

1 Introduction

Dance training at a pre- and professional level is a highly demanding physical pursuit coupled with pressures for dancers to conform to aesthetic ideals. Rehearsal and performance volumes in professional ballet (mean 19.1 to 27.5 h per week and as high as 40 h per week) are much higher than training and competitions in elite sport and are likely to underpin end-of-season performance reductions and high rate of burnout among ballet dancers (1–3). While training demands are high in dance at a professional level (e.g., full-time role in a company), dancers training at a pre-professional level (such as at a full-time vocational or tertiary institution) are also faced with high volumes of physical training, along with juggling external stressors such as academic workload and living away from home for the first time (4).

Low energy availability (LEA) is defined as "any mismatch between dietary energy intake and energy expended in exercise that leaves the body's total energy needs unmet, that is, there is inadequate energy to support the functions required by the body to maintain optimal health and performance" (5). High training and performance volumes in dance coupled with inadequate dietary intake can place a dancer at risk of low energy availability (6). Among dancers, there is reportedly a high prevalence of disordered eating (including excessive exercise in fear of gaining weight) and eating disorders (7, 8), restricting energy intake or engaging in excessive exercise can increase the risk of LEA. Recent studies have highlighted the importance of health and nutrition education among dancers to promote knowledge and awareness to potentially reduce LEA, disordered eating patterns, and injury risk while also assessing the supports needed for dancers entering into higher education (9, 10).

Despite LEA being first described in female dancers, the literature has been more broadly applied to other sports. Relative energy deficiency in sport (REDs) in particular, has gained the attention of the International Olympic Committee (IOC) and has recently been redefined as "impaired physiological and/or psychological functioning experienced by female and male athletes that is caused by exposure to problematic (prolonged and/or severe) low energy availability. The detrimental outcomes include, but are not limited to, decreases in energy metabolism, reproductive function, musculoskeletal health, immunity, glycogen synthesis and cardiovascular and haematological health, which can all individually and synergistically lead to impaired wellbeing, increased injury risk and decreased sports performance" (5). The key precipitator of this syndrome is LEA. The estimated prevalence of LEA/REDs in female athletes is 23 to 79.5% and in male athletes, 15 to 70% (5). These rates are across a wide range of sports with broad prevalence ranges due to inconsistent definitions and research methodologies (5). Previous studies in elite dance report 65% of female vocational (or pre-professional) ballet dancers (11), 40% of professional female dancers (12), and 57% female and 29% of male pre-professional, professional, and advanced amateur dancers (6) to be at risk of LEA or REDs. Among Norwegian jazz and contemporary university dance students, a reported 20 to 60% of dancers indicated symptoms of LEA, anxiety or depression, and/or symptoms of eating disorders and disordered eating behaviours (8).

Benchmarking health status and preventative health knowledge of those entering a pre-professional programme may help inform the required educational strategies to promote longevity in careers and optimal long-term health among dancers. Previous findings from a study among dancers suggest that the incidence of LEA and subsequent risk of REDs is higher (particularly among females) than awareness of these terms (6). Despite health and nutrition being highly rated by dancers in terms of interest, many pre-professional dance training institutions do not commonly include curriculum in these areas (10).

The aim of this research was to establish a baseline measure of students at a single Australian pre-professional institution based on a range of metrics focused on current health, nutrition, lifestyle, and wellbeing while also gauging knowledge of longer-term implications. The opportunity was to obtain insights for educational opportunities of dancers more generally, which could also be applied in younger age groups of vocational dancers where patterns of behaviour are established.

2 Materials and methods

2.1 Study design

A cross-sectional study design was adopted. Data collection took place in September 2022 via a self-administered anonymous online survey. The survey was based on the Dance-Specific Energy Availability Questionnaire (DEAQ) developed by Keay et al. (6). The DEAQ has been developed and administered by Keay et al. (6) specifically for dancers aimed at measuring and understanding the prevalence of LEA (6) and forms a useful screening tool for assessing risk of REDs. The DEAQ draws on three validated questionnaires, the Low Energy Availability in Females Questionnaire (LEAF-Q), a Sports Specific Energy Availability Questionnaire (SEAQ-I), and the IOC REDs clinical assessment tool (REDs CAT), and includes questions addressing the recognised physiological indicators of LEA, other potential correlates with LEA such as injury or illness and a basic nutrition assessment (6). Questions in the DEAQ relating to key food groups and serves of dairy were adapted in alignment with the Australian Guide to Healthy Eating (13). Vaping was added to the question related to smoking. An additional question was added to separate out the number of days of dancing missed due COVID-19 from other illness.

The anonymous online survey was developed in Qualtrics XM Platform™ (Provo, UT, USA) and pilot tested with five external dancers. The final 15-min survey consisted of 62 questions and used an open and closed questionnaire design with free text, multiple-choice responses and Likert scale questions. The survey was divided into six parts: (1) brief demographic information and dance background; (2) anthropometric data and dance training; (3) medical history, medications, and lifestyle; (4) smoking status; (5) injuries and sick days; (6) general health and nutrition knowledge.

Abbreviations: BMI, body mass index; DEAQ, Dance-Specific Energy Availability Questionnaire; IOC, International Olympic Committee; LEA, low energy availability; METs, metabolic equivalent of task; REDs, relative energy deficit in sport; WAAPA, Western Australian Academy of Performing Arts.

Scored questions (20/62) included weight/height (for calculation of body mass index; BMI), hormonal indicators specific to males and females, limitations on dietary patterns (including key food groups), smoking status, injuries, sleep patterns, and control tendencies including past medical history of eating disorders. Two questions that were scored within the REDs risk score related to how controlling weight and controlling eating affects dancers. These were assessed on a scale from 0 ("doesn't affect me") to 6 ("affects me greatly"). See Keay et al. (6) for specific DEAQ questions and REDs risk scoring tool. Ethical approval for this study was obtained through HREC at Edith Cowan University (REMS NO: 2022-03575-NICHOLAS) and HREC at the University of New South Wales.

2.2 Participants and procedure

Participation was open to all dance students at the Western Australian Academy of Performing Arts (WAAPA) regardless of age or year of study. Students were enrolled in either a ballet or contemporary-focused full-time dance programme, however, they are trained in both dance styles as well as other genres. Participation was optional and voluntary, and not linked with academic attainment. Written parental consent was sought for all participants < 18 years. Participant consent was obtained from all participants via the survey tool. An online survey link was distributed to the whole cohort via standard student communication channels including online announcements and weekly meetings. Participants were able to complete the survey in their own time using the link provided, a studio space was also provided to students following weekly meetings.

2.3 Data analysis

Participant response data was exported from Qualtrics (Provo, UT, United States) to a Microsoft Excel^{TM} spreadsheet (Version 16.53, Washington, DC, USA) for calculation of LEA and REDs score. Descriptive statistics (means, standard deviation, ranges, and frequencies) for quantitative data were calculated using IBM SPSS (Version 29.0, Armonk, NY, USA). A summary report of free text response was also generated by Qualtrics (Provo, UT, United States) to assist with the qualitative data analysis. Free text responses were reviewed and collated and where necessary, content analysis was conducted and assigned to reoccurring themes by one researcher and independently checked by the other.

3 Results

3.1 Participant demographics and dance background

The survey was attempted by N = 69 of 116 enrolled dance students, providing a response rate of 59.5%. The survey was completed in full by n = 63, providing a completion rate of 91%. Due to the independent nature of each question, all survey responses, inclusive of those partially completed, were included in the final analysis with varied participation numbers reflected in the results. The majority of respondents were female (88.8%, n = 56) and aged between 16 and 24 years (mean 19.24 years) with most (51.6%, n = 33) from the first-year cohort. **Table 1** provides detailed demographic characteristics and dance background of study participants. There was a large range in the age when participants first commenced dance training, from 1 to 17 years (mean age 5.2 years), with a mean transition to full time training at 16.5 years (10 to 21 years of age).

3.2 Anthropometric measures and activity exposure

Anthropometric and activity exposure data is displayed in **Table 2**. Self-reported body weight for males ranged from 68 to 85 kg and for females ranged from 43 to 73 kg. Mean body mass index (BMI) for all participants was 20.5 kg/m^2 (within the healthy weight range). Mean BMI for males was 23.3 kg/m^2 (range 19.9 to 26.7 kg/m^2) and for females was 20.2 kg/m^2 (range 16.5 to 25.3 kg/m2), with 47% of female dancers reporting a BMI < 20. Participants were also asked to report their lowest body weight for current height. At their lightest weight, BMI for female dancers ranged from 14.6 to 25 kg/m^2 with 61% reporting a BMI < 20.

When asked about how often they weighed themselves, most dancers reported they did not weigh themselves (62.3%). Of those who did weigh, the mean frequency was 4.8 days/week and 17.4% reported they weighed themself less than weekly. Very few reported being weighed at a previous studio (n = 2/69), with weigh-in frequency being weekly. More than one-third of dancers (n = 24/69) had been told to lose weight at some point prior to commencing at WAAPA, mostly by past teachers.

When asked about what weight they dance best at, 34% either stated their current weight or provided the same numerical measure as their current weight, whereas 62% stated a lower weight from 0.5 to 7 kg lower than their current stated weight. Two dancers provided a higher weight in comparison to their current stated weight. Linked with this, 62.5% believe a lower weight was more likely to help them attain a leading role. Almost a third of dancers (28%) dancers reported not knowing or not weighing themselves and therefore did not provide a response to this question.

Participants reported dancing an average of 21.4 h per week (range 5 to 40 h), the large range in volume may have been impacted by dancers who were injured and not dancing at the time of data collection. Additionally, training and performance requirements differed greatly between cohorts at the time of data collection. Most dancers (81.5%) reported doing additional supplementary fitness or cross training for an average of 2.8 h per week (range 1 to 10 h).

3.3 Hormones and menstrual cycle

Male dancers made up a small portion of respondents (n = 5), all reported morning erections, mostly 3 to 5 days per week.

Among females (n = 58), menarche was reported by 67.2% between the ages of 12 and 14 years, with 24.1% delayed until after

TABLE 1 Demographic characteristics including dance background of participants.

Demographic variable	Mean ± SD	Count (%)
Gender (n = 63)		
Female		56 (88.8)
Male		4 (6.3)
Non-binary		2 (3.2)
Not listed or other		0 (0)
Prefer not to say		1 (1.6)
Biological sex (n = 63)		
Female		58 (92.1)
Male		5 (7.9)
Age in years (*n* = 67)	19.2 ± 1.4	
16–17 years		4 (6.0)
18–20 years		50 (74.6)
21–24 years		13 (19.4)
Age started dancing (*n* = 67)	5.2 ± 3.1	
1–5 years		46 (68.7)
6–10 years		18 (26.9)
11–17 years		3 (10.4)
Age transitioned to full-time training (*n* = 67)	16.5 ± 2.3	
Current year of study (n = 64)		
First		33 (51.6)
Second		17 (26.6)
Third		13 (20.3)
Fourth		1 (1.6)
Prior residential location (n = 68)		
Australian Capital Territory		5 (7.4)
New South Wales		9 (13.2)
Northern Territory		2 (2.9)
Queensland		5 (7.4)
South Australia		3 (4.4)
Tasmania		0 (0.0)
Victoria		7 (10.3)
Western Australia		37 (54.4)
Other/overseas		0 (0.0)
Current main dance genres (n = 68)		
Classical ballet		53 (76.8)
Contemporary/modern		64 (92.8)
Jazz/musical theatre		5 (7.2)
Hip hop/urban/commercial		1 (1.4)
Other (text entry–tap)		1 (1.4)

≥ 15 years (primary amenorrhea). No dancers reported not having commenced menstruation. Over one-third of dancers (34.5%) had experienced oligomenorrhea (i.e., less than 9 periods per year), with two dancers (3.4%) currently experiencing secondary amenorrhea (i.e., periods stopped for three or more consecutive months, not due to pregnancy or contraceptive pill) and 31% experiencing this previously. Only 35% of dancers reported that healthcare practitioners had asked about periods, and this was only reportedly addressed in 42.1% (*n* = 8/19) cases where lack of periods were reported.

The oral contraceptive pill was taken by 27.6% of female dancers at the time of the survey, mostly for contraception (93.8%), however, reduction of menstrual pain, bleeding, and regulation of periods were also considerations. An equal number of dancers (46.6%, *n* = 27/58) knew that the hormones in the contraceptive pill were not equivalent to the hormones produced by the body, as those that did not know. A very small number (6.9%, *n* = 4/58) believed hormones produced by the body were equivalent to that provided in contraceptives.

When asked about dance health knowledge and beliefs (*n* = 63), 18.8% perceived it to be normal for female dancers to not have periods (i.e., menstruate) and 12.7% "did not know." Furthermore, 11.1% were not aware of any negative effects of not menstruating and 7.9% did not know of any associated problems (*n* = 5).

3.4 Illness and injuries

Seventy percent (*n* = 44/63) reported sustaining a soft tissue injury in the past year (i.e., muscle, ligament, tendon, and joint injuries excluding fractures). There was an average of 1.6 injuries per dancer in the preceding year, 37% of dancers did not miss any days due to injury, and a further 35% missed ≤ 5 days. There were a small group of dancers (11.3%, *n* = 7/62) who had missed significantly more days of dance due to injury (28 to 210 days). Of the soft tissue injuries, 54% of participants reported that they were recurrent (i.e., in the same location, or same type of injury).

Over a third (34.9%, *n* = 22/63) had experienced a bone injury since starting full-time dance training. Where dancers had experienced a fracture, the most prevalent anatomical regions were the feet (28.6%, *n* = 18), followed by arm (17.5%, *n* = 11), leg (7.9%, *n* = 5), and spine (4.8%, *n* = 3).

General illness resulted in an average of 9.9 days absence affecting 85% of the cohort, and COVID-19 a further 12.2 days. Ten dancers had not experienced COVID-19 at the time of data collection.

When asked about missing classes or rehearsals, 65.2% of dancers felt "worried or anxious" to miss a class or rehearsal, 23.2% felt "relieved to have a day to rest," 30.4% indicated they "understood these things happen." A small number of dancers expressed feeling guilty, lazy, self-critical, judged, and expressed feelings of self-hatred.

3.5 Eating habits, supplements, and nutrition advice

Dancers were asked about particular dietary choices, with 21.9% (*n* = 14/63) reporting they chose a vegetarian dietary pattern, with just two (3.2%) selecting vegan. Almost half (43.7%) reported excluding food groups. Excluded food groups or categories included: carbohydrate foods (1.6%, *n* = 1/63), meat (20.6%, *n* = 13),

TABLE 2 Anthropometric measures and activity exposure.

	Biological sex		Combined	
	Female (n = 58)	Male (n = 5)	Mean ± SD	Range (min–max)
Anthropometrics				
Height (m) (n = 63)	1.67 ± 0.68	1.78 ± 0.10	1.68 ± 0.1	1.45–1.9
Weight (kg) (n = 53)	56.3 ± 7.2	73.8 ± 6.7	58.0 ± 8.7	43.0–85.0
BMI (kg/m^2) (n = 52)	20.2 ± 1.9	23.3 ± 2.8	20.5 ± 2.2	16.5–26.7
Weight variability (%) * (n = 51)	14.6 ± 6.8	10.1 ± 5.8	14.5 ± 7.0	4.0–38.0
Activity exposure (hours) (n = 65)				
Class	21.8 ± 6.1	18.0 ± 4.5	21.1 ± 6.5	0.0–40.0
Rehearsal	6.8 ± 3.3	6.0 ± 2.9	6.7 ± 3.3	0.0–15.0
Performance	1.5 ± 2.5	1.0 ± 2.2	1.4 ± 2.4	0.0–10.0
Supplemental training	2.9 ± 2.2	1.8 ± 2.7	2.8 ± 2.3	0.0–10.0
Total hours	33.0 ± 8.8	26.8 ± 2.0	31.9 ± 9.4	0.0–53.0

*Weight variability calculated for current height by dividing the difference between maximum and minimum weight by current weight, then multiplied by 100.

fish (19.0%, n = 12), dairy (11.1%, n = 7), and gluten (9.5%, n = 6). Nearly one-quarter of dancers (23.8%, n = 15/63) had been told to exclude particular foods by teachers in the past, with a range of advice provided:

> *"In the past it had been suggested for me to only eat light salads, and cruskits with cottage cheese to lose weight."*

> *"I've been told many things in the past, e.g., only eat raw foods as cooking reduces nutritional value, try an [Emma] Ware fast diet as it cleanses the gut."*

> *"Meat, dairy and bread. 'To avoid bulking up'."*

> *"Sugary foods, carbs, fats, dairy, gluten."*

> *"Carbohydrates, fats, any processed foods."*

With dancers noting that the advice was aimed at weight concerns:

> *"For body image"; "To lose weight"; "Was told to cut carbs to reduce puffiness in body"; "Don't eat/drink sugary things, you will be fat. Don't eat lunch before class, the teacher says she can see our lunch."*

Caffeine as caffeinated beverages were consumed by 58% (n = 39/67). The mean consumption was 1 cup per day (range 0–4 cups per day). Dairy consumption was varied with 42.2% consuming 2–3 serves/day, 12.6% (n = 8/63) consuming no dairy, and 51.6% consuming below the recommended serves/day (i.e., 0–1 serves/day). Supplements were used by 68% of participants, mostly iron supplements (60%) and magnesium (51%), with fewer (14%) taking calcium, zinc, Vitamin C, or a multivitamin. Vitamin B was taken by 19% and Vitamin D by 12%. More than half (53%) were taking more than two supplements and a list of 12 other supplements were provided.

The most common sources of nutrition advice in descending order were the internet (42.2%), the advice of friends (28.1%), and 25% sought advice from a qualified dietitian. Almost one-third (29%) had not sought any form of nutrition advice despite this same proportion had been told to lose weight at some point in their dance training. A follow-on question about the impact of social media was also asked, with 81.3% of dancers reporting that social media makes them feel like they should "sometimes" or "always" try and lose weight.

Five dancers reported smoking (7.9%, n = 5/63) for social reasons, stress management, or due to addiction. Three out of five reported trying to stop.

3.6 Wellbeing and psychological factors

A range of questions were asked to gauge wellbeing among the dancers. Dancers were asked to rate their freshness or energy level on a scale of 0 ("extremely tired all the time") to 5 ("no fatigue at all"), with 84.4% providing a score $\geq$ 2 indicating higher levels of energy with two dancers reporting they were extremely fatigued. Similarly, when asked about sleep, most (64%) provided a score $\geq$ 3, with just three dancers reporting they hardly ever had a good night's sleep in the past year. When prompted about sleeping difficulties, the key issues were difficulty falling asleep, disrupted sleep, and waking too early.

Digestive problems were also rated, with a small number of dancers (6.3%, n = 4/63) indicating continuous problems, usually linked with medical issues. Further prompt questions revealed bloating (66.6%, n = 42), constipation (33.3%, n = 21) and discomfort (50.8%, n = 32) were the top ranked issues among dancers with 11.1% (n = 7/63) reporting no such issues. Allergies and intolerances were reported by 40.6% of dancers, which may be linked with the issues regarding digestion, the core foods excluded, and supplement use described earlier. Medications were reported by 27%, with antidepressant used by 10.5% (n = 7) of the participants.

Ten dancers (15.6%) had previously been diagnosed with an eating disorder, including four with anorexia nervosa, two with bulimia, one reported a combination of anorexia nervosa and bulimia, another reported avoidant/restrictive food intake disorder, and another was "not specified." We noted that one additional participant did not respond to the question regarding type of eating disorder.

3.7 Awareness and REDs risk score

REDs risk scores were calculated for 49 participants (45 females). REDs risk scores ranged from −16 to +16 points with negative scores indicating LEA and a higher risk of REDs. The average score for the cohort was 2.1 (SD = 5.9) for females and 5.25 (SD = 3.9) for males, indicating males in the study group were not at risk. Negative scores (< 0) were seen in female dancers with 33.3% scoring in the negative range.

Just over half of all participants were aware of REDs (53.1%) and female athlete triad (56.3%), and a large proportion acknowledged an awareness of LEA (73.4%). There was a high degree of awareness about disordered eating (92.2%) and just over a third were aware of orthorexia (35.9%).

The REDs risk score included questions about how controlling weight and controlling dietary intake affects dancers. These questions utilised a scale as described from 0 ("doesn't affect me") to 6 ("affects me greatly"), while 17.5% reported no issues, 60.3% of dancers rated controlling what they eat as 3–5/6, and no dancers gave a rating of 6/6. Controlling weight was more problematic, with 47.6% scoring 3–5/6 and an additional 20.6% scoring this question 6/6 indicating it "affects me greatly." Control of body weight was of no concern for 24% of participants (n = 15/62).

4 Discussion

This study was based on the DEAQ by Keay et al. (6), a tool combining three validated questionnaires to better capture information relevant to dance, with a REDs scoring tool embedded. The current research was conducted with dance students at a single training institution to help understand current health status, nutrition habits, lifestyle, and wellbeing, while also gauging knowledge of LEA and REDs to inform specific curriculum related to dancer health and wellbeing. A third of female dancers in this study received a negative REDs risk score indicating low energy availability and risk of developing REDs. No males scored negatively, noting that males made up just 6.3% of the participant group. The risk of LEA and REDs among dancers in this study was less than reported in previous studies among elite dancers: 65% of female vocational ballet dancers (11), 40% of professional female dancers (12), and 57% female and 29% of male pre-professional, professional, and advanced amateur dancers (6).

Dance, particularly ballet, as a physical pursuit is unique. Exertion within a typical ballet class builds gradually over an hour to several hours from almost stationary movements at the barre, to grand allegro, with intermittent bursts of athletic jumps and sequences using the entire studio space or length of the room (14). A performance may last 3 to 7 min (14), and performers within a full ballet cycle on and off stage, or to the outer rim of the stage area, holding postures for extended periods of time. Performances involve high volumes of plies, lifts, and jumps eliciting near-maximal metabolic responses (3). With the metabolic demand of ballet classes being less than in performance, it is unlikely that ballet training alone provides metabolic and musculoskeletal adaptations (3). In contemporary dance, dance classes and rehearsals alone are reportedly inadequate in preparing dancers for the cardiorespiratory demands of performance (15). Although elite dance training involves high volumes of activity, professional ballet dancers spend half of recorded day-to-day activity at below 3 METs (metabolic equivalent of task) which is less than a moderate level of exercise intensity (3, 16). As dance classes alone are unlikely to induce adequate metabolic, musculoskeletal, and cardiorespiratory training effects, it is recommended dancers incorporate supplementary fitness training into their schedule (3). Supplementary fitness training can help promote strength, balance, proprioception, and cardiovascular endurance to promote fatigue-resistance and reduce injury risk (10, 17). Despite this suggestion, ballet training is intensive, beginning at a young age, most often as children begin school, as was typical within this study. Young vocational dancers may attend classes daily, with pre-professional dancers taking classes "full-time" 9 am to 5 pm on up to 5–6 days per week from about 16 years of age, with 20 h (on average) up to 40 h a week reported in this study, leaving very little time for the suggested supplementary training or food consumption between classes (18). Managing nutrient requirements within a tighter dietary energy intake when there is discomfort while dancing, or while being lifted, may further encourage dancers to limit food intake (food volume and/or kilojoules) to maintain a lower body mass and body fat percentage (14, 18). As a result, lower energy availability may become chronic, and malnutrition may also become an issue. In a recent study among Australian elite dancers (19), the reported average daily energy intake was lower than the typical intake reported in studies of elite dancers from the UK and Scotland (11, 18) and in particular, lower carbohydrate intakes. Furthermore, over an 8-week period a decline in iron (sFER) levels was observed in these dancers which was attributed to lower than adequate energy intake (19).

In pre-professional dance programmes, nutrition education may be absent or limited (20), unlike in elite sporting contexts other athletic sports programmes where nutrition education and qualified sports dietitians may be more accessible (21, 22). Only 25% of participants had sought individualised advice from a qualified dietitian, despite many following vegetarian (22%) and vegan (3.2%) eating patterns. This alteration in dietary pattern places an even greater focus on adequate energy intake, adequacy of iron, zinc, Vitamin B12, calcium, and omega-3 due to plant sources having reduced bioavailability (particularly iron) and insufficient plant-based B12 sources (18). It is therefore important for dancers to have the required knowledge, skills in planning, and perhaps professional guidance to ensure adequate intake enough of the nutrients that are more easily consumed through animal food sources. The number of dancers following non-meat diets also far exceeds that within the general Australian population with representative studies suggesting 4.3% of adults are vegetarian and 1.6% are vegan (23). As a consequence, meat and fish were the most frequently excluded food items. Additionally, more than half of the dancers in this study were not consuming adequate serves of dairy per day thereby leading to reduced calcium intake from food sources. Supplements were widely used, with more than half taking multiple nutrition supplements, mostly iron and magnesium. Fewer were taking Vitamin D supplements, yet this vitamin may be at low levels primarily due to lack of exposure to sunshine and the extended time spent exercising and training inside, rather than outside (14). There are recommendations for dancers in the northern hemisphere to monitor Vitamin D status and use supplementation (24), however, this would need further investigation among Australian dancers.

Nutrition education is included in the curriculum at WAAPA, focused on building awareness of the Government guidance regarding the Australian Dietary Guidelines, the Guide to Healthy Eating and serves of foods. Dance and sport-specific evidence-based resources such as publications developed by the International Association for Dance Medicine and Science and the Australian Institute of Sport are also emphasised. Several studies have evaluated the importance of incorporating health and nutrition education into elite dance programmes with improvements in health, nutrition, and food literacy reported (20, 25, 26). Despite nutrition education being delivered at WAAPA, many participants reported consulting the internet or friends for advice, and there was a high prevalence of dancers feeling like social media made them feel like they should lose weight. These are important considerations for future curriculum design. Due to the higher risk of LEA and REDs in dancers, Civil et al. (11) suggest that a qualified sports dietitian is involved with dance programmes to enhance knowledge, and personalise advice that is safe and sustainable. This may be even more useful if students are screened for LEA and REDs on admission and annually as a general health check, as there are known challenges transitioning to higher education (10). An understanding of those dancers most at risk at the pre-professional level could help prevent shorter term issues with performance, illness and injury, but also help protect longer term health issues particularly those of a reproductive nature, protection of mental health, and provide enhanced self-worth and assist with longevity of the dance career (27). Similar to the research by Civil et al. (11), further research related to the dietary patterns of this group would be valuable. Particularly in consideration of the vegan and vegetarian diet patterns where grain and legume foods are high in dietary fibre, but low biological value proteins, and also the tendency use supplementation which may be in place of nutrient-dense foods potentially further encouraging LEA. Dietitians were utilised by a quarter of the sample, however, their services could be beneficial to a larger portion of dancers by providing practical and individualised advice, assisting dancers make food choices that provide greater gastrointestinal comfort during dance training and performance (which was a common concern), maintain lean muscle mass, improve recovery, and help better match intake with rehearsal and performance schedules (11). Hypothetically, the provision of such services would normalise the acceptability of dietitians within dance, add significant promotional value for programmes offering this type of support, and form an ongoing important focus on health and safety. This is particularly important as poor knowledge and misinformation from dance teachers and internet sources could have a negative affect on dancers, including impacts on food selection and eating patterns.

Previous studies have found that dancer knowledge of LEA was understood, more so than REDs (6). Indeed, dancers in this study also indicated a greater awareness of LEA (73.4%) compared with REDs (56.3%). Perhaps the concept of having "low energy" available is more easily understood, as it can be related to day-to-day feelings of fatigue and tiredness, whereas REDs is the result of chronic and more complex interactions between dietary intake, hormonal, and bone health. Linked with an understanding of REDs, 19% of dancers in this sample perceived the absence of menstruation as normal for female dancers and were either unaware of negative consequences or any associated problems with a lack of menstruation. In dancer health training, it is worth educators or teachers explaining REDs to dance students, including REDs Health Conceptual Model and REDs Performance Conceptual Model proposed by Mountjoy et al. (5) along with raising awareness of symptoms such as amenorrhea, low libido, pain from bone stress injury, hunger, and training plateaus. Discussing the conceptual models and symptoms of REDs could provide an opportunity to discuss eating patterns, the known risks of amenorrhea or lack of morning erections for males, which can then be monitored by the dancer as a personal sign of health, along with longer-term problems associated with REDs such as osteoporosis. Importantly, under extreme circumstances, some of the negative consequences of REDs have proven difficult to reverse even with pharmacological means, placing even greater responsibility on the dance community to protect dancers of all ages. Of concern is the low level of awareness of menstrual disturbance and LEA among healthcare practitioners reported by dancers in this study. Despite a number of specific resources being developed for healthcare practitioners and the dance community (28) in recent years, there remains a lack of awareness among doctors (29).

In this study there was a reported 1.6 injuries per dancer in the past year, a similar finding to research among modern dancers [1.31 injuries per dancer (30)] yet less than in ballet [6.8 injuries per dancer (31)]. Soft tissue injuries were relatively common, with more than half being recurrent in nature. Results align with other research which reported soft tissue injuries [i.e., muscle and tendon inflammation (36%), and strains and sprains (27%)] as the most frequent injuries among a similar pre-professional group (N = 76) (8). Over one-third of dancers had sustained a bone injury since starting full-time dance, with the foot being the most common fracture site. These results align with previous research which indicates bone injuries in the feet are common in dancers (30, 32, 33). Previous research has identified that dancers with LEA are more likely to sustain a soft tissue injury and that training volume (exposure time) is associated with a higher risk of bone injuries (34). Similarly, in a study of Norwegian university dance students there was an increased odds of having an injury if symptoms of low LEA were prevalent (8). In another study, high training volume among professional ballet dancers was linked to insufficient bone remodelling, potentially leading to an increased risk of bone stress injury (35). It is well established that LEA contributes to impaired bone health (36), and previous research among dancers has highlighted the importance of adequate nutrition and controlled training volumes to optimise energy availability and reduce the prevalence of injuries (34).

In this study, 22% of dancers did not respond to the question about their body weight. This could be interpreted in a number of ways. Either they did not know or have not recently weighed, or they did not want to provide this information. Body weight was an important measure to estimate health status and was utilised within the REDs score to understand risk, but realising the issues among dancers and sensitivity to reporting weight, the data from this response may be highly unreliable. However, body weight data, together with the calculated BMI, was reflected in hormonal changes reported by this group with a third of dancers experiencing oligomenorrhea and 31% experiencing secondary amenorrhea previously. Dancers also reported their lowest body weight for current height with BMI calculations indicating as many as 61% of dancers having a BMI < 20 (below the healthy range) when they were at their lower weight. Sixteen percent of dancers had a previous history of eating disorders, which is within the prevalence range of 12 to 26.5% described in the literature across all dance genres (8). Future research should aim to understand the issues of weighing and weight, as there may be other factors involved. Rejection of body mass as a single measure is a point of discussion, and this is an important issue for the future of dance where rudimentary cut-offs have been applied for entry to competitions, programmes, and at the professional level in the past. The recently published 2023 IOC Consensus Statement on REDs (5) recommend avoiding body composition assessment and monitoring in athletes < 18 years, with the exception of medical purposes (37, 38). Further, the supporting Best Practice Guidelines highlight the importance of sport federations addressing competition requirements which perpetuate unhealthy practices around body composition and report multiple sports (including figure skating, gymnastics, and artistic swimming) modifying rules to reduce the risk of developing REDs associated with participation (38).

Other health concerns within this cohort can be drawn from information supplied relating to medication use, sleeping issues, digestive problems, and the way both eating and weight affect the dancer's sense of self. There are known links between nutrition and mental health, depression and eating disorders, sleep disturbance, and physiological functions of the body such as digestive issues (39). Further, emerging adulthood has been highlighted as a key period of development in terms of both diet quality and mental health (39). Comparable studies of pre-professional dancers have reported 20 to 60% having anxiety or depression, symptoms of LEA and/or eating disorders (8). Importantly, one-third of dancers in this study ranked the questions relating to "controlling what they eat" and "controlling what they weigh" at the highest end of the scale (as 4 or 5 on a six-point scale), indicating that both factors affect the way they feel about themselves. Others have pointed to issues particularly related to dancers including "self-oriented perfectionism" whereby self-worth is highly linked with performance and feedback as well as the ability to push their body to artistic limits and constant improvement (8). Striving for perfectionism, however, can cause anxiety, stress, or compulsiveness which are reportedly potential comorbidities to eating disorders (40).

A large portion of dancers in this study reported the belief that a lighter body weight would increase chances of getting a lead role and many reported the belief that they dance best at a weight lower than their current weight. These findings align with previous studies reporting a desire for thinness and body dissatisfaction in dance (6), which are factors linked with an increased risk of disordered eating, eating disorders, and LEA (40–43). In a recent study exploring dance science knowledge, dancer educators identified mental health and psychology as topics requiring more research (44). Furthermore, recent recommendations by Reece et al. (45) state: "Treatment of LEA requires addressing the underlying cause. The gold standard for treatment of disordered eating/eating disorders is a multidisciplinary, collaborative approach. Education for athletes, parents, coaches, and athletic trainers is imperative for the detection and prevention of LEA and disordered eating/eating disorders" (45). Indeed, in the last decade there has been an increase in eating disorder prevention and nutrition education interventions developed for athletes and dancers. Education programmes focused on promoting body acceptance [e.g., the Body Project (46) and the Body Project with dancers (47)] and incorporating behaviour change such as using cognitive-behaviour-dissonance (48) show promise in reducing body dissatisfaction, reducing the risk of eating disorders, and in increasing the number of athlete's seeking professional advice regarding female triad symptoms (46). Despite progress in health education programmes for dancers [e.g., (47, 49)], addressing education at a broader level including dance teachers and industry, along with a culture shift away from thinness idealisations in dance is required (4, 10). Safeguarding the health and welfare of high-performance athletes has been flagged as a priority in all sporting environments, including among young athletes (21), and the importance of sports medicine within performing arts has been acknowledged (11, 50). Recommendations for principles and functions of health services within Australian high-performance sport have recently been proposed (21) including for disordered eating (51). However, despite the promising progress in elite sport, guidelines for healthcare services within the context of dance are lacking in Australian educational programmes.

4.1 Limitations and future research

Several limitations are worth noting. First, the sample only included dancers from one Australian tertiary institution. However, dancers originated from states and territories across Australia with half of all participants in first year of study, and as such, the findings reflect Australian dancers embarking on full-time training. Second, the small number of male participants in this study limited the ability to analyse and compare differences between male and female dancers across all measures. We were, however, able to analyse BMI, LEA, and risk of REDs, with which males were at lower risk than female dancers. Nonetheless, education remains important to promote career longevity among males as it does females (27) and is an important consideration in future research. Third, the DEAQ self-report tool has been validated among dancers previously and in this study it was adapted for the Australian environment. Inclusion of additional health measures such as blood work and bone mineral density such as those outlined in the 2023 IOC REDs Consensus Statement (5) would allow for a greater understanding of LEA and REDs indicators among dancers. Fourth, alcohol intake was not measured in this study. A study of professional dancers has compared weekday energy intake to weekends and dancers do increase their energy intake over weekends from fat and alcohol, reducing carbohydrate and protein when they are not dancing (18). As alcohol influences energy intake, potentially fat mass (52), and macro and micronutrient absorption (53) it is recommended alcohol intake is considered in future studies among dancers. Lastly, the current study was descriptive in nature. To provide greater insight into understanding reasons for certain choices or behaviours (such as supplement use or eliminating specific foods) a range of questions could be used to probe certain issues. Additionally, focus groups or clinical interviews would allow a more comprehensive understanding of the needs of dancers, dance teachers, and those involved in professional companies along with the perceived barriers and facilitators of health and nutrition education in elite dance.

4.2 Practical implications

Recognising dancers as athletes may be the first step in helping the dance community move forward on a range of issues related to dancer health and wellbeing. For training institutions, the focus needs to be on ensuring curriculum and education standards reflect current knowledge in dance and sport science as well as facilitating access to a range of health professionals, with health promotion and prevention of injury at the forefront of the dance training philosophy. Evaluation of dancer knowledge of preventative health and correcting misinformation will help break the cycle of misinformation, as today's pre-professional dancers will likely become the teachers and artistic directors of the future. Screening in a manner that engages the dancer rather than punishing their self-esteem may also be necessary to adequately risk assess and manage the needs of each cohort. Early screening will help facilitate earlier intervention and protect and promote longevity in dance careers. Screening questions could be incorporated into existing physical and health screenings [e.g., (54)] or built into early curriculum to raise dancer self-awareness.

5 Conclusion

This research has helped establish an understanding of dance students' knowledge of current health, nutrition issues, lifestyle, wellbeing, and understanding of longer-term health implications. As a crude indication, one-third of female dancers had a negative REDs risk score. A similar proportion had a BMI < 20, previous fractures, a history of secondary amenorrhea, and had experienced oligomenorrhea. Awareness of REDs and the connection with menstrual issues was relatively low among dancers despite this being a good indicator of overall current physical health. Broad engagement of the dance community is called to action, to acknowledge the need to develop programmes for dancers of all ages and level of training that help protect and empower dancers in relation to health, nutrition, fitness, and wellbeing. This study also highlights the importance of adequate healthcare within dance, including access to relevant healthcare practitioners within elite training environments, along with improved awareness and education of the physical and psychological demands of dancing and LEA and REDs among all practitioners working with dancers.

Data availability statement

The datasets presented in this article are not readily available because ethical approval and participant consent was not obtained for sharing data. Requests to access the datasets should be directed to JN, j.nicholas@ecu.edu.au.

Ethics statement

Ethics approval for this study was granted by the Edith Cowan University Human Research Ethics Committee (REMS NO: 2022-03575-NICHOLAS) and University of New South Wales Human Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Where participants were <18 years of age, written informed consent was provided by the participants' legal guardians/next of kin.

Author contributions

JN: Conceptualization, Formal analysis, Investigation, Methodology, Writing–original draft, Writing–review and editing. SG: Conceptualization, Formal analysis, Investigation, Methodology, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors planned and conducted the research independently and prior to obtaining funding from the Royal Academy of Dance Australia for open access publication fees. The Royal Academy of Dance were not involved in any part of the research.

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References

1. Koutedakis Y, Jamurtas A. The dancer as a performing athlete. *Sports Med.* (2004) 34:651–61. doi: 10.2165/00007256-200434100-00003

2. Wyon M. Preparing to perform: periodization and dance. *J Dance Med Sci.* (2010) 14:67–72. doi: 10.1177/1089313X1001400205

3. Shaw J, Mattiussi A, Brown D, Springham M, Pedlar C, Tallent J. The activity demands and physiological responses observed in professional ballet: a systematic review. *J Sport Exerc Sci.* (2021) 5:254–69.

4. Blevins P, Erskine S, Hopper L, Moyle G. Finding your balance: an investigation of recovery–stress balance in vocational dance training. *J Dance Educ.* (2020) 20:12–22. doi: 10.1080/15290824.2018.1532571

5. Mountjoy M, Ackerman K, Bailey D, Burke L, Constantini N, Hackney A, et al. 2023 International Olympic Committee's (IOC) consensus statement on Relative Energy Deficiency in Sport (REDs). *Br J Sports Med.* (2023) 57:1073–97. doi: 10.1136/bjsports-2023-106994

6. Keay N, Overseas A, Francis G. Indicators and correlates of low energy availability in male and female dancers. *BMJ Open Sport Exerc Med.* (2020) 6:e000906. doi: 10.1136/bmjsem-2020-000906

7. Arcelus J, Witcomb G, Mitchell A. Prevalence of eating disorders amongst dancers: a systemic review and meta-analysis. *Eur Eat Disord Rev.* (2014) 22:92–101. doi: 10.1002/erv.2271

8. Fostervold Matheson T, Sundgot-Borgen C, Anstensrud B, SundgotBorgen J. Mental health, eating behaviour and injuries in professional dance students. *Res Dance Educ.* (2022) 23:108–25. doi: 10.1080/14647893.2021.1993171

9. Fostervold Mathisen T, Sundgot-Borgen C, Anstensrud B, Sundgot-Borgen J. Intervention in professional dance students to increase mental health- and nutrition literacy: a controlled trial with follow up. *Front Sports Act Living.* (2022) 4:727048. doi: 10.3389/fspor.2022.727048

10. Paschali A, Araújo L. *Health Promotion among Dance Students in Higher Education: a Qualitative Investigation of Experiences and Perceptions of Health and Well-Being.* London: Informa UK Limited (2023). doi: 10.1080/14647893.2023.2199197

11. Civil R, Lamb A, Loosmore D, Ross L, Livingstone K, Strachan F, et al. Assessment of dietary intake, energy status, and factors associated with RED-S in vocational female ballet students. *Front Nutr.* (2019) 5:136. doi: 10.3389/fnut.2018.00136

12. Staal S, Sjödin A, Fahrenholtz I, Bonnesen K, Melin A. Low RMR ratio as a surrogate marker for energy deficiency, the choice of predictive equation vital for correctly identifying male and female ballet dancers at risk. *Int J Sport Nutr Exerc Metab.* (2018) 28:412–8. doi: 10.1123/ijsnem.2017-0327

13. National Health and Medical Research Council. *Australian Guide to Healthy Eating.* (2013). Available online at: https://www.eatforhealth.gov.au/sites/default/files/2022-09/n55a_australian_dietary_guidelines_summary_131014_1.pdf (accessed July 12 2023).

14. Sousa M, Carvalho P, Moreira P, Teixeira V. Nutrition and nutritional issues for dancers. *Med Probl Perform Art.* (2013) 28:119–23. doi: 10.21091/mppa.2013.3025

15. Wyon M, Redding E. Physiological monitoring of cardiorespiratory adaptations during rehearsal and performance of contemporary dance. *J Strength Cond Res.* (2005) 19611–4. doi: 10.1519/14233.1

16. Garber C, Blissmer B, Deschenes M, Franklin B, Lamonte M, Lee I, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults. *Med Sci Sports Exerc.* (2011) 43:1334–59. doi: 10.1249/MSS.0b013e318213fefb

17. Rodrigues-Krause J, Krause M, Reischak-Oliveira Á. Cardiorespiratory considerations in dance: from classes to performances. *J Dance Med Sci.* (2015) 19:91–102. doi: 10.12678/1089-313X.19.3.91

18. Brown M, Howatson G, Quin E, Redding E, Stevenson E. Energy intake and energy expenditure of pre-professional female contemporary dancers. *PLoS One.* (2017) 12:e0171998. doi: 10.1371/journal.pone.0171998

19. Attwell C, McKay A, Sim M, Dugan C, Nicholas J, Hopper L, et al. Timing is everything, but does it really matter? Impact of 8-weeks morning versus evening iron supplementation in ballet and contemporary dancers. *Eur J Sport Sci.* (2023). [Online ahead of print]. doi: 10.1080/17461391.2023.2224285

20. Kozai A, Ambegaonkar J. Health literacy for collegiate dancers: provision and perceptions of health-related education in university dance programs. *J Dance Med Sci.* (2020) 24:118–25. doi: 10.12678/1089-313X.24.3.118

21. Drew M, Toohey L, Smith M, Baugh C, Carter H, McPhail S, et al. Health systems in high-performance sport: key functions to protect health and optimize performance in elite athletes. *Sports Med.* (2023) 53:1479–89. doi: 10.1007/s40279-023-01855-8

22. Trakman G, Forsyth A, Hoye R, Belski R. Australian team sports athletes prefer dietitians, the internet and nutritionists for sports nutrition information. *Nutr Dietetics.* (2019) 76:428–37. doi: 10.1111/1747-0080.12569

23. Malek L, Umberger W. Distinguishing meat reducers from unrestricted omnivores, vegetarians and vegans: a comprehensive comparison of Australian consumers. *Food Qual Prefer.* (2021) 88:104081. doi: 10.1016/j.foodqual.2020.104081

24. Wyon M, Koutedakis Y, Wolman R, Nevill A, Allen N. The influence of winter vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: a controlled study. *J Sci Med Sport.* (2014) 17:8–12. doi: 10.1016/j.jsams.2013.03.007

25. Yannakoulia M, Sitara M, Matalas A. Reported eating behavior and attitudes improvement after a nutrition intervention program in a group of young female dancers. *Int J Sport Nutr Exerc Metab.* (2002) 12:24–32. doi: 10.1123/ijsnem.12.1.24

26. Doyle-Lucas A, Davy B. Development and evaluation of an educational intervention program for pre-professional adolescent ballet dancers: nutrition for optimal performance. *J Dance Med Sci.* (2011) 15:65–75. doi: 10.1177/1089313X1101500203

27. Kim H, Tasker S, Shen Y. How to persevere in a ballet performance career: exploring personal wisdom of retired professional ballet dancers. *Res Dance Educ.* (2022) 23:425–50. doi: 10.1080/14647893.2020.1837765

28. Keay N. *Raising Awareness of RED-S in Male and Female Athletes and Dancers.* (2018). Available online at: https://blogs.bmj.com/bjsm/2018/10/30/raising-awareness-of-red-s-in-male-and-female-athletes-and-dancers/ (accessed July 12 2023).

29. British Association of Sports & Exercise Medicine. *Concerning' Lack of Awareness of How Best to Reduce Risk of Stress Fractures in Female Athletes and Dancers, a Year after Change in NICE Guidelines.* (2023). Available online at: https://basem.co.uk/concerning-lack-of-awareness-of-how-best-to-reduce-risk-of-stress-fractures-in-female-athletes-and-dancers-a-year-after-change-in-nice-guidelines/ (accessed June 26, 2023).

30. Bronner S. Injury characteristics in pre-professional modern dancers: prospective study of differences due to sex, training year, and external causal mechanisms. *J Dance Med Sci.* (2021) 25:117–30. doi: 10.12678/1089-313X.061521g

31. Allen N, Nevill A, Brooks J, Koutedakis Y, Wyon M. Ballet injuries: injury incidence and severity over 1 year. *J Orthop Sports Phys Ther.* (2012) 42:781–A781. doi: 10.2519/jospt.2012.3893

32. Albisetti W, Perugia D, De Bartolomeo O, Tagliabue L, Camerucci E, Calori G. Stress fractures of the base of the metatarsal bones in young trainee ballet dancers. *Int Orthop.* (2010) 34:51–5. doi: 10.1007/s00264-009-0784-3

33. Iafrate J, Townsend C, Scott C, Yun H, Ventola A, Semanson S. Diagnosis and management of foot and ankle injuries in dancers. *Curr Phys Med Rehabil Rep.* (2021) 9:47–56. doi: 10.1007/s40141-021-00313-8

34. Prus D, Mijatovic D, Hadzic V, Ostojic D, Versic S, Zenic N, et al. (Low) energy availability and its association with injury occurrence in competitive dance: cross-sectional analysis in female dancers. *Medicina.* (2022) 58:853. doi: 10.3390/medicina58070853

35. Bladt F, Varaeva Y, Benjamin J, Patel S, Courtney A, Holloway P, et al. Preliminary study of bone biomarkers in elite female ballet dancers. *Curr Issues Sport Sci.* (2023) 8:004. doi: 10.36950/2023.1ciss004

36. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the Female Athlete Triad—Relative Energy Deficiency in Sport (RED-S). *Br J Sports Med.* (2014) 48:491–7. doi: 10.1136/bjsports-2014-093502

37. Ackerman K, Stellingwerff T, Elliott-Sale K, Baltzell A, Cain M, Goucher K, et al. #REDS (Relative Energy Deficiency in Sport): time for a revolution in sports culture and systems to improve athlete health and performance. *Br J Sports Med.* (2020) 54:369–70. doi: 10.1136/bjsports-2019-101926

38. Fostervold Mathisen T, Ackland T, Burke L, Constantini N, Haudum J, Macnaughton L, et al. Best practice recommendations for body composition considerations in sport to reduce health and performance risks: a critical review, original survey and expert opinion by a subgroup of the IOC consensus on Relative Energy Deficiency in Sport (REDs). *Br J Sports Med.* (2023) 57:1148–58. doi: 10.1136/bjsports-2023-106812

39. Collins S, Dash S, Allender S, Jacka F, Hoare E. Diet and mental health during emerging adulthood: a systematic review. *Emerg Adulthood.* (2022) 10:645–59. doi: 10.1177/2167696820943028

40. Torres-McGehee T, Emerson D, Pritchett K, Moore E, Smith A, Uriegas N. Energy availability with or without eating disorder risk in collegiate female athletes and performing artists. *J Athletic Train.* (2020) 56:993–1002. doi: 10.4085/JAT0502-20

41. Martinsen M, Bratland-Sanda S, Eriksson AK, Sundgot-Borgen J. Dieting to win or to be thin? A study of dieting and disordered eating among adolescent elite athletes and non-athlete controls. *Br J Sports Med.* (2010) 44:70. doi: 10.1136/bjsm.2009.068668

42. Sundgot-Borgen J, Torstveit M. Aspects of disordered eating continuum in elite high-intensity sports. *Scand J Med Sci Sports.* (2010) 20(Suppl 2):112–21. doi: 10.1111/j.1600-0838.2010.01190.x

43. Wasserfurth P, Palmowski J, Hahn A, Krüger K. Reasons for and consequences of low energy availability in female and male athletes: social environment, adaptations, and prevention. *Sports Med Open.* (2020) 6:44. doi: 10.1186/s40798-020-00275-6

44. Hawke J, Bredin S. Examining the preferences and priorities of dance educators for dance science information: a pilot study. *J Dance Med Sci.* (2023) 27:107–15. doi: 10.1177/1089313X231178079

45. Reece L, Farnsworth N, Whitney K, Ackerman K. Eating disorders and relative energy deficiency in sport (RED-S). In: Christino M, Pluhar E, Micheli L editors. *Psychological Considerations in the Young Athlete: A Multidisciplinary Approach.* Cham: Springer International Publishing (2023). p. 59–79. doi: 10.1007/978-3-031-25126-9_3

46. Becker C, McDaniel L, Bull S, Powell M, McIntyre K. Can we reduce eating disorder risk factors in female college athletes? A randomized exploratory investigation of two peer-led interventions. *Body Image.* (2012) 9:31–42. doi: 10.1016/j.bodyim.2011.09.005

47. Gorrell S, Schaumberg K, Boswell J, Hormes J, Anderson D. Female athlete body project intervention with professional dancers: a pilot trial. *Eat Disord.* (2021) 29:56–73. doi: 10.1080/10640266.2019.1632592

48. Larsen T. *A Systematic Review of Eating Disorder Prevention and Preventative Protocols for Elite Female Athletes in Individual Sports [Psy.D.].* Tucson, AZ: University of Arizona Global Campus (2022).

49. Nordin-Bates S, Lundström P, Melin A, Sundgot-Borgen J, Edlund K. Preventing disordered eating in teenage ballet students: evaluation of dancexcellent, a combined CBT and nutrition education intervention. *Med Probl Perform Art.* (2023) 38:71–9. doi: 10.21091/mppa.2023.2009

50. Dick R, Berning J, Dawson W, Ginsburg R, Miller C, Shybut G. Athletes and the arts — the role of sports medicine in the performing arts. *Curr Sports Med Rep.* (2013) 12:397–403. doi: 10.1249/JSR.0000000000000009

51. Wells K, Jeacocke N, Appaneal R, Smith H, Vlahovich N, Burke L, et al. The Australian Institute of Sport (AIS) and National Eating Disorders Collaboration (n.d.) position statement on disordered eating in high performance sport. *Br J Sports Med.* (2020) 54:1247–58. doi: 10.1136/bjsports-2019-101813

52. Kwok A, Dordevic A, Paton G, Page M, Truby H. Effect of alcohol consumption on food energy intake: a systematic review and meta-analysis. *Br J Nutr.* (2019) 121:481–95. doi: 10.1017/S0007114518003677

53. Butts M, Sundaram V, Murughiyan U, Borthakur A, Singh S. The influence of alcohol consumption on intestinal nutrient absorption: a comprehensive review. *Nutrients.* (2023) 15:1571. doi: 10.3390/nu15071571

54. Matiussi A, Shaw J. Data at the ballet. *Significance.* (2023) 20:14–7. doi: 10.1093/jrssig/qmad042

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The effect of menthol rinsing before intermittent exercise on physiological, physical, and thermo-behavioral responses of male football referees in hot and humid environment

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Introduction: In the current experiment, we aimed to evaluate whether eliciting pre-exercise non-thermal cooling sensations would alter perceptual measures, and physical and physiological responses in football referees.
Methods: Nine highly trained male football referees undertook two 45-minute intermittent exercise protocols in hot and humid conditions (34.2 ± 0.6°C, 62.5 ± 1.0% relative humidity). In a randomized counterbalanced crossover design, 1 of 2 beverages were given before the warm-up: a 0.01% menthol solution or a placebo noncaloric solution. Physical performance was quantified as total distance covered in each of the three 15-minute exercise blocks. Core temperature, heart rate, thermal sensation and thermal comfort were measured at rest and after each exercise block.
Results: No changes were observed between trials and over time for distance covered. No main effect of mouth rinse was observed for core temperature and heart rate, but both increased over time in all conditions ($P < 0.001$). Thermal sensation and thermal comfort were significantly improved with menthol after mouth-rinsing ($P < 0.05$), but with no differences at any other time-point.
Discussion: These results indicate that non-thermal cooling oral stimuli provide immediate behavioral changes but may not influence physiological or physical responses in football referees, during intermittent exercise in hot and humid environments.

Clinical Trial Registration: www.clinicaltrials.gov, identifier NCT05632692.

KEYWORDS

cooling, football referees, heat stress, intermittent exercise, oral menthol, thermal perception

1 Introduction

A competitive football match play is regulated by a referee, two assistant referees, and a sideline official. In a whole sports season, 1.3 million referees enter the football pitch every week to regulate players' behavior and to regulate the rules of the game (1). Despite these statistics and the important role in ensuring that players and others involved maintain the laws of the game, very little scientific literature is available on football refereeing, especially when compared with players (2).

Intermittent exercise efforts (i.e., sprinting) in the heat is recognized to augment thermoregulatory strain (3), reducing physical performance (e.g., distance covered) and disturbing motivation levels (4), when compared to similar exercise in temperate conditions, concretely in football players. In fact, substantial changes to football players' performance as a consequence of exercising in the heat are observed, with a reduced number of sprints performed by players (−10%), as well as a reduction in the distance covered at high intensity (5) and total distance covered during games (6). These changes result from anticipatory pacing to mitigate excessive increases in core and muscle temperature, as well as in thermal sensation (7, 8).

Considering that football players and football referees are allowed the same strategies to cope with the heat, it is plausible that the extreme conditions may also affect referees' physical and physiological performance, compromising their skills to monitor and control important events during the game (9, 10). In addition, referees cover 7.5–11.5 km per match play (11, 12), and an elite football referee spends 42% of the match running at high intensity (18.1–24 km h^{-1}) (13, 14). Therefore, it can be anticipated that the activity profile of elite referees is demanding and they must be considered as athletes (15). This way, it is fair that their training and performance may be given with due consideration from sport sciences, particularly regarding heat-related fatigue and strategies to cope with the heat, such as cooling interventions.

Menthol is an organic compound that incites non-thermal cooling effects on target receptors, throughout the body regions, depending on if it is applied internally or externally (16). Although menthol does not prevent heat gain or reduce core temperature, it seems to help the athlete to feel perceptually cool, being further able to perform for a longer period of time (17–19) or at a higher power output (20–22). Specifically, menthol activates the transient receptor potential cation channel subfamily M member 8 (TRPM8), which responds to cold stimuli. This activation is responsible for the cold sensation experienced from this compound and for the eventual improvements in thermal sensation, thermal comfort, rating of perceived exertion (RPE), and performance (23, 24). A recent systematic review concluded that mouth rinsing with a menthol solution (0.01%) during exercise in the heat significantly improved physical performance, mainly in continuous exercises (25). Also, considering the current body of evidence, a recent consensus statement found that research regarding the effect of menthol on intermittent exercise and on elite athletes was insufficient, while providing greater attention to endurance efforts and recreational participants (26). Adding this to the fact that menthol is simply transportable and low cost, it is worth understanding if menthol mouth rinse might be a viable non-thermic alternative to improve intermittent exercise in the heat (27).

In addition to the fact that very few studies have evaluated the ergogenic effect of menthol in intermittent exercise protocols, no study has evaluated the effects of internal cooling strategies on football referees. Accordingly, the primary purpose of the current study is to analyze the effects of a menthol solution mouth rinse on football referees' physical performance and perceptions of the environment, during a standardized laboratory exercise protocol performed in the heat. We hypothesized that menthol mouth rinsing will improve perceptual responses and consequently increase physical performance without increasing physiological strain in football referees, while exercising in the heat.

2 Materials and methods

The study has been approved by the Ethics Committee of the Faculty of Nutrition and Food Sciences of the University of Porto, Portugal (Report 112/22 CEFCNAUP 2022), conducted in accordance to the Declaration of Helsinki for human studies (28) and developed considering the guidelines from Consolidated Standards of Reporting Trials (CONSORT) (29). Also, the protocol of the trial was registered on www.ClinicalTrials.gov (NCT05632692; 20 November 2022).

2.1 Participants

Nine healthy non-heat-acclimated highly trained (30) male football referees registered in the Portugal Football Federation participated in the study (Table 1). The participants were recommended to maintain training regimes from one trial to the following and abstain from exercise 24 h before each trial. To minimize the difference in muscle glycogen levels and respiratory exchange ratio (RER) between trials, participants were advised to consume their habitual diet and to repeat pre-exercise food intakes from one trial to the next. The participants were also asked to ingest 2–3 L of water in the day before each session.

For sample size and statistical power calculations, power analysis was based on changes in thermal sensation after the internal

TABLE 1 Participants characteristics.

Age (years)	33.4 ± 5.2
Height (m)	1.8 ± 0.1
Weight (kg)	73.2 ± 6.2
Body mass index (kg/m^2)	22.4 ± 1.1
Body fat (%)	11.9 ± 3.6
Lean body mass (kg)	64.3 ± 6.0
Hours of training per week (h)	7.6 ± 2.1
Experience as a referee (years)[a]	16.7 ± 5.1
Experience as an elite referee (years)[b]	7.4 ± 2.3

Data are presented as mean ± SD (*n* = 9).

[a]Experience as a referee considers the period (number of years) since the beginning of professional activity.

[b]Experience as an elite referee considers the period (number of years) of professional activity in C1 or C2 elite categories.

administration via mouth rinsing or ingestion of menthol solution. A type I error of 5% and a power of 85%, with a statistical significance (P-value ≤ 0.05) and a moderate effect size of 0.54 [differences in thermal sensation were considered following Jeffries and Waldron results (31)], were considered, using G*Power 3.1.9.2®. A sample size of at least eight participants was determined. Inclusion criteria required participants to be: highly trained male field football referees registered in Portuguese Football Federation, aged ≥18 and ≤45 years, with normal weight (body mass index ≥18.5 and ≤24.9 kg/m^2), and available to participate in the familiarization session and in the two experimental sessions. Participants were excluded from the present study if they were under the influence of any medications that may affect urinary parameters, thermoregulation mechanisms, circulatory system, thyroid and pituitary function, or metabolic status; had injury, diabetes, autoimmune disease, cardiovascular disease, or obstructive disease of the gastrointestinal tract (e.g., diverticulitis, inflammatory bowel disease); were diagnosed with schizophrenia, bipolar disorder, or other psychotic disorders, as well as eating disorders; and had a magnetic resonance imaging scan scheduled within 48 h after the experimental trials (32).

2.2 Study design

A randomized single-blinded, counterbalanced, crossover trial with two conditions was performed. After fulfilling the eligibility criteria, and carrying one introductory meeting and a familiarization session, the participants were ascribed to two experimental days for undergoing two different randomly ordered experimental conditions. Each condition was comprised of a 45 min football protocol [intermittent Soccer Aerobic Fitness Test (SAFT-45)] (33), with the administration of one of two beverages before warm-up (pre-cooling). There was a minimum washout period of 7 days between the familiarization session and the first trial, as well as between the first and second trials, to reduce carryover effects from the previous condition and to assure an adequate exercise recovery. The trials took place in an experimental room, with temperatures ranging from 33.6 to 35.4°C and relative humidity ranging from 58.0% to 63.5%.

The main researcher assigned the allocation sequence for the order of the trial conditions to every participant, recurring to individual randomization from a computer-generated random order. The main researcher was not blinded to the type of study condition, had access to the allocation sequence list, and retrieved the randomization code prior to each experiment to prepare the beverages. All other investigators and the outcome surveyors were blinded to the study condition and group allocation, and the participants were blinded to the beverages' composition and aim of the study.

2.3 Introductory meeting

The research team presented the study details to the participants, before the experimental trials. All stages of the study as well as the tools and procedures that were going to be implemented were described. After understanding and accepting to share their information, the participants signed the informed consent.

2.4 Familiarization session

All participants underwent a familiarization session. All experimental procedures were entirely described and tested, so that the familiarization trial was as close as possible to the experimental trials, replicating the exercise protocol.

2.5 Exercise protocol

The SAFT-45 is an adaptation of the original SAFT-90, consisting of sets of 15 min of predetermined intermittent football-specific protocol (34). The protocol imitates the intermittent and multi-directional nature of football match play, with regular changes in direction and activity (35). It is based on time–motion analysis data from the English Championship–level match play acquired during the 2007 season (33) and has the goal to simulate the activity demands and physiological reactions of a football game (36). Participants navigate around a 20-m agility course in an intermittent fashion via standing (0 km h^{-1}), walking (5.5 km h^{-1}), jogging (10.7 km h^{-1}), striding (15.0 km h^{-1}), or sprinting (maximal effort) (37). The protocol is divided into equivalent 15-min activity profiles, lasting 45 or 90 min, and can be performed indoors. The type of movement activity and intensity is controlled using verbal signals from an audio MP3 file (33). The participants of the present study conducted a normal pre-match routine regarding rest and nutrition, and a 5-min warm-up preceded the 45-min protocol.

2.6 Solution formulation

The participants were given one of two beverages: Beverage A—menthol solution was formulated by crushing non-caloric menthol lozenges into small pieces (Halls Extra Strong, Mondelez International, Birmingham, United Kingdom) weighed to obtain a concentration of 0.05% and dissolved in warm deionized water (32). After complete dissolution, the menthol solutions were diluted to a 0.01% concentration (i.e., 20 ml of the 0.05% solution were diluted in 80 ml of deionized water) (17, 18, 38); Beverage B—placebo solution was prepared using a non-caloric berry-flavored sweetener consisting of sucralose (Crystal Light, Don Mills, Ontario, Canada) (32). Prior to use, both solutions were prepared for mouth rinse and warmed at room temperature. The subjects were given, in a pre-cooling mode, 75 ml of the solution to rinse prior to the warm-up, divided into three equal parts (25 ml each). Each part was made available to the participant every 1 min (3 min of pre-cooling) and rinsed for 10 s.

2.7 Physiological responses

2.7.1 Heart rate

Heart rate was measured through heart rate monitors (Polar H10 Heart Rate Sensor, USA) continuously throughout the exercise period and reported at baseline (before warm-up) and after each 15-min exercise block. Data were visualized and exported recurring to Polar Vantage V and Polar Flow Sync software and calculated by minutes. PolarH10 can accurately measure the mean heart rate and low-frequency oscillations (up to 0.15 Hz) of heart rate at rest and during the exercise (39).

2.7.2 Core temperature

Core temperature was evaluated continuously through a telemetric pill ingested 60 min prior the start of the session (BodyCap®, Hérouville-Saint-Clair, France) and reported at baseline (before warm-up) and after each 15-min exercise block. Given the satisfactory precision, the ability to measure in field-based situations, and being non-invasive, the ingestible telemetric temperature pill was suitable to assess the core temperature during exercise in different settings (40).

2.7.3 Sweating rate

Sweating rate was estimated according to the following equation (41):

$$\frac{\text{Pre-exercise body weight} - \text{post-exercise body weight} + \text{fluid intake} - \text{urine volume}}{\text{exercise time in hours}}$$

Pre-exercise body weight was measured after participants emptied their bladder. Body weight was assessed on a digital platform scale (InBody 270, InBody CO., LTD, South Korea), with minimal clothing. Post-exercise body weight was recorded also with minimal clothing, after participants' towel off themselves, at the end of the protocol. Urinary excretion was not considered since the protocol was continuous (no half-time) and therefore the participants did not go to the bathroom. Fluid intake was evaluated via the measurement of mass change to the nearest 0.1 ml of the individual bottles, provided to the participants, at the warm-up and collected at the end of the session (Seca, Hamburg, Germany). The research team advised the participants not to spit out the fluids at any time of the protocol. Also, fluid intake was only allowed in the 30th minute of the exercise protocol, to simulate "cooling break–water break" (32) rule implemented by FIFA. FIFA's guidelines for extreme heat conditions (i.e., wet bulb globe temperature > 32°C) refer cooling breaks to be mandatory in both halves of a match, around the 30th minute and 75th minute, so that football players and referees may rehydrate (42).

2.7.4 Blood glucose and lactate levels

Blood lactate and glucose levels were assessed via a fingertip sample before and after the exercise protocol, recurring to a Blood Lactate Meter (Lactate Pro 2, Arkray, Ltd., Koka-shi, Shiga, Japan) and a Glucometer (FreeStyle Precision Neo, Abbott Laboratories, USA), respectively. Both devices have been validated previously (43, 44).

2.7.5 Hydration status

Hydration status was evaluated through urine-specific gravity (USG), recurring to urine test strips (Combur10 Test M, Roche, Switzerland) and a Urisys 1100® analyzer (Roche, Switzerland) before the start and at the end of the session.

2.8 Perceptual measures

2.8.1 RPE, thermal sensation, thermal comfort, and perceived thirst

RPE was recorded through the CR-10 Borg scale (45) from 0 ("rest") to 10 ("maximal effort"). Thermal sensation was recorded with a 9-point scale (33) from −4 ("very cold") to 4 ("very warm") (46). Thermal comfort was assessed according to a 6-point scale from to −3 ("very uncomfortable) to 3 ("very comfortable") (47). Perceived thirst was evaluated recurring to a 7-point scale from 1 ("not thirsty at all") to 7 ("very, very thirsty") (48).

2.9 Physical parameters

2.9.1 Distance covered

Distance covered was calculated based on the number of shuttle run routes taken by the participants, multiplying the number of routes by the length of each route – 20 m (51). The number of shuttle run routes was measured using an app (https://simplecounter.app/). Counting was started after the warm-up, at the beginning of the 45-min exercise protocol.

2.10 Experimental procedure

Upon arrival at the laboratory, having abstained from vigorous exercise in the 24 h before the test, and after the telemetric pill ingestion, a urine sample was collected for a USG test to check hydration status. Both trials were schedule at the same time of the day to control for circadian variations. Body mass was assessed to gauge sweat loss, and water bottles were weighed after this (and immediately after the end of the exercise protocol).

After body mass assessment, heart rate and core temperature measurement devices were activated. Then, pre-exercise blood lactate and glucose levels evaluation occurred, through finger prick to capillary blood draws.

Next, participants were taken to the experimental room where the exercise protocol took place. Despite the short duration of the exercise protocol, internal temperature was closely monitored to ensure prevention of heat-induced illness.

When the participants arrived at the room, pre-cooling began. So, beverages (previously prepared and stored at room temperature) were distributed to the referee. Total volume of beverages for pre-cooling previously described (75 ml of Beverage A or B) (49) were divided into three equal parts and made available to the participant every 1 min. Participants were instructed to swill both beverages for 10 s before spitting into a bowl without swallowing. Perceptual measures (thermal sensation, thermal comfort, and perceived thirst) were evaluated before the beginning of the pre-cooling and after the last mouth rinse. Afterward, the participants began to warm-up, performing the same exercise protocol for 5 min. A water bottle was offered to the participants, and they were instructed to drink *ad libitum* but only in the "water break" (at the end of the second 15-min block), in order to mimic FIFA's "cooling–water" break. The temperature of the water was measured before the water break, using a digital thermometer (YSI 409B, Yellow Springs Instruments, Ohio, USA). During the warm-up, as well as after each 15-min block, environmental conditions were measured according to the Kestrel 5400 Heat Stress Tracker (Kestrel Instruments®, Boothwyn, PA, USA) (32).

The exercise protocol then started and perceptual measures were evaluated at the end of each 15-min block. At the end, under normothermic conditions, blood lactate and glucose levels were measured again and afterward, the RPE scale was applied to the participants. Figure 1 provides an overview of the measurements and the study protocol.

2.11 Statistical analysis

All statistical analyses were performed using SPSS (IBM SPSS Statistics 28 Inc., USA). Statistical significance was accepted at $P < 0.05$. Normal distribution was tested using the Shapiro–Wilk test. Data are presented as mean ± SD, unless otherwise indicated. Single time point data were examined for within-group effects across conditions using a one-way repeated-measures analysis of variance (ANOVA). A two-way repeated-measures ANOVA was used to test for within-group effects across time in both conditions. If sphericity was violated, a Greenhouse–Geisser correction was applied. When a significant difference was found for main effects (trial or time), *post-hoc* pairwise comparisons were made incorporating a Bonferroni adjustment. The magnitude of effect was calculated with partial eta-squared (η_p^2) according to the following criteria: 0.02, small difference; 0.13, moderate difference; and 0.26, large difference (49). A paired *t*-test was used to compare single parameter differences and magnitude of effect calculated (Cohen's *d*) according to the following criteria: 0.2, small difference; 0.5, moderate difference; and 0.8, large difference (49).

3 Results

3.1 Trial conditions and pre-exercise biochemical measures

The menthol and placebo trials did not differ for environmental conditions (menthol: temperature 34.2°C ± 0.6°C, relative humidity 62.5% ± 1.0%, wet bulb globe temperature 30.3 ± 0.5°C; placebo: temperature 34.7°C ± 0.7°C, relative humidity 60.4% ± 2.4%, wet bulb globe temperature 30.4 ± 0.4°C; $P \geq 0.05$), or pre-trial USG levels (menthol: 1.013 ± 0.01 USG, placebo: 1.012 ± 0.01 USG, $P = 0.347$). Also, no differences were

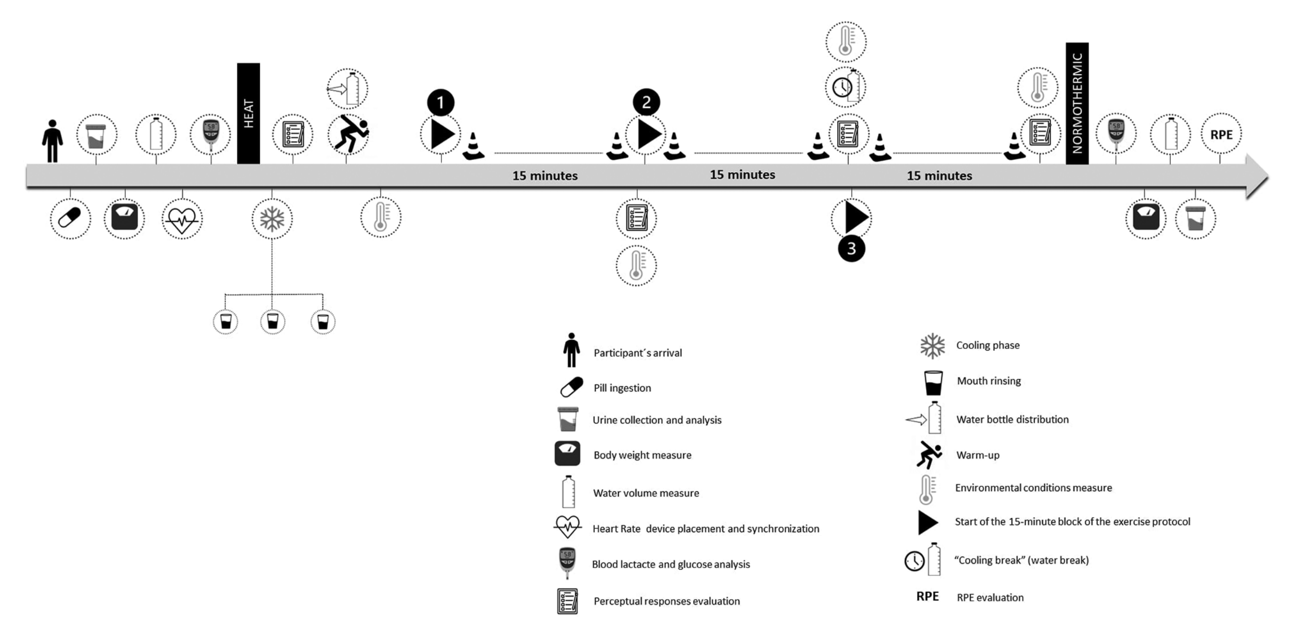

FIGURE 1
Schematic representation of the experimental trial.

TABLE 2 Biochemical measures before and after exercise, across menthol and placebo trials.

Parameter	Pre-test ($n = 9$)			Post-test ($n = 9$)		P-value
	Menthol	Placebo	P-value	Menthol	Placebo	
USG	1.013 ± 0.01	1.012 ± 0.01	0.347	1.013 ± 0.05	1.012 ± 0.05	0.282
Blood glucose (mg/dl)	97.6 ± 19.6	93.1 ± 10.2	0.406	99.2 ± 12.5	92.7 ± 10.2	0.119
Blood lactate (mmol/L)	2.5 ± 0.6	1.9 ± 0.6	0.111	2.7 ± 0.5	2.1 ± 0.4	0.321

Data are presented as mean ± SD ($n = 9$).

detected for pre-trial glucose and lactate levels (menthol: glucose 97.7 ± 19.2 mg/dl, placebo: 93.1 ± 10.2 mg/dl, $P = 0.406$; menthol: lactate 2.48 ± 0.62 mmol/L, placebo: 1.90 ± 0.61 mmol/L, $P = 0.111$) (Table 2).

3.2 Physiological responses to mouth rinse

There was no main effect of mouth rinse on core temperature, heart rate, sweating rate, post-exercise hydration status, and *ad libitum* water intake, as well as an interaction effect between mouth rinse and time on core temperature and heart rate (core temperature: menthol: $F_{(1,8)} = 0.26$, $P = 0.622$, $\eta_p^2 = 0.032$, menthol × time: $F_{(3,24)} = 1.1$, $P = 0.290$, $\eta_p^2 = 0.441$; heart rate: menthol: $F_{(1,8)} = 2.64$, $P = 0.143$, η_p^2 0.248, menthol × time: $F_{(3,24)} = 1.83$, $P = 0.212$, $\eta_p^2 = 0.186$; sweating rate: menthol: $F_{(1,8)} = 1.61$, $P = 0.240$, $\eta_p^2 = 0.168$; hydration status: menthol: $F_{(1,8)} = 1.33$, $P = 0.282$, $\eta_p^2 = 0.143$; water intake: menthol: $F_{(1,8)} = 0.13$, $P = 0.724$, $\eta_p^2 = 0.016$). Blood lactate and glucose levels at the end were also not significantly different between conditions (lactate: $F_{(1,8)} = 2.91$, $P = 0.321$, $\eta_p^2 = 0.619$; glucose: $F_{(1,8)} = 3.02$, $P = 0.119$, $\eta_p^2 = 0.276$). Physiological responses are presented in Table 2 and Figure 2.

Core temperature (°C) and heart rate (b min^{-1}) significantly increased over time (core temperature: $F_{(15.75,0.33)} = 17.34$, $P < 0.001$, $\eta_p^2 = 0.979$; heart rate: $F_{(1.268,10.143)} = 151.06$, $P < 0.001$, $\eta_p^2 = 0.950$). Heart rate was higher at every time point than the previous time point (all $P < 0.01$, all $d > 1.1$), as well as core temperature (all $P < 0.01$, all $d > 0.9$), except for the last 15-min block ($P = 0.073$, $d = 0.18$), where core temperature decreased for both conditions (Figures 2A,B).

3.3 Perceptual responses to mouth rinse

There was no main effect of mouth rinse on thermal sensation, thermal comfort, and perceived thirst (thermal sensation: $F_{(1,8)} = 1.22$, $P = 0.302$, $\eta_p^2 = 0.132$; thermal comfort: $F_{(1,8)} = 1.07$, $P = 0.332$, $\eta_p^2 = 0.118$; perceived thirst: $F_{(1,8)} = 0.42$, $P = 0.537$, $\eta_p^2 = 0.049$).

However, there was an interaction effect between mouth rinse and time on thermal sensation and thermal comfort but not on perceived thirst (thermal sensation: $F_{(4,32)} = 9.51$, $P = 0.037$, $\eta_p^2 = 0.135$; thermal

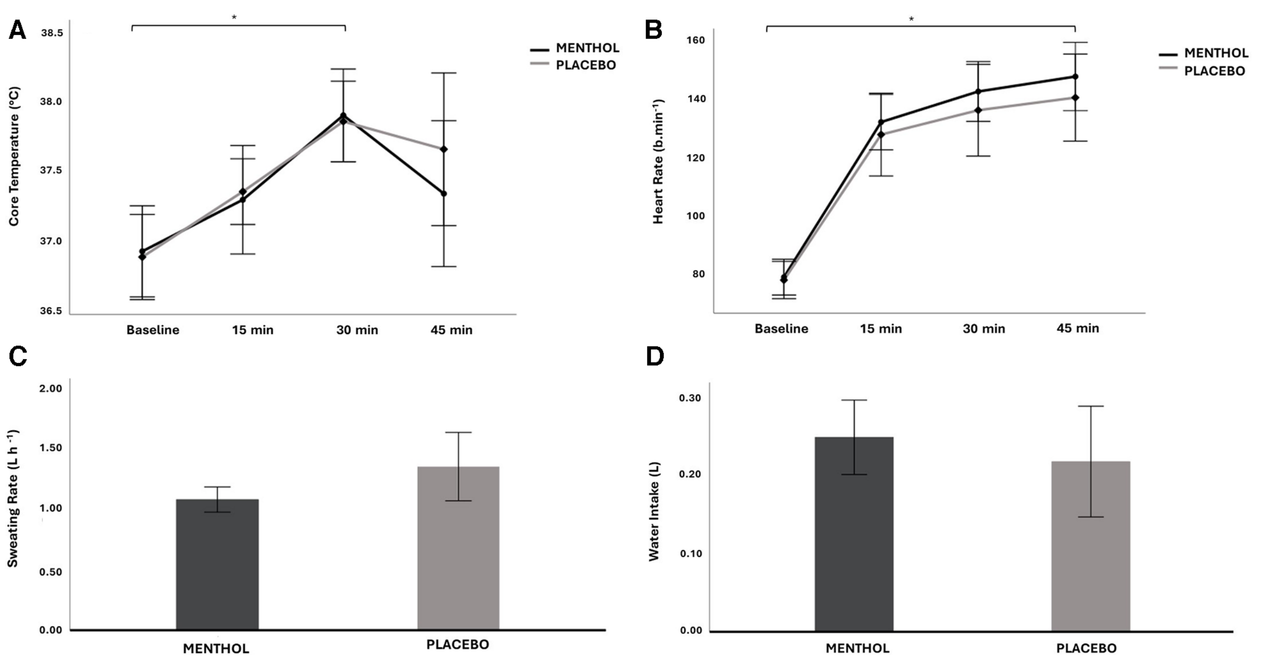

FIGURE 2
Mean ± 95% CI for core temperature (A), heart rate (B), sweating rate (C) and water intake (D) across the menthol and placebo conditions, for all time points. *Significant difference between exercise blocks, with no difference between groups.

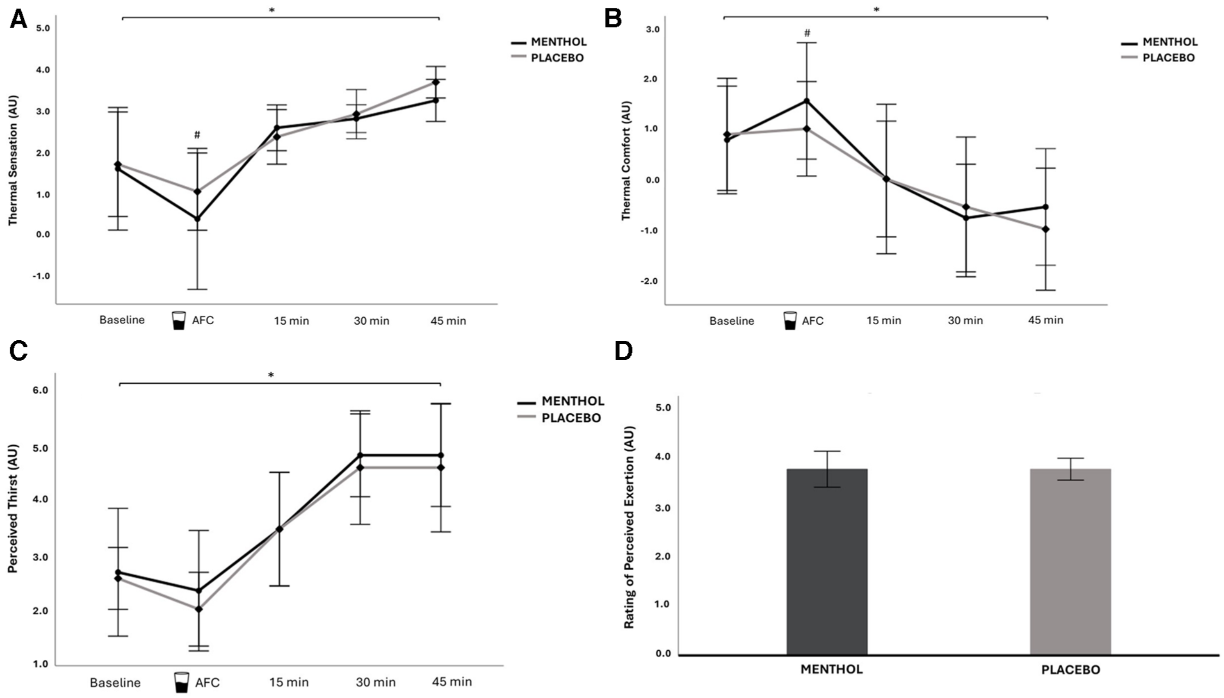

FIGURE 3
Mean ± 95% CI for thermal sensation (A), thermal comfort (B), perceived thirst (C) and RPE (D) across the menthol and placebo conditions, for all time points. *Significant difference between exercise blocks, with no difference between conditions. #Significant difference between conditions. AFC, after cooling; AU, arbitrary units.

comfort: $F_{(4,32)} = 8.70$, $P = 0.041$, $\eta_p^2 = 0.126$; perceived thirst: $F_{(4,32)} = 2.95$, $P = 0.089$, $\eta_p^2 = 0.021$). Pairwise analysis confirmed that thermal sensation was significantly lower in menthol trial after cooling ($P = 0.022$, $d = 0.41$) and thermal comfort was significantly higher also after cooling in the menthol condition ($P = 0.044$, $d = 0.37$). Thermal sensation and thermal comfort did not differ between menthol and placebo conditions at baseline and at the first, second, and third exercise blocks (all $P \geq 0.05$).

Thermal sensation, thermal comfort, and perceived thirst significantly changed through each time point (thermal sensation: $F_{(1.27,10.215)} = 14.10$, $P = 0.002$, $\eta_p^2 = 0.638$; thermal comfort: $F_{(1.35,10.603)} = 12.20$, $P = 0.003$, $\eta_p^2 = 0.604$; perceived thirst: $F_{(2.255,18.043)} = 29.38$, $P < 0.001$, $\eta_p^2 = 0.786$). Thermal sensation significantly decreased from baseline to after cooling and then significantly increased over time (all $P < 0.05$, all $d > 0.8$). Thermal comfort significantly increased from baseline to after cooling and significantly decreased over time (all $P < 0.05$, all $d > 0.6$), except for the last exercise block, where thermal comfort was not significantly higher than the previous block ($P = 1.00$, $d = 0.1$). Perceived thirst significantly increased from baseline and after cooling to the second and third exercise blocks, as well as from the first to second and third exercise blocks (all $P < 0.05$, all $d > 0.4$), with no differences between any other time points. Perceptual responses are presented in Figures 3A–C.

Finally, there was no main effect of mouth rinse on RPE [$F_{(1,8)} = 0.0$, $P = 1.00$, $\eta_p^2 = 0.000$], as a matter of fact mean RPE in the menthol condition was equal to the placebo ($P = 1.00$) (Figure 3D).

3.4 Effect of mouth rinse on exercise performance

There was no main effect of mouth rinse, or interaction effect between mouth rinse and time on distance covered (Figure 4) (menthol: $F_{(1,8)} = 1.24$, $P = 0.298$, $\eta_p^2 = 0.134$; menthol × time: $F_{(2,16)} = 0.23$, $P = 0.801$, $\eta_p^2 = 0.027$). The distance covered during each of the 15-min exercise block was not significantly different between the three blocks [$F_{(2,16)} = 0.16$, $P = 0.855$, $\eta_p^2 = 0.019$] in both conditions.

4 Discussion and implications

The purpose of the present study was to evaluate the effect of pre-cooling with menthol solution mouth rinse on perceptual measures and distance covered in male football referees, while performing an exercise protocol that mimics a football game. Our main findings revealed that mouth rinsing with a menthol solution ameliorated perceptual measures, enhancing thermal sensation and thermal comfort immediately after rinsing, but with no long-term effect. Also, no changes were observed in physical performance, not even in the first exercise block,

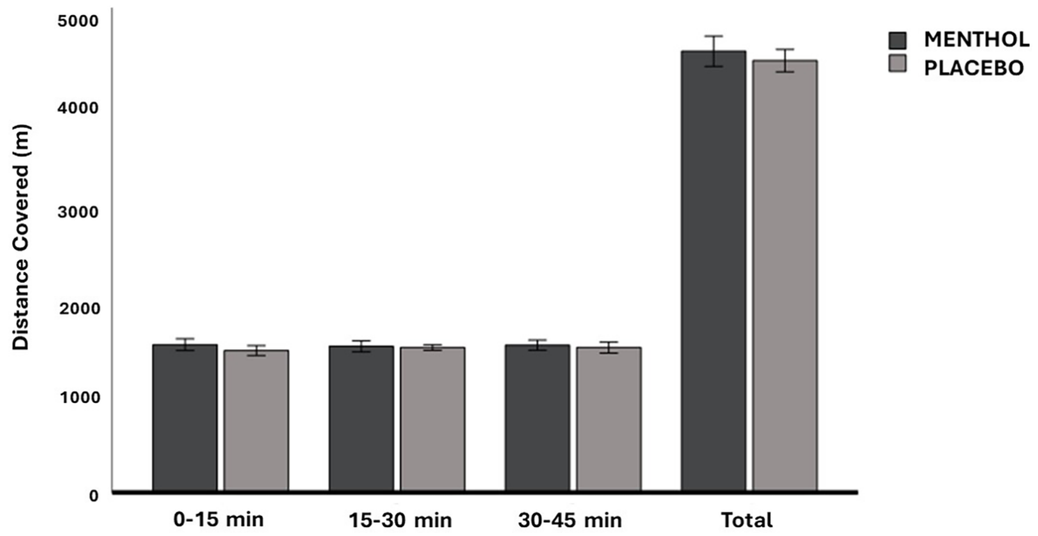

FIGURE 4
Mean ± 95% CI of the distance covered (in each exercise block and total) across the menthol and placebo conditions.

refuting our initial hypothesis that an eventual improvement in the perceptual measures would benefit physical performance.

4.1 Effect of mouth rinse on exercise performance

Physical performance measured by the distance covered did not significantly differ between conditions, which is in accordance with previous findings that revealed no improvements in intermittent exercise performance with oral menthol, even when thermal comfort was higher, as occurred in our study (50, 51). This may be due to the fact that exercise performed in an intermittent intensity mode, interspersing high-intensity bouts with periods of rest, causes less heat stress than the same amount of continuous exercise, where stored heat is greater and core temperature and thermal sensation rise faster (52–54). We may also speculate that, despite the observed increases in heart rate and core temperature along our exercise protocol, the exercise task may have not increased over time, since the distance covered in each block was similar, with no deterioration in performance over time. This may explain the fact that menthol did not have a significant impact on physical performance in our study, which could have been detected with a more demanding exercise protocol. In addition, it was previously observed that a cooling intervention using ice slurry reduced core temperature and heart rate at the end of an intermittent exercise protocol performed in hot conditions, which was translated to an increased peak power (+4%) and work done (+2%) in the intervention group (55, 56). These performance data, combined with no differences in RPE or thermal sensation between cooling and control trials, suggested that the performance ability during intermittent sprints in the heat was more closely related to physiological responses, rather than perceived temperature. This highlights an explanation for the unchanged intermittent exercise performance in the present study, despite improved thermal sensation and thermal comfort, as a non-thermal cooling method was used. Other study showed that peak power only improved in heat (+2%) when the intervention induced reductions in core temperature, heart rate, and thermal sensation (24). For that reason, thermal internal cooling techniques may best suit intermittent exercise protocols. These findings indicates that physiological intervention might be worthy for intermittent sprinting in the heat, while physiological and perceptual manipulations should be discussed for continuous endurance exercise in heat (25).

Furthermore, even though we observed a significant increase in thermal sensation in both conditions during our exercise protocol, thermal sensation points were higher during exercise in other trials with continuous endurance efforts (18, 21, 38, 57). This also may explain why we did not find a performance improvement with oral menthol, since the "thermal stress" felt by the participants may not have been high enough to benefit from non-thermal cooling.

4.2 Physiological responses to mouth rinse

Consistent with the "non-thermal" mechanistic basis of menthol's cooling effects (31), there were no changes in core temperature or heart rate between conditions. Actually, our work showed lower core temperature values (menthol: 37.6 ± 0.4°C), compared to what was achieved (~39.5°C) in other studies, where a continuous endurance exercise protocol was performed (19, 57, 58). Oral menthol significantly improved physical performance in those studies, which may be due to the higher heat storage observed. It is unlikely that the smaller increase in core temperature found in our protocol is due to atmospheric conditions, as other studies carried out under similar atmospheric conditions have higher core temperature values

(18, 22, 27). Perhaps, exercise intensities achieved in our study were not sufficiently high, which is supported by the lower heart rate values (menthol: 135.5 ± 11.1 b min^{-1}), compared to other findings (19, 21, 22), as well as an average RPE of 4 points, corresponding to "somewhat hard."

It is still important to point out that core temperature increased throughout the exercise protocol but decreased in the last block possibly as a consequence of the "water break" (Figure 2A). It is expected that the increase in core temperature will be attenuated when participants hydrate themselves with sufficient fluid volumes, even if the water was at room temperature (59), as was the case.

Regarding water intake, previous studies demonstrated a larger consumption when the fluid was cold (≤5°C) compared to a control (16–19°C) (24, 60). In fact, we used a beverage temperature similar to deep body temperature to minimize the influence of visceral temperature modulation, but other studies used cooler mouth rinses (57, 61). However, as menthol exerts its cooling effect by activating subsequent stimuli (inspired air, water consumed) feel cool (17), water may be understood as cooler in the menthol trial, which may have contributed to increase the volume ingested (even though not significant).

4.3 Perceptual responses to mouth rinse

Menthol, by stimulating the trigeminal system, seems to directly activate reward centers in the brain to increase "central drive" and enhance work capacity (62). Activation of these areas in the brain, such as the insula/frontal operculum, the orbitofrontal cortex, and the striatum, may lower perceived exertion (63) and help improve motivation during exercise performance (64). Nonetheless, in our study, there were no significant differences in RPE between conditions. To the best of our knowledge, none of the studies that applied oral menthol in intermittent exercise found improvements in RPE. In those studies (50, 51), core temperature and heart rate values were similar to those observed in our work, supporting that menthol may not affect RPE in this type of exercise protocols, possibly due to a less physically demanding exercise task.

Our findings revealed an improvement in thermal sensation and thermal comfort after cooling, but not in subsequent time points. Another study where an intermittent exercise protocol with similar duration was performed, thermal sensation and thermal comfort were also immediately improved after menthol mouth rinse (50). However, this improvement continued throughout the exercise because oral menthol was administered every 10 min. It is highly likely that multiple moments of per-cooling during our exercise protocol would have led to an extension of the improvement in the perceptual measures. Anyhow, we intended to make our protocol as realistic as possible, getting closer to the cooling and hydration timings that exist in a football game.

Despite being expected to find differences between conditions in perceived thirst (65), it was not lower in the menthol trial. Oral menthol rising is presumed to increase the drive to breathe and ventilation, and to decrease thirst, as well as promote sensations of coolness and freshness (23, 66). Menthol stimulates oral cold receptors and may subsequently have the same effects on thirst and the hedonic process as cold water (67). As perceived thirst was not lower in the menthol trial, perhaps a greater concentration of menthol or a higher frequency of mouth rinses during the exercise may be necessary.

The findings of the current study suggest that menthol stimulation of the TRPM8 ion channel enhanced thermal comfort and sensation following mouth rinsing, suggesting that our participants were more "perceptually tolerant" to physiological heat stress. The improvement in perceptual measures right after rinsing would be expected as it was found in previous research (68). However, improvements in perception did not extend throughout the exercise, possibly indicating that a higher frequency of menthol mouth rinsing should have been experimented. This, along with a higher demanding exercise protocol, could have contributed to a possible ergogenic action of menthol. Future research should explore the influence of menthol mouth rinsing also in the advanced stages of exercise, when fatigue is traditionally high, concretely in long-lasting moderate-to-high-intensity exercise protocols.

4.4 Limitations

As there is no validated exercise protocol that mimics the activity of football referees, the SAFT-45 was chosen, although it has only been validated for football players. The exercise protocol was performed in an experimental room with hot and humid environments to simulate conditions of high temperature and humidity, while still ensuring stability between measures. The closed task of SAFT-45 possibly provokes an experimental artifact, because participants are not able to sprint and perform freely (either in frequency or duration). Future work should consider the benefits of a "free" task (e.g., devising a protocol with an undefined sprint duration), or participant-regulated sprint frequency, where participants could pace independently in response to non-thermal cooling interventions in the heat. This approach may enable different responses due to the elevation in pacing associated with these tasks, differentiating it from the SAFT-45, which applies a fixed duration and fixed frequency of sprinting. In this study, we opted to shorten the original SAFT-90 and used the SAFT-45, to increase adherence and ensure the crossover design with the second visit of the participants. Conversely, this may have been a major limitation of the study, because the exercise protocol lasts only 45 min and, therefore, only replicates the first half of a football match. Future work should extend the task duration to more closely replicate the team sport of interest (69), in high-level team sport players or referees.

Another possible limitation of our work was the non-use of menthol crystals to prepare the solution, as in other studies (16, 19, 20, 22). However, Kalantzis et al. showed that menthol significantly changes thermal sensory thresholds in the oral cavity (68) when the participants were asked to suck a lozenge (Halls Extra Strong menthols, Mondelez International, Birmingham, United Kingdom) immediately before repeating the measurement of the thermal thresholds on the right dorsal surface of the tongue, indicating that this substrate can also be used to trigger changes in thermal perception, being much more practical than preparing the beverage with menthol crystals, which requires a lot of time in advance.

Finally, although with the aim of understanding whether menthol would influence the perception of thirst and, consequently, the amount of water ingested, not fixing the volume of water that the referees drank during the exercise in both trials could have conditioned the perceptual results observed in the last block of exercise. However, this was not observed, since no significant differences were found for water intake and no statistical effects were observed between conditions for perceptual measurements after the 30th-min water break.

5 Conclusions

Mouth rinsing with a menthol solution improved thermal sensation and comfort immediately after the administration but had no long-term effect during a 45-min intermittent exercise protocol, in heat, in male football referees. In opposition of what was hypothesized, no changes were also detected in the physical performance or physiological responses. Pre-cooling with oral menthol in a hot and humid environment may promote an immediate change on perceptual measures, but not enough to improve intermittent exercise performance in football referees.

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 Ethics Committee of the University of Porto. 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

MR: Conceptualization, Investigation, Methodology, Writing – original draft. JB: Conceptualization, Methodology, Software, Writing – review & editing. FT: Methodology, Software, Writing – review & editing. KS: Methodology, Software, Writing – review & editing. VT: Conceptualization, Methodology, Supervision, Writing – review & editing.

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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.

Publisher's note

References

1. Reilly T, Williams AM, Nevill A, Franks A. A multidisciplinary approach to talent identification in soccer. *J Sports Sci.* (2000) 18(9):695–702. doi: 10.1080/02640410050120078

2. Moen C, Andersen TE, Clarsen B, Madsen-Kaarød G, Dalen-Lorentsen T. Prevalence and burden of health problems in top-level football referees. *Sci Med Football.* (2022) 7(2):1–8. doi: 10.1080/24733938.2022.2055782

3. Duffield R, Coutts AJ, Quinn J. Core temperature responses and match running performance during intermittent-sprint exercise competition in warm conditions. *J Strength Cond Res.* (2009) 23(4):1238–44. doi: 10.1519/JSC.0b013e318194e0b1

4. Bandelow S, Maughan R, Shirreffs S, Ozgünen K, Kurdak S, Ersöz G, et al. The effects of exercise, heat, cooling and rehydration strategies on cognitive function in football players. *Scand J Med Sci Sports.* (2010) 20(Suppl 3):148–60. doi: 10.1111/j.1600-0838.2010.01220.x

5. Nassis GP, Brito J, Dvorak J, Chalabi H, Racinais S. The association of environmental heat stress with performance: analysis of the 2014 FIFA world cup Brazil. *Br J Sports Med.* (2015) 49(9):609–13. doi: 10.1136/bjsports-2014-094449

6. Racinais S, Mohr M, Buchheit M, Voss SC, Gaoua N, Grantham J, et al. Individual responses to short-term heat acclimatisation as predictors of football performance in a hot, dry environment. *Br J Sports Med.* (2012) 46(11):810–5. doi: 10.1136/bjsports-2012-091227

7. Tucker R, Marle T, Lambert EV, Noakes TD. The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion. *J Physiol.* (2006) 574(Pt 3):905–15. doi: 10.1113/jphysiol.2005.101733

8. Gagge AP, Stolwijk JA, Hardy JD. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environ Res.* (1967) 1(1):1–20. doi: 10.1016/0013-9351(67)90002-3

9. Dicks M, Davids K, Button C. Individual differences in the visual control of intercepting a penalty kick in association football. *Hum Mov Sci*. (2010) 29 (3):401–11. doi: 10.1016/j.humov.2010.02.008

10. Orth D, Davids K, Araújo D, Renshaw I, Passos P. Effects of a defender on run-up velocity and ball speed when crossing a football. *Eur J Sport Sci*. (2014) 14(Suppl 1): S316–23. doi: 10.1080/17461391.2012.696712

11. Costa EC, Vieira CM, Moreira A, Ugrinowitsch C, Castagna C, Aoki MS. Monitoring external and internal loads of Brazilian soccer referees during official matches. *J Sports Sci Med*. (2013) 12(3):559–64. PMID: 24149165; PMCID: PMC3772602.

12. Weston M, Drust B, Gregson W. Intensities of exercise during match-play in FA premier league referees and players. *J Sports Sci*. (2011) 29(5):527–32. doi: 10.1080/02640414.2010.543914

13. Krustrup P, Mohr M, Bangsbo J. Activity profile and physiological demands of top-class soccer assistant refereeing in relation to training status. *J Sports Sci*. (2002) 20 (11):861–71. doi: 10.1080/026404102320761778

14. Castagna C, Abt G, D'Ottavio S. Physiological aspects of soccer refereeing performance and training. *Sports Med*. (2007) 37(7):625–46. doi: 10.2165/00007256-200737070-00006

15. Casajus JA, Castagna C. Aerobic fitness and field test performance in elite spanish soccer referees of different ages. *J Sci Med Sport*. (2007) 10(6):382–9. doi: 10.1016/j.jsams.2006.08.004

16. Crosby S, Butcher A, McDonald K, Berger N, Bekker PJ, Best R. Menthol mouth rinsing maintains relative power production during three-Minute maximal cycling performance in the heat compared to cold water and placebo rinsing. *Int J Environ Res Public Health*. (2022) 19(6):3527. doi: 10.3390/ijerph19063527

17. Mündel T, Jones DA. The effects of swilling an L(-)-menthol solution during exercise in the heat. *Eur J Appl Physiol*. (2010) 109(1):59–65. doi: 10.1007/s00421-009-1180-9

18. Flood TR, Waldron M, Jeffries O. Oral L-menthol reduces thermal sensation, increases work-rate and extends time to exhaustion, in the heat at a fixed rating of perceived exertion. *Eur J Appl Physiol*. (2017) 117(7):1501–12. doi: 10.1007/s00421-017-3645-6

19. Jeffries O, Goldsmith M, Waldron M. L-Menthol mouth rinse or ice slurry ingestion during the latter stages of exercise in the heat provide a novel stimulus to enhance performance despite elevation in mean body temperature. *Eur J Appl Physiol*. (2018) 118(11):2435–42. doi: 10.1007/s00421-018-3970-4

20. Gavel EH, Logan-Sprenger HM, Good J, Jacobs I, Thomas SG. Menthol mouth rinsing and cycling performance in females under heat stress. *Int J Sports Physiol Perform*. (2021) 16(7):1014–20. doi: 10.1123/ijspp.2020-0414

21. Stevens CJ, Thoseby B, Sculley DV, Callister R, Taylor L, Dascombe BJ. Running performance and thermal sensation in the heat are improved with menthol mouth rinse but not ice slurry ingestion. *Scand J Med Sci Sports*. (2016) 26(10):1209–16. doi: 10.1111/sms.12555

22. Riera F, Trong TT, Sinnapah S, Hue O. Physical and perceptual cooling with beverages to increase cycle performance in a tropical climate. *PloS One*. (2014) 9(8): e103718. doi: 10.1371/journal.pone.0103718

23. Eccles R. Role of cold receptors and menthol in thirst, the drive to breathe and arousal. *Appetite*. (2000) 34(1):29–35. doi: 10.1006/appe.1999.0291

24. Mündel T, King J, Collacott E, Jones DA. Drink temperature influences fluid intake and endurance capacity in men during exercise in a hot, dry environment. *Exp Physiol*. (2006) 91(5):925–33. doi: 10.1113/expphysiol.2006.034223

25. Roriz M, Brito P, Teixeira FJ, Brito J, Teixeira VH. Performance effects of internal pre- and per-cooling across different exercise and environmental conditions: a systematic review. *Front Nutr*. (2022) 9:959516. doi: 10.3389/fnut.2022.959516

26. Barwood MJ, Gibson OR, Gillis DJ, Jeffries O, Morris NB, Pearce J, et al. Menthol as an ergogenic aid for the Tokyo 2021 Olympic games: an expert-led consensus statement using the modified delphi method. *Sports Med*. (2020) 50 (10):1709–27. doi: 10.1007/s40279-020-01313-9

27. Gavel EH, Hawke KV, Bentley DJ, Logan-Sprenger HM. Menthol mouth rinsing is more than just a mouth wash-swilling of menthol to improve physiological performance. *Front Nutr*. (2021) 8:691695. doi: 10.3389/fnut.2021.691695

28. World Medical Association. World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. (2013) 310 (20):2191–4. doi: 10.1001/jama.2013.281053

29. Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 Statement: extension to randomised crossover trials. *Br Med J*. (2019) 366:l4378. doi: 10.1136/bmj.l4378

30. McKay AKA, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL, et al. Defining training and performance caliber: a participant classification framework. *Int J Sports Physiol Perform*. (2022) 17(2):317–31. doi: 10.1123/ijspp.2021-0451

31. Jeffries O, Waldron M. The effects of menthol on exercise performance and thermal sensation: a meta-analysis. *J Sci Med Sport*. (2019) 22(6):707–15. doi: 10.1016/j.jsams.2018.12.002

32. Roriz M, Brito J, Teixeira FJ, Spyrou K, Teixeira VH. Menthol mouth rinsing and performance in elite football referees in the heat: a study protocol for a randomized crossover trial. *Contemp Clin Trials Commun*. (2023) 35:101202. doi: 10.1016/j.conctc.2023.101202

33. Small K, McNaughton L, Greig M, Lovell R. The effects of multidirectional soccer-specific fatigue on markers of hamstring injury risk. *J Sci Med Sport*. (2010) 13(1):120–5. doi: 10.1016/j.jsams.2008.08.005

34. Gonçalves B, Hegyi A, Avela J, Cronin N. How does a simulated soccer match affect regional differences in biceps femoris muscle architecture? *Br J Sports Med*. (2017) 51(4):322. doi: 10.1136/bjsports-2016-097372.98

35. Barrett S, Guard A, Lovell R. *SAFT90 Simulates the internal and external loads of competitive soccer match-play. Science and Football VII: Proceedings of the Seventh World Congress on Science and Football*; Routledge (2013). Vol. 43. p. 95–100.

36. Lovell R, Midgley A, Barrett S, Carter D, Small K. Effects of different half-time strategies on second half soccer-specific speed, power and dynamic strength. *Scand J Med Sci Sports*. (2013) 23(1):105–13. doi: 10.1111/j.1600-0838.2011.01353.x

37. Marshall P, Lovell R, Jeppesen G, Andersen K, Siegler J. Hamstring muscle fatigue and central motor output during a simulated soccer match. *PloS One*. (2014) 9:e102753. doi: 10.1371/journal.pone.0102753

38. Stevens CJ, Bennett KJ, Sculley DV, Callister R, Taylor L, Dascombe BJ. A comparison of mixed-method cooling interventions on preloaded running performance in the heat. *J Strength Cond Res*. (2017) 31(3):620–9. doi: 10.1519/jsc.0000000000001532

39. Hernández-Vicente A, Hernando D, Marín-Puyalto J, Vicente-Rodríguez G, Garatachea N, Pueyo E, et al. Validity of the polar H7 heart rate sensor for heart rate variability analysis during exercise in different age, body composition and fitness level groups. *Sensors*. (2021) 21(3):902. doi: 10.3390/s21030902

40. Bongers CC, Hopman MT, Eijsvogels TM. Using an ingestible telemetric temperature pill to assess gastrointestinal temperature during exercise. *J Vis Exp*. (2015) 104:53258. doi: 10.3791/53258

41. Armstrong LE. Assessing hydration status: the elusive gold standard. *J Am Coll Nutr*. (2007) 26(5 Suppl):575s–84s. doi: 10.1080/07315724.2007.10719661

42. FIFA. Football development, players' health. Playing in the heat. Available online at: http://www.fifa.com/development/medical/players-health/minimising-risks/heat.html (accessed November 7, 2022).

43. Raa A, Sunde G, Bolann B, Kvåle R, Bjerkvig C, Eliassen H, et al. Validation of a point-of-care capillary lactate measuring device (Lactate Pro 2). *Scand J Trauma Resusc Emerg Med*. (2020) 28:83. doi: 10.1186/s13049-020-00776-z

44. Brannan C. Evaluation of the FreeStyle precision pro blood glucose and β-ketone monitoring system. *Point Care*. (2015) 28:4–13. doi: 10.1097/POC.0000000000000128

45. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*. (1970) 2(2):92–8. doi: 10.2340/1650197719702239298

46. Zhang H, Huizenga C, Arens E, Wang D. Thermal sensation and comfort in transient non-uniform thermal environments. *Eur J Appl Physiol*. (2004) 92 (6):728–33. doi: 10.1007/s00421-004-1137-y

47. Bedford T. *The Warmth Factor in Comfort at Work: A Physiological Study of Heating and Ventilation*. London: HMSO (1936).

48. Engell DB, Maller O, Sawka MN, Francesconi RN, Drolet L, Young AJ. Thirst and fluid intake following graded hypohydration levels in humans. *Physiol Behav*. (1987) 40(2):229–36. doi: 10.1016/0031-9384(87)90212-5

49. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. New York: Routledge (1988). Available online at: https://doi.org/10.4324/9780203771587

50. Gibson OR, Wrightson JG, Hayes M. Intermittent sprint performance in the heat is not altered by augmenting thermal perception via L-menthol or capsaicin mouth rinses. *Eur J Appl Physiol*. (2019) 119(3):653–64. doi: 10.1007/s00421-018-4055-0

51. Best R, Temm D, Hucker H, McDonald K. Repeated menthol mouth swilling affects neither strength nor power performance. *Sports*. (2020) 8(6):90. doi: 10.3390/sports8060090

52. Mora-Rodriguez R, Del Coso J, Estevez E. Thermoregulatory responses to constant versus Variable-intensity exercise in the heat. *Med Sci Sports Exercise*. (2008) 40:1945–52. doi: 10.1249/MSS.0b013e31817f9843

53. Ekblom B, Greenleaf CJ, Greenleaf JE, Hermansen L. Temperature regulation during continuous and intermittent exercise in man. *Acta Physiol Scand*. (1971) 81 (1):1–10. doi: 10.1111/j.1748-1716.1971.tb04871.x

54. Kraning KK 2nd, Gonzalez RR. Physiological consequences of intermittent exercise during compensable and uncompensable heat stress. *J Appl Physiol*. (1991) 71(6):2138–45. doi: 10.1152/jappl.1991.71.6.2138

55. Morito A, Inami T, Hirata A, Yamada S, Shimomasuda M, Haramoto M, et al. Ice slurry ingestion improves physical performance during high-intensity intermittent exercise in a hot environment. *PloS One*. (2022) 17(9):e0274584. doi: 10.1371/journal.pone.0274584

56. Castle PC, Macdonald AL, Philp A, Webborn A, Watt PW, Maxwell NS. Precooling leg muscle improves intermittent sprint exercise performance in hot,

humid conditions. *J Appl Physiol*. (2006) 100(4):1377–84. doi: 10.1152/japplphysiol.00822.2005

57. Riera F, Trong TT, Rinaldi K, Hue O. Precooling does not enhance the effect on performance of midcooling with ice-slush/menthol. *Int J Sports Med*. (2016) 37 (13):1025–31. doi: 10.1055/s-0042-107597

58. de Camargo R, Vilaça A, Mota GR, Elsangedy HM, Haile L, Sasaki JE. The influence of L-menthol on time trial running performance in recreational runners. *Res Q Exerc Sport*. (2022) 94(2):1–9. doi: 10.1080/02701367.2021.2014049

59. Akerman AP, Lucas SJE, Katare R, Cotter JD. Heat and dehydration additively enhance cardiovascular outcomes following orthostatically-stressful calisthenics exercise. *Front Physiol*. (2017) 8:756. doi: 10.3389/fphys.2017.00756

60. Sandick BL, Engell DB, Maller O. Perception of drinking water temperature and effects for humans after exercise. *Physiol Behav*. (1984) 32(5):851–5. doi: 10.1016/0031-9384(84)90205-1

61. Trong T T, Riera F, Rinaldi K, Briki W, Hue O. Ingestion of a cold temperature/menthol beverage increases outdoor exercise performance in a hot, humid environment. *PloS One*. (2015) 10(4):e0123815. doi: 10.1371/journal.pone.0123815

62. Hensel H, Zotterman Y. The effect of menthol on the thermoreceptors. *Acta Physiol Scand*. (1951) 24(1):27–34. doi: 10.1111/j.1748-1716.1951.tb00824.x

63. Nybo L. Exercise and heat stress: cerebral challenges and consequences. *Prog Brain Res*. (2007) 162:29–43. doi: 10.1016/s0079-6123(06)62003-7

64. Pitsiladis YP, Strachan AT, Davidson I, Maughan RJ. Hyperprolactinaemia during prolonged exercise in the heat: evidence for a centrally mediated component of fatigue in trained cyclists. *Exp Physiol*. (2002) 87(2):215–26. doi: 10.1113/eph8702342

65. Meamarbashi A, Rajabi A. The effects of peppermint on exercise performance. *J Int Soc Sports Nutr*. (2013) 10(1):15. doi: 10.1186/1550-2783-10-15

66. Sant'Ambrogio FB, Anderson JW, Sant'Ambrogio G. Effect of l-menthol on laryngeal receptors. *J Appl Physiol*. (1991) 70(2):788–93. doi: 10.1152/jappl.1991.70.2.788

67. Schäfer K, Braun HA, Isenberg C. Effect of menthol on cold receptor activity. Analysis of receptor processes. *J Gen Physiol*. (1986) 88(6):757–76. doi: 10.1085/jgp.88.6.757

68. Kalantzis A, Robinson PP, Loescher AR. Effects of capsaicin and menthol on oral thermal sensory thresholds. *Arch Oral Biol*. (2007) 52(2):149–53. doi: 10.1016/j.archoralbio.2006.09.001

69. Turner G, Gibson OR, Maxwell NS. Simulated moderate hypoxia reduces intermittent sprint performance in games players. *J Sports Med Phys Fitness*. (2014) 54(5):566–74. PMID: 25270776

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Investigating the interrelationships among food habits, sports nutrition knowledge, and perceived barriers to healthy eating: a study of adolescent swimmers

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This cross-sectional study aims to explore the relationships between food habits, perceived barriers to healthy eating, and sports nutrition knowledge among adolescent swimmers. The study focuses on understanding how these factors interact and influence dietary choices in young athletes. A cohort of 52 adolescent swimmers aged 12–18 from Al Hussein Youth Club in Al-Hussein Sport City participated in the study. Data were collected through surveys assessing food habits, perceived barriers to healthy eating, and sports nutrition knowledge. Pearson Correlation analysis was employed to examine associations between variables, and stepwise regression analysis was used to identify predictors of food habits. The analysis revealed a significant positive association between food habits and sports nutrition knowledge (r=0.393, p=0.004). Knowledge emerged as a significant positive predictor of food habits (β=0.393, p=0.004), highlighting the influential role of sports nutrition knowledge in shaping the dietary choices of adolescent swimmers. However, the introduction of Barriers lacked significance, and individual predictors did not reach statistical significance. These findings underscore the importance of targeted interventions aimed at enhancing nutritional education among adolescent swimmers. Understanding the interplay between knowledge, barriers, and food habits provides valuable insights into the complex dynamics that influence the dietary choices of young athletes. Addressing these factors through tailored educational programs can promote healthier eating habits and optimize performance among adolescent swimmers. This study highlights the critical role of sports nutrition knowledge in shaping the dietary behaviors of adolescent swimmers. By addressing knowledge gaps and overcoming perceived barriers, targeted interventions can help improve food habits and enhance the overall health and performance of young athletes.

KEYWORDS

food habits, sports nutrition knowledge, perceived barriers, healthy eating, adolescents swimmers

Introduction

Insufficient understanding of nutrition among athletes has the potential to impact their performance (1, 2). It is crucial for adolescent athletes to embrace a healthy eating regimen to adequately fulfill their nutritional requirements, foster well-being, and optimize their athletic performance (3, 4). While sports organizations worldwide emphasize the significance of continuous nutritional education for young athletes, coaches, and parents (5, 6), the extent of nutrition education among adolescent athletes, particularly swimmers, remains inadequately explored.

Nonetheless, the necessity for athlete education is apparent, as evidenced by a study involving elite athletes, a substantial proportion of them were aged under 18 years. These athletes exhibited lower scores in the section about 'nutrient sources', as compared to individuals who are not athletes (7). Additionally, a comprehensive analysis of nutrition knowledge (NK) among competitive, recreational, or elite athletes aged 13 and above revealed that while athletes' understanding of nutrition was on par with or superior to that of non-athletes, it lagged behind when compared to non-athlete comparison groups (8). Several research studies have demonstrated inadequate compliance of athletes with dietary guidelines, coupled with insufficient consumption of essential micronutrients (9, 10). Enhancing the Food habits (FH) of adolescent swimmers (ASs) could potentially involve bolstering their understanding of nutrition.

The Consensus Statement on 'Nutrition for Swimming' from The International Journal of Sport Nutrition and Exercise Performance proposes achieving this objective by implementing a carefully designed nutrition education program. This initiative is intended to systematically enhance participants' understanding of nutrition principles (11). It is widely acknowledged that achieving peak athletic performance can be significantly influenced by optimal nutrition (12). Nonetheless, several obstacles have the potential to impede athletes from attaining the best possible dietary practices. These challenges encompass constraints such as inadequate time and kitchen facilities to cook meals, limited financial means, deficient abilities in meal planning and preparation, and demanding travel schedules (13).

Studies on nutrition education have echoed comparable outcomes, showcasing its efficacy in enhancing individuals' understanding of food, their dietary behaviors, and their mindfulness about nutrition. These results underscore the necessity and effectiveness of nutrition education in augmenting knowledge about nutrition, self-confidence in making dietary choices, and fostering favorable shifts in perceptions (14).

Studies involving children have indicated that interventions encompassing multiple components and involving parental participation have demonstrated the greatest success in enhancing FH (15). However, nutrition education programs of this nature are relatively underexplored in the context of adolescent athletes. Only a few isolated cases have yielded mixed outcomes in this regard (16, 17). Despite research indicating that numerous athletes tend to underestimate their nutrient requirements (18) and frequently fall short of meeting the nutrition guidelines essential for optimal performance (19–21), limited attention has been given to exploring the potential barriers to healthy eating among ASs.

During the adolescent phase, the nutritional demands for sports involvement become more complex due to the simultaneous requirements of growth, physical development, and psychological maturation. Inadequacies in nutrition during this period can lead to enduring repercussions extending into adulthood (22). Adolescence serves as a crucial juncture for establishing dietary patterns (22). Consequently, special attention is needed for the FH and NK of teenage athletes (23).

The dietary routines of young swimmers can be influenced by their substantial time commitment to clubs and training centers, necessitating meals outside their homes. Frequently, they are accountable for their food choices, which can pose challenges in maintaining a nutritious and balanced diet to counterbalance the energy expended in their athletic pursuits. Considering the level of autonomy adolescent athletes possess over their diet and physical activities, having a grasp of nutrition can play a pivotal role in cultivating appropriate eating behaviors in this group. Nevertheless, research in diverse cultural settings has exposed gaps in athletes' nutritional understanding, encompassing both general nutritional aspects (24) and comprehension of their sport-specific requirements (23, 25). These knowledge deficiencies can be amplified by misconceptions about dietary practices (23) that may stem from superstitions or unreliable advice.

In order to foster more healthful dietary practices among adolescent athletes, it is crucial to confront these hurdles and equip them with precise nutritional instruction. This instruction should not solely concentrate on fundamental nutritional principles but also extend to the specific dietary prerequisites linked to their sport. This approach empowers athletes to make knowledgeable decisions that bolster their performance and overall well-being. Given the demanding schedules of ASs, which involve training and school responsibilities, it becomes crucial to comprehend the barriers they face when making nutritional choices.

The aim of this study was to explore the correlations between FH, perceived obstacles to maintaining a healthy diet, and the level of SNK among ASs. The study examined potential links between the FH of ASs and their PBHE to adopting a healthy diet.

The first hypothesis proposes that there is a positive association between the quality of FH and the level of SNK among ASs, resulting in a lower PBHE. The second hypothesis suggests that various demographic factors, including swimmer category, gender, training experience, and BMI, may act as moderators in the relationship between FH, PBHE, and SNK among ASs. It is postulated that these demographic variables influence how the aforementioned relationships manifest.

Materials and methods

Participants

In the conducted study, a cross-sectional design was implemented to explore the interplay between dietary habits, PBHE, and SNK among ASs associated with Al Hussein Youth Club in Al-Hussein Sport City. The participant recruitment process commenced in August 2023, coinciding with researchers' visits to the Olympic swimming pool in Al-Hussein Sport City. During these visits, 71 swimmers aged between 12 and 18 years, classified into the second, third, fourth, and fifth categories of both genders according to the International Swimming Federation (FINA), were informed about the research's purpose and invited to volunteer. A total of 52 swimmers (73.2%) willingly participated, with assurances of confidentiality and no incentives provided. Informed consent from participants and parent/guardian assent was obtained prior to their involvement in the study.

The data collection phase occurred concurrently with the Jordanian Clubs Swimming Championship at the Olympic Pool in Al-Hussein Sport City, spanning from August 23, 2023, to August 27, 2023. While coaches did not partake in the study, they, along with researchers, were available to assist participants in clarifying questionnaire-related queries. The completion of surveys took an average of 45 min. The study protocol received approval from NAMA Strategic Intelligence Solutions (No. M-SH/1/523).

The criteria for inclusion and exclusion from the group in the conducted study were defined to ensure the relevance and reliability of the research findings. Inclusion criteria encompassed participants aged between 12 and 18 years who were affiliated with Al Hussein Youth Club in Al-Hussein Sport City and fell into the second, third, fourth, or fifth categories according to the International Swimming Federation (FINA). Additionally, voluntary participation and informed consent were essential prerequisites for inclusion. Conversely, exclusion criteria encompassed individuals outside the specified age range, those not associated with the mentioned club, and those not classified within the designated swimmer categories by FINA. These criteria were established to maintain the study's focus on a specific population group namely, ASs from Al Hussein Youth Club and to mitigate potential confounding variables while adhering to ethical guidelines regarding informed consent and participant confidentiality.

This diverse study population, as evidenced by the distribution of participants across various demographic variables, contributes to a comprehensive understanding of the research objectives. The majority of participants fell into the third category, representing 40.4%, followed by the second category at 13.5%. Gender distribution revealed a slight majority of male participants (53.8%), while 46.2% identified as female. Participants' Body Mass Index (BMI) varied, with the largest proportion falling within the 25th Percentile range (44.2%). Experience levels were also diverse, with 5–7 years being the most common bracket at 38.5%.

Procedures

The procedures employed for data collection in this study involved the use of a self-administered printed questionnaire to gather information on participants' demographic details, PBHE, SNK, and FH. Specifically, for ASs, the research team conducted direct measurements of height and weight using the Health-o-Meter portable digital floor scale. To standardize body weight data collection, swimmers were instructed to wear a swimsuit.

Participants' height and weight data were utilized to calculate body mass index (BMI) in kilograms per square meter (kg/m^2). Studies have shown a strong correlation between self-reported and measured BMIs (26). Subsequently, the height and weight data underwent comparison with the Centers for Disease Control (CDC) growth charts to identify any instances of self-reported values that deviated excessively from the norm (27).

Instruments

Perceived barriers to healthy eating (PBHE)

A set of questions addressing barriers to maintaining a healthy diet was adapted from a prior study focusing on barriers to weight management (28). Participants were presented with five response options: 'strongly disagree,' 'disagree,' 'neither agree nor disagree,' 'agree,' and 'strongly agree'. Each option was assigned a numerical value ranging from 1 to 5, indicating higher barriers with increasing values. However, in the section covering social and environmental barriers, a modification was made to the original paragraph. Instead of 'Not having time to prepare or eat healthy foods due to 'job,'' the revised statement reads, 'Not having time to prepare or eat healthy foods due to school.' This adjustment is more applicable to adolescents who are primarily engaged in education rather than work. Similarly, the phrase 'Not having time to prepare or eat healthy foods because of my family commitment' was revised to 'Not having time to prepare or eat healthy foods because of my commitment to training'. This modification is made to clarify that this category pertains to other obligations, such as training, whereas family commitments are more relevant to adults. The internal consistency of measurement scale was assessed based on a survey sample of 25 participants. The PBHE Scale, comprising 11 items, demonstrated a moderate level of reliability with a Cronbach's Alpha of 0.724. These reliability statistics suggest that the items within scale consistently measure the intended constructs, providing a reliable foundation for further analyses in exploring PBHE within the surveyed sample (see Table 1).

Sports nutrition knowledge (SNK)

The survey utilized to assess the nutritional knowledge (NK) of athletes was the NK for Athletes survey, chosen for its validated suitability for this specific purpose. This survey, having undergone rigorous validation procedures, offers attributes such as minimal time burden, updated content, and user-friendliness, particularly suitable for early adolescents. The survey comprises 59 items that cover various aspects of sports nutrition, including macronutrients, micronutrients, hydration, and the frequency of food intake (29). Participants were asked to provide responses to these items, and each correct answer was awarded +1 point, incorrect answers were given a score of −1 point, and unanswered items received 0 points. Higher scores on the survey indicated a higher level of nutrition knowledge, with the maximum achievable score being 59 points. The SNK Scale, a more extensive measure with 59 items, maintained a moderate level of internal consistency, reflected by a Cronbach's Alpha of 0.731. These reliability statistics suggest that the items within scale consistently measure the intended constructs, providing a reliable foundation for further analyses in exploring SNK within the surveyed sample (see Table 1).

Food habits (FH)

Food habits: encompassing a set of 14 inquiries. One question has been excised: Is wine or beer commonly consumed during meals? This omission stems from the study's focus on adolescents aged 12–18,

TABLE 1 Internal consistency assessment of scales.

Scale	Cronbach's Alpha	Number of items
Perceived barriers to healthy eating (PBHE)	0.724	11
Food habits (FH)	0.724	13
Sports nutrition knowledge (SNK)	0.731	59

making the question incongruous for this demographic. Consequently, the total number of questions stands at 13.

The aim of this segment was to examine the FH of adolescents, with a particular focus on aspects like the composition of breakfast, frequency of daily meals, regular intake of fruits and vegetables, and consumption patterns of soft beverages.

Seven of the questions had the following response categories: "always," "often," "sometimes," and "never." Conversely, the remaining six questions employed distinct structures featuring four response categories. Each response was allocated a score between 0 and 3, where the highest score indicated the healthiest choice and the lowest denoted the least healthy. The cumulative score attainable for this section amounted to 39. The FH Scale, consisting of 13 items, exhibited comparable reliability with a Cronbach's Alpha of 0.724. These reliability statistics suggest that the items within scale consistently measure the intended constructs, providing a reliable foundation for further analyses in exploring FH within the surveyed sample (see Table 1).

Statistical analyses

The study employed a variety of statistical analyses to thoroughly examine different aspects of the collected data. Initially, an internal consistency assessment was conducted using Cronbach's Alpha, a widely accepted measure for evaluating scale reliability. The study employed descriptive statistics and categorical analysis, presenting counts and row percentages for various categorical variables related to the scales. Furthermore, correlation analysis was applied with Pearson Correlation coefficients calculated to explore relationships between different scales. Finally, a stepwise regression analysis was performed to identify predictors influencing food habits. Together, these analyses provide a comprehensive and insightful examination of scale reliability, inter relationships between variables, and factors influencing FH within the surveyed sample.

Results

Table 2 presents a breakdown of categorical variables related to different scales, each classified into low, moderate, and high levels. The categories include food habits.cat, personal Barriers.cat, social Barriers.cat, environmental Barriers.cat, barriers.cat, and Sports Nutrition Knowledge.cat. The counts and row percentages for each level are provided, offering insights into the distribution of participants across the specified categorical variables.

Table 3 explores the interconnections among various scales, specifically examining the relationships between food habits, personal barriers, social barriers, environmental barriers, overall barriers, and knowledge. The table presents Pearson Correlation coefficients along with corresponding two-tailed significance levels (Sig.). Additionally, the sample size (N) for each correlation is provided, enhancing the understanding of statistical relationships based on data collected from 52 participants. Notably, a significant positive correlation is evident between the 'Food Habits' and 'Knowledge' scales, indicated by a Pearson Correlation coefficient of 0.393 and a noteworthy two-tailed p-value of 0.004.

Table 4 presents a comprehensive overview of a stepwise regression analysis aimed at identifying predictors influencing food habits. In the initial step, SNK emerges as a statistically significant positive predictor ($\beta = 0.393$, $p = 0.004$), indicating a positive association with FH. However, introducing PBs in Step 2 results in a statistically significant positive relationship ($\beta = -0.173$, $p = 0.187$). In the absence of a statistical indicator of barriers to eating healthy food, the positive association between SNK and FH seems to lead to a reduction in barriers to eating healthy food.

Moving to Step 3, additional predictors, including Category, Gender, BMI, and Experience, are introduced. However, none of these individual predictors demonstrates a statistically significant relationship with FH at this step Category ($\beta = 0.103$, $p = 0.481$), Gender ($\beta = 0.041$, $p = 0.785$), BMI ($\beta = -0.164$, $p = 0.223$), and Experience ($\beta = 0.010$, $p = 0.949$).

Despite the lack of individual predictor significance, the overall model remains statistically significant across all steps (Step 1: $F = 9.153$, $p = 0.004$; Step 2: $F = 5.543$, $p = 0.007$; Step 3: $F = 2.173$, $p = 0.063$). The R2 values indicate the proportion of variance explained, progressively increasing from 0.155 in Step 1 to 0.250 in Step 3. The incremental change in R2 is 0.029 in Step 2 and 0.066 in Step 3, suggesting notable enhancements in the model's explanatory power upon the inclusion of predictors.

Discussion

This study investigated the correlations between FH, PBHE, and the SNK among ASs, while also considering the moderating role of demographic factors in connecting FH, PB, and SNK. Two hypotheses

TABLE 2 Categorical variables describing scales.

	Low		Moderate		High	
	Count	Row N %	Count	Row N %	Count	Row N %
Food habits.cat	14	26.9%	26	50.0%	12	23.1%
Personal barriers.cat	18	34.6%	21	40.4%	13	25.0%
Social barriers.cat	16	30.8%	27	51.9%	9	17.3%
Environmental barriers.cat	15	28.8%	27	51.9%	10	19.2%
Perceived barriers.cat	15	28.8%	27	51.9%	10	19.2%
Sports nutrition knowledge.cat	13	25.0%	28	53.8%	11	21.2%

are proposed: the first suggests a positive association between FH and SNK, leading to a lower perception of barriers; the second proposes that demographic factors may moderate the relationships between FH, PBHE, and SNK among ASs.

Nutrition understanding significantly impacts athletes' performance, emphasizing the importance of a healthy eating regimen to meet nutritional requirements and optimize athletic performance. Despite global recognition of the importance of continuous nutritional education for young athletes (5, 6), the precise exploration of fundamental dietary patterns among ASs remains insufficient within the existing body of literature. This study aims to fill this gap by examining SNK, FH, and PBHE among ASs, particularly in Jordanian athletes.

TABLE 3 Correlations between scales-investigating Pearson correlations and significance levels.

		Food. Habits
Personal barriers	Pearson Correlation	−0.097
	Sig. (2-tailed)	0.492
	N	52
Social barriers	Pearson Correlation	−0.209
	Sig. (2-tailed)	0.136
	N	52
Environmental barriers	Pearson Correlation	−0.097
	Sig. (2-tailed)	0.492
	N	52
Perceived barriers	Pearson Correlation	−0.183
	Sig. (2-tailed)	0.193
	N	52
Sports nutrition knowledge(SNK)	Pearson Correlation	0.393**
	Sig. (2-tailed)	0.004
	N	52

**Statistical significance $p < 0.05$.

The study revealed a significant positive correlation between FH and SNK, indicating that an increase in SNK among ASs correlates with an improvement in the quality of their FH.

This aligned with previous research showing a positive association between nutrition knowledge and practices among adolescent athletes (30–32), the positive correlation between dietary intake and SNK among Adolescent Soccer Players supports this finding (33). Notably, our research outcomes are contrasts with a study demonstrating that possessing knowledge does not always result in the adoption of good dietary practices (34). The positive association between SNK and FH emphasizes the pivotal role of education in shaping athletes' dietary choices (14, 35). Educational interventions targeting SNK enhancement could lead to positive changes in the dietary practices of young athletes (36). For example, Foo et al. (37) conducted a nutrition education intervention among highly trained ASs, showing a significant improvement in SNK scores, indicating the positive influence of targeted education on athletes' knowledge.

The results offer a nuanced perspective on the factors influencing food habits, shedding light on the relationships between SNK, PBs, and various demographic factors. Initially, a statistically significant positive association between SNK and FH ($\beta = 0.393$, $p = 0.004$) aligns with existing literature emphasizing the positive impact of nutrition knowledge on dietary choices (31, 38, 39). This underscores the role of education in fostering healthier eating habits among participants.

The introduction of PBs in Step 2 of the analysis reveals a noteworthy finding. The statistically significant positive relationship between PBs and FH suggests that as SNK increases, there is a concurrent reduction in personal barriers to healthy eating among ASs. This unexpected relationship can be seen as a positive outcome, indicating that higher knowledge levels empower individuals to overcome perceived obstacles, fostering the adoption of healthier dietary habits. Further exploration into the specific nature of these PBs and how increased knowledge contributes to their mitigation is warranted like targeted education initiatives that focus on increasing nutritional knowledge and practical skills have the potential to effectively address barriers to healthy eating. By empowering individuals to make informed choices, develop essential cooking skills, and cultivate a positive mindset toward nutrition, these initiatives can

TABLE 4 Stepwise regression analysis of predictors influencing FH.

Variables	Step 1			Step 2			Step 3		
	B	*β*	*p*	*B*	*β*	*p*	*B*	***B***	*p*
Sports nutrition knowledge(SNK)	0.136	0.393	0.004	0.134	0.389	0.004	0.124	0.360	0.010
Perceived barriers(PB)	–	–	–	−0.095	−0.173	0.187	−0.104	−0.188	0.163
Category	–	–		–	–	–	0.273	0.103	0.481
Gender	–	–	–	–		–	0.284	0.041	0.785
BMI	–	–	–	–	–	–	−0.665	−0.164	0.223
Experience	–	–	–	–	–	–	0.040	0.010	0.949
R2	0.155			0.184			0.250		
Model fit	$F = 9.153$ $p = 0.004$			$F = 5.543$ $p = 0.007$			$F = 2.173$ $p = 0.063$		
R2 change				0.029			0.066		

Statistical significance $p < 0.05$.

facilitate long-term behavior change and promote healthier lifestyles among athletes. The observed reduction in PBs with enhanced SNK underscores the potential effectiveness of targeted education initiatives. Studies by Brauman et al. (30) have identified significant barriers to healthy eating, such as lack of time, easy access to unhealthy foods, cost, lack of knowledge, and cooking skills. Additional research has shown that adolescents identify a lack of willpower and a hectic lifestyle as primary obstacles to embracing a healthy diet (33). These findings highlight the positive impact of targeted education initiatives in addressing barriers to healthy eating among athletes (40). By providing athletes with the knowledge and skills to navigate challenges such as time constraints, limited access to healthy foods, and financial considerations, educational interventions contribute to creating an environment conducive to healthier dietary choices (41).

In Step 3 of the analysis, additional predictors including category, gender, BMI, and experience are introduced. Despite their inclusion, none of these individual predictors demonstrates a statistically significant relationship with FH in this step. This lack of significance suggests that, in the context of this study, these demographic factors may not independently influence participants' dietary behaviors. However, it is crucial to acknowledge the potential interplay and cumulative effect of these factors, which might collectively contribute to shaping FH.

The overall model's persistence in statistical significance across all steps highlights the collective explanatory power of the included predictors. The increasing R-squared (R^2) values signify the progressively improved ability of the model to explain the variance in food habits. The incremental change in R2 between steps indicates notable enhancements in the model's explanatory power upon the inclusion of predictors, reinforcing the notion that a combination of SNK, PB, and demographic factors collectively contributes to the understanding of FH among participants. In accordance with our findings, another study also supported the results presented in this paper by detecting no differences based on sex or category (42). Similarly, a study found no significant correlations between sex, BMI, SNK, and FH (43). Additionally, another study showed no differences between SNK and gender (18). Conversely, a study validated that knowledge was notably linked to the age, gender, and duration of sports training among participants. Meanwhile, only age and BMI exhibited significant associations with FH (44).

The study's identification of a significant positive relationship between PBHE and FH, coupled with the unexpected reduction in personal barriers as SNK increases, underscores the potential effectiveness of targeted educational initiatives. The results suggest that enhancing knowledge empowers individuals to overcome obstacles, fostering the adoption of healthier dietary habits. Further exploration into the specific nature of these barriers and how increased knowledge contributes to their mitigation could provide valuable insights for future interventions. The introduction of demographic factors, including category, gender, BMI, and experience, did not show individual significance in influencing food habits. However, the study acknowledges the potential cumulative effect and interplay of these factors, emphasizing the collective explanatory power of SNK, PB, and demographics in understanding participants' FH. In light of these findings. The head coach, along with assistant coaches, plays a crucial role in reinforcing proper dietary practices among swimmers. This comprehensive approach extends beyond just working with the athletes; it involves engaging with both the swimmers and their parents. This strategy emphasizes that the SNK acquired is not just theoretical but is actively implemented in the athletes' daily lives. Numerous studies underscore the importance of involving parents in the nutritional education process. This inclusion recognizes the influential role parents play in shaping their child's dietary habits, ensuring a holistic approach to nutrition education and the major parental role in strengthening NK among adolescent competitive swimmers (45). This inclusion recognizes the influential role parents play in shaping their child's dietary habits, ensuring a holistic approach to nutrition education and the major parental role in strengthening nutrition knowledge among adolescent competitive swimmers (46). Additionally, coaches act as mentors and facilitators in the athletes' journey toward optimal nutrition, providing guidance and oversight. This involvement serves not only to educate but also to reinforce positive nutritional behaviors. By actively participating in tracking food habits, coaches contribute to the overall well-being of the athletes under their guidance, fostering a culture of health and performance. However, it's crucial to acknowledge that athlete perceptions of coaches helping them track food intake can be highly controversial. Some athletes may feel subverted or like they are being too closely monitored by coaches, which can lead to negative outcomes such as developing a strained relationship with food and body image issues. This integrated approach, encompassing both parental involvement and coach-led monitoring, aligns with the broader sports community's understanding of the interconnected factors influencing athletes' nutritional choices and performance outcomes.

It is evident that targeted education initiatives play a crucial role in addressing barriers and fostering healthier dietary choices among young athletes. Crafting suitable nutrition education strategies using online resources and mobile applications can effectively bolster both nutritional knowledge and practices among athletes (47). Research indicates that mobile applications are especially proficient in augmenting nutrition knowledge (48). By integrating mobile applications, there exists substantial potential to elevate nutritional understanding (49) consequently fostering healthier dietary habits and advancing nutritional knowledge even further (50). Our study calls for continued efforts in providing athletes with the knowledge and skills to navigate challenges, such as time constraints, limited access to healthy foods, and financial considerations. Moreover, our study emphasizes the need for tailored educational programs to address the specific context of adolescent swimmers, contributing to the optimization of nutritional practices and, consequently, athletic performance.

It's imperative to acknowledge several potential limitations associated with the dataset utilized in our study. Firstly, the sample size of the dataset may be limited, which could hinder the generalizability of our findings to a broader population of athletes. Secondly, our study may have employed a cross-sectional design, gathering data at a singular time point. This approach limits our ability to establish causality or track changes in dietary behaviors over time. Lastly, the conclusions drawn from our study may be specific to the context of adolescent swimmers. Consequently, the generalizability of our results to other athlete populations or different settings may be constrained. Recognizing these potential limitations is crucial for interpreting the implications of our study accurately and for guiding future research endeavors aimed at addressing the complexities of nutrition education among athletes.

Conclusion

In conclusion, this study sheds light on the relationships among FH, PBHE, and SNK within a cohort of 52 ASs aged 12–18 from Al Hussein Youth Club in Al-Hussein Sport City. The significant positive association between FH and SNK underscores the importance of SNK in shaping the dietary choices of young athletes. However, the lack of significance in introducing PB and individual predictors suggests that the influence of barriers and demographic factors may not be as straightforward in determining FH in this specific population. While individual predictors did not achieve statistical significance, the overall model remained significant, emphasizing the multifaceted nature of factors influencing FH among ASs. These findings contribute valuable insights into the nuanced dynamics of nutritional behaviors in ASs, highlighting the need for targeted interventions to enhance nutritional education. Some targeted interventions that could be considered include, Firstly, Implementing nutrition education workshops specifically tailored to the needs and challenges of adolescent swimmers, addressing topics such as meal planning, nutrient timing, and hydration strategies. Secondly, providing access to online resources and mobile applications designed to enhance nutrition knowledge and promote healthy eating habits among adolescent swimmers. Additionally, collaborating with nutritionists or dietitians to offer personalized dietary counseling and guidance to athletes, considering their individual nutritional needs and goals. Lastly, Incorporating nutrition education into the overall training program for adolescent swimmers, emphasizing the importance of proper nutrition for performance, recovery, and overall health. The study advocates for the development of tailored educational programs that address the specific nutritional needs and challenges faced by ASs. By enhancing SNK, interventions can empower these athletes to make informed dietary choices, ultimately optimizing their performance and overall well-being. Future research endeavors should delve deeper into the diverse factors contributing to FH in adolescent athletes, considering contextual and individual variables that may influence nutritional behaviors. Such insights will aid in the formulation of more precise and effective interventions, fostering a culture of healthy FH among ASs. However, it's important to note that with a small sample size, the findings of the study may not be representative of the broader population of ASs. The demographics, experiences, and health behaviors of the participants might not be reflective of other groups, limiting the generalizability of the 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

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

WA: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. SA: Data curation, Writing – original draft.

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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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References

1. Amawi AT, Moualla DS, Alshuwaier GO, Al-Nuaim AA, Bursais AK, Aljaloud KS, et al. Knowledge and attitude of dietary supplements among Arab Olympic athletes and coaches in preparation program for Tokyo 2020 Olympic games. *Int J Hum Mov Sports Sci*. (2023) 11:368–77. doi: 10.13189/saj.2023.110214

2. Amawi A, Alshuwaier G, Alaqil A, Alkasasbeh WJ, Bursais A, Al-Nuaim A, et al. Exploring the impact of dietary factors on body composition in elite Saudi soccer players: a focus on added sugars, salt, and oil consumption. *Int J Hum Mov Sports Sci*. (2023) 11:1305–12. doi: 10.13189/saj.2023.110614

3. Desbrow B, McCormack J, Burke LM, Cox GR, Fallon K, Hislop M, et al. Sports dietitians Australia position statement: sports nutrition for the adolescent athlete. *Int J Sport Nutr Exerc Metab*. (2014) 24:570–84. doi: 10.1123/ijsnem.2014-0031

4. Rodriguez NR, DiMarco NM, Langley S. Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. *J Am Diet Assoc*. (2009) 109:509–27. doi: 10.1016/j.jada.2009.01.005

5. Mountjoy M, Armstrong N, Bizzini L, Blimkie C, Evans J, Gerrard D, et al. IOC consensus statement: "training the elite child athlete". *Br J Sports Med*. (2008) 42:163–4. doi: 10.1136/bjsm.2007.044016

6. Meyer F, O'Connor H, Shirreffs SM. Nutrition for the young athlete. *J Sports Sci*. (2007) 25:S73–82. doi: 10.1080/02640410701607338

7. Spendlove JK, Heaney SE, Gifford JA, Prvan T, Denyer GS, O'Connor HT. Evaluation of general nutrition knowledge in elite Australian athletes. *Br J Nutr*. (2012) 107:1871–80. doi: 10.1017/S0007114511005125

8. Heaney S, O'Connor H, Michael S, Gifford J, Naughton G. Nutrition knowledge in athletes: a systematic review. *Int J Sport Nutr Exerc Metab*. (2011) 21:248–61. doi: 10.1123/ijsnem.21.3.248

9. Martínez S, Pasquarelli BN, Romaguera D, Arasa C, Tauler P, Aguiló A. Anthropometric characteristics and nutritional profile of young amateur swimmers. *J Strength Cond Res*. (2011) 25:1126–33. doi: 10.1519/JSC.0b013e3181d4d3df

10. de Sousa EF, Da Costa THM, Nogueira JAD, Vivaldi LJ. Assessment of nutrient and water intake among adolescents from sports federations in the Federal District. *Br J Nutr*. (2008) 99:1275–83. doi: 10.1017/S0007114507864841

11. Burke L. Nutrition for swimming. *Int J Sports Nutr Exerc Metab*. (2014) 24:360–72. doi: 10.1123/ijsnem.2014-0015

12. Alkasasbeh WJ, Amawi AT. Impact of eating habits on the psychological state of Jordanian athletes: a descriptive study. *Food Sci Technol*. (2023) 11:168–81. doi: 10.13189/fst.2023.110305

13. Bm M, Rf O, Aj C, Ab C, Ab C. Summer league college baseball players: do dietary intake and barriers to healthy eating differ between game and non-game days? *SMART J*. (2007) 3:23–34.

14. Paschoal VCP, Amancio OMS. Nutritional status of Brazilian elite swimmers. *Int J Sport Nutr Exerc Metab*. (2004) 14:81–94. doi: 10.1123/ijsnem.14.1.81

15. Evans CEL, Christian MS, Cleghorn CL, Greenwood DC, Cade JE. Systematic review and meta-analysis of school-based interventions to improve daily fruit and vegetable intake in children aged 5 to 12 y. *Am J Clin Nutr*. (2012) 96:889–901. doi: 10.3945/ajcn.111.030270

16. Reading KJ, McCargar LJ, Marriage BJ. Adolescent and young adult male hockey players: nutrition knowledge and education. *Can J Diet Pract Res*. (1999) 60:166–9.

17. Doyle-Lucas AF, Davy BM. Development and evaluation of an educational intervention program for pre-professional adolescent ballet dancers: nutrition for optimal performance. *J Dance Med Sci*. (2011) 15:65–75. doi: 10.1177/1089313X1101500203

18. Jagim AR, Fields JB, Magee M, Kerksick C, Luedke J, Erickson J, et al. The influence of sport nutrition knowledge on body composition and perceptions of dietary requirements in collegiate athletes. *Nutrients*. (2021) 13:2239. doi: 10.3390/nu13072239

19. Posthumus L, Fairbairn K, Darry K, Driller M, Winwood P, Gill N. Competition nutrition practices of elite male professional rugby union players. *Int J Environ Res Public Health*. (2021) 18:5398. doi: 10.3390/ijerph18105398

20. Hanson J, Dole A. Potential nutrition contributions to exercise-associated muscle cramping in four recreational half-marathoners: a case series. *Int J Strength Cond*. (2021) 1:1–9. doi: 10.47206/ijsc.v1i1.43

21. Condo D, Lohman R, Kelly M, Carr A. Nutritional intake, sports nutrition knowledge and energy availability in female Australian rules football players. *Nutrients*. (2019) 11:971. doi: 10.3390/nu11050971

22. Ascensión MD. Alimentación y valoración del estado nutricional de los adolescentes españoles (Estudio AVENA). Evaluación de riesgos y propuesta de intervención. I. Descripción metodológica del proyecto* The AVENA group** Conclusión: Este proyecto incluye la actividad coordi-NTS (AVENA Study). Assessment Of Risks And Intervention Proposal. *Nutr Hosp*. (2003) 1:15–28.

23. Cotugna N, Vickery CE, McBee S. Sports nutrition for young athletes. *J Sch Nurs*. (2005) 21:323–8. doi: 10.1177/10598405050210060401

24. Kunkel ME, Bell LB, Luccia BH. Peer nutrition education program to improve nutrition knowledge of female collegiate athletes. *J Nutr Educ*. (2001) 33:114–5. doi: 10.1016/s1499-4046(06)60175-9

25. Amawi A, Alkasasbeh W, Jaradat M, Almasri A, AlObaidi S, Abuhammad A, et al. Athletes' nutritional demands: a narrative review of nutritional requirements. *Front Nutr*. 10:1331854. doi: 10.3389/fnut.2023.1331854

26. Elgar FJ, Roberts C, Tudor-Smith C, Moore L. Validity of self-reported height and weight and predictors of bias in adolescents. *J Adolesc Health*. (2005) 37:371–5. doi: 10.1016/j.jadohealth.2004.07.014

27. Kuczmarski RJ. 2000 CDC growth charts for the United States: methods and development. *Vital Health Stat*. (2002) 246:1–190.

28. Andajani-Sutjahjo S, Ball K, Warren N, Inglis V, Crawford D. Perceived personal, social and environmental barriers to weight maintenance among young women: a community survey. *Int J Behav Nutr Phys Act*. (2004) 1:15. doi: 10.1186/1479-5868-1-15

29. Vázquez-Espino K, Fernández-Tena C, Lizarraga-Dallo MA, Farran-Codina A. Development and validation of a short sport nutrition knowledge questionnaire for athletes. *Nutrients*. (2020) 12:1–14. doi: 10.3390/nu12113561

30. Brauman K, Achen R, Barnes JL. The five most significant barriers to healthy eating in collegiate student-athletes. *J Am Coll Health*. (2023) 71:578–83. doi: 10.1080/07448481.2021.1899186

31. Spronk I, Kullen C, Burdon C, O'Connor H. Relationship between nutrition knowledge and dietary intake. *Br J Nutr*. (2014) 111:1713–26. doi: 10.1017/S0007114514000087

32. Debnath M, Chatterjee S, Bandyopadhyay A, Datta G, Dey SK. Prediction of athletic performance through nutrition knowledge and practice: a cross-sectional study among young team athletes. *Sport Mont*. (2019) 17:13–20. doi: 10.26773/smj.191012

33. Noronha DC, Santos MIAF, Santos AA, Corrente LGA, Fernandes RKN, Barreto ACA, et al. Nutrition knowledge is correlated with a better dietary intake in adolescent soccer players: a cross-sectional study. *J Nutr Metab.* (2020) 2020:1–7. doi: 10.1155/2020/3519781

34. Jusoh N, Low Fook Lee J, Tengah RY, Azmi SH, Suherman A. Association between nutrition knowledge and nutrition practice among Malaysian adolescent handball athletes. Malays. *J Nutr*. (2021) 27:27. doi: 10.31246/mjn-2020-0113

35. Kosendiak A, Król M, Ligocka M, Kepinska M. Eating habits and nutritional knowledge among amateur ultrarunners. *Front Nutr*. (2023) 10:10. doi: 10.3389/fnut.2023.1137412

36. Tam R, Beck KL, Manore MM, Gifford J, Flood VM, O'Connor H. Effectiveness of education interventions designed to improve nutrition knowledge in athletes: a systematic review. *Sports Med*. (2019) 49:1769–86. doi: 10.1007/s40279-019-01157-y

37. Foo WL, Faghy MA, Sparks A, Newbury JW, Gough LA. The effects of a nutrition education intervention on sports nutrition knowledge during a competitive season in highly trained adolescent swimmers. *Nutrients*. (2021) 13:2713. doi: 10.3390/nu13082713

38. Crites SL, Aikman SN. Impact of nutrition knowledge on food evaluations. *Eur J Clin Nutr*. (2005) 59:1191–200. doi: 10.1038/sj.ejcn.1602231

39. Krešić G, Kenđel Jovanović G, Pavičić Žeželj S, Cvijanović O, Ivezić G. The effect of nutrition knowledge on dietary intake among Croatian university students. *Coll Antropol*. (2009) 33:1047–56.

40. Judge LW, Kumley RF, Bellar DM, Pike KL, Pierson EE, Weidner T, et al. Hydration and fluid replacement knowledge, attitudes, barriers, and behaviors of NCAA division 1 American football players. *J Strength Cond Res*. (2016) 30:2972–8. doi: 10.1519/JSC.0000000000001397

41. Alawamleh T, AlKasasbeh W. Exploring the landscape of eHealth in promoting physical activity and healthy dietary intake. *Univers J Public Health*. (2024) 12:120–7. doi: 10.13189/ujph.2024.120113

42. Andrews A, Wojcik JR, Boyd JM, Bowers CJ. Sports nutrition knowledge among mid-major division I university student-athletes. *J Nutr Metab*. (2016) 2016:1–5. doi: 10.1155/2016/3172460

43. Ahmadi F, Ebrahimi M, Kashani V. Sports nutritional knowledge, attitude, and practice of adolescent athletes in Tehran, Iran. Asian. *J Sports Med*. (2022) 13:e131584. doi: 10.5812/asjsm-131584

44. Bakhtiar M, Masud-ur-Rahman M, Kamruzzaman M, Sultana N, Rahman SS. Determinants of nutrition knowledge, attitude and practices of adolescent sports trainee: a cross-sectional study in Bangladesh. *Heliyon*. (2021) 7:e06637. doi: 10.1016/j.heliyon.2021.e06637

45. Philippou E, Middleton N, Pistos C, Andreou E, Petrou M. The impact of nutrition education on nutrition knowledge and adherence to the Mediterranean diet in adolescent competitive swimmers. *J Sci Med Sport*. (2017) 20:328–32. doi: 10.1016/j.jsams.2016.08.023

46. White HJ, Harwood CG, Wiltshire G, Plateau CR. Parents' experiences of family food routines in adolescent elite-level swimming. *Psychol Sport Exerc*. (2022) 62:102237. doi: 10.1016/j.psychsport.2022.102237

47. O'brien L, Collins K, Amirabdollhian F. Exploring sports nutrition knowledge in elite gaelic footballers. *Nutrients*. (2021) 13:1081. doi: 10.3390/nu13041081

48. AlKasasbeh WJ, Tawfiq AA. The effectiveness of using Mobile learning application on undergraduates' intrinsic motivation and their general nutrition knowledge. *Int J Interact Mob Technol*. (2023) 17:19–37. doi: 10.3991/ijim.v17i17.40959

49. Scarry A, Rice J, O'connor EM, Tierney AC. Usage of Mobile applications or Mobile health technology to improve diet quality in adults. *Nutrients*. (2022) 14:2437. doi: 10.3390/nu14122437

50. Vasiloglou MF, Christodoulidis S, Reber E, Stathopoulou T, Lu Y, Stanga Z, et al. What healthcare professionals think of ″nutrition & diet″ apps: an international survey. *Nutrients*. (2020) 12:1–17. doi: 10.3390/nu12082214

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Beet supplementation mitigates post-exercise inflammation

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Objectives: This study investigated the efficacy of a mixed beet-based supplement (BEET) versus placebo (PL) in countering inflammation during recovery from 2.25 h of intensive cycling in 20 male and female cyclists. A multi-omics approach was used that included untargeted proteomics and a targeted oxylipin panel.

Methods: A randomized, placebo-controlled, double-blind, crossover design was used with two 2-week supplementation periods and a 2-week washout period. Supplementation periods were followed by a 2.25 h cycling bout at close to 70%VO_{2max}. The BEET supplement provided 212 mg of nitrates per day, 200 mg caffeine from green tea extract, 44 mg vitamin C from Camu Camu berry, B-vitamins from quinoa sprouts (40% Daily Value for thiamin, riboflavin, niacin, and vitamin B6), and 2.5 g of a mushroom blend containing Cordyceps sinensis and Inonotus obliquus. Six blood samples were collected before and after supplementation (overnight fasted state), immediately post-exercise, and at 1.5 h-, 3 h-, and 24 h-post-exercise.

Results: The 2.25 h cycling bout increased plasma levels of 41 of 67 oxylipins detected. BEET supplementation significantly increased plasma nitrate (NO_3^-) and nitrite (NO_2^-) (sum, $NO_3^- + NO_2^-$) concentrations (interaction effect, $p < 0.001$) and two anti-inflammatory oxylipins [18-hydroxyeicosapentaenoic acid (18-HEPE) and 4-hydroxy-docosahexanoic acid (4-HDoHE)]. The untargeted proteomics analysis identified 616 proteins (458 across all times points), and 2-way ANOVA revealed a cluster of 45 proteins that were decreased and a cluster of 21 that were increased in the BEET versus PL trials. Functional enrichment supported significant BEET-related reductions in inflammation-related proteins including several proteins related to complement activation, the acute phase response, and immune cell adhesion, migration, and differentiation.

Discussion: Intake of a BEET-based supplement during a 2-week period was linked to higher plasma levels of $NO_3^- + NO_2^-$, elevated post-exercise levels of two anti-inflammatory oxylipins, and a significant decrease in a cluster of proteins involved in complement activation and inflammation. These data support that 2-weeks intake of nitrate from a mixed beet-based supplement moderated protein biomarkers of exercise-induced inflammation in athletes.

KEYWORDS

beets, exercise, proteomics, oxylipins, inflammation

Introduction

Nitrate is found in some root vegetables such as beets and in green leafy vegetables such as spinach and lettuce. The typical Western diet provides about 110 mg/day nitrate (1, 2). After ingestion, nitrate is metabolized to nitric oxide and other bioactive nitrogen oxides through a complex pathway. Nitrate is absorbed in the small intestine and peak plasma levels can be measured 30–60 min post-intake with an effective half-life of about six hours (3). Nitrate is cleared by the kidneys, but about 25% of the nitrate is taken up and secreted by the salivary glands and is subsequently reduced to nitrite by commensal bacteria in the mouth (4). The nitrite is then swallowed, absorbed through the intestinal tract, and further reduced to nitric oxide and other nitrite intermediates by enzymatic and nonenzymatic mechanisms in the blood and tissues. The use of mouthwashes, antacids, and chewing gum can interfere with the microbiome in the mouth and the conversion of nitrate to nitrite.

Dietary nitrate has been linked to several health-related benefits including blood pressure reduction, improved vascular function, and modulation of inflammatory processes and immune cell function (3). Nitric oxide acts as a signaling molecule during exercise and has physiological effects including vasodilation to increase blood flow regulation of muscle contraction and glucose uptake regulation of cellular respiration. The primary performance benefit of nitrate supplementation appears to be a reduction in the energy cost of exercise and a corresponding modest improvement in endurance capacity (5–7). Studies reporting performance benefits used acute nitrate doses of 5–6 mmol (or about 300 mg), or chronic doses at about half that amount (8). Chronic nitrate supplementation has not been consistently linked to improvements in aerobic capacity or performance (9). Concerns have been raised about high nitrate intake by athletes and the formation of carcinogenic N-nitroso compounds, a process that may be inhibited by vitamin C (10–13). An acceptable daily intake (ADI) level of 3.7 mg nitrates per kg body weight has been recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (14). This recommendation includes all nitrate sources including plant foods, drinking water, and cured and processed meats, although a different approach is recommended for nitrate from plant food sources (15). Co-supplementation of nitrate with caffeine, vitamin C, and other food components may enhance potential beneficial effects from moderate intake of nitrate and minimize risks from N-nitroso compounds (16).

Nitric oxide is considered a potent anti-inflammatory mediator and inhibitor of leukocyte recruitment, a key feature of inflammatory responses (3). However, most of the evidence comes from rodent-based studies. Human studies investigating the effects of nitrate or nitrate-rich beetroot juice on exercise-induced inflammation and muscle damage have produced mixed results (8, 17–19). Part of the problem is that only a few basic outcome measures related to inflammation (e.g., C-reactive protein, IL-6, IL-8) have been utilized in these studies. This study used a multi-omics approach centered on targeted oxylipins and untargeted proteomics analysis to investigate the influence of 2-weeks dietary nitrate ingestion from a beet-based mixed supplement on exercise-induced inflammation. Oxylipins are upstream regulators of inflammation, increase strongly after prolonged and vigorous exercise, and exert both pro- and anti-inflammatory effects depending on the fatty acid substrate and enzyme system (20–24). Untargeted proteomics studies indicate that long endurance cycling and running cause perturbations in numerous immune system and inflammatory proteins (25–27). Previous studies by our research group indicate that exercise-induced changes in inflammatory oxylipins and proteins can be moderated through nutrition-based interventions (21–24, 28, 29). We hypothesized that a multi-omics approach would reveal that 2-weeks ingestion of a beet-based supplement would mitigate exercise-induced inflammation.

Methods

Study participants

Male and female cyclists were invited to take part in this study if they met the inclusion criteria including 18 to 60 years of age, capable of cycling 2.25 h in a laboratory setting at 70% maximal oxygen consumption rate (VO_{2max}), and a willingness to avoid supplements and medications such as non-steroidal anti-inflammatory drugs (NSAIDs) with a potential to influence inflammation. Participants also agreed to limit intake of nitrate-rich vegetables during the study to less than 1 cup per day. These vegetables included spinach, lettuce, beets, beetroot juice, celery, and cabbage. Participants also agreed to avoid the use of mouthwashes, antacids, and chewing gum during the entire 6-week study and the 2-week period prior to the study. During the 3-day period prior to the 2.25 h cycling session, subjects agreed to taper exercise training and ingest a moderate-carbohydrate diet using a food list restricting high fat foods and visible fats.

A total of 46 participants were assessed for eligibility and 25 were entered into the study, with 20 completing all aspects of the protocol (Figure 1). The study participant number provided more than 80% power to detect a difference in pro-inflammatory oxylipins with an effect size 1.06 at alpha 0.05 using two-sample *t*-tests (23). Participants voluntarily signed the informed consent, and procedures were approved by the university's Institutional Review Board. Trial Registration: ClinicalTrials.gov, U.S. National Institutes of Health, identifier: NCT05907135.

Study design

This study employed a randomized, placebo controlled, double-blind, crossover design with two 2-week supplementation periods and a 2-week washout period. The study included seven lab visits at the Appalachian State University Human Performance Laboratory (HPL) at the North Carolina Research Campus, Kannapolis, NC.

During the first two lab visits prior to the 2-week supplementation period, study participants were given a complete orientation to the study protocol, signed the consent form, provided an overnight fasted blood sample, reported demographics and training histories using questionnaires, and recorded responses to the delayed onset of muscle soreness (DOMS) 1–10 scale questionnaire (30). Height and body weight were assessed, with body composition measured using the BodPod system (Cosmed, Rome, Italy). Study participants were tested for maximal aerobic capacity (VO_{2max}) during a graded, cycling test with the Lode cycle ergometer (Lode B.V., Groningen, Netherlands) and the Cosmed CPET metabolic cart (Cosmed, Rome, Italy).

The beet-based (BEET) and placebo (PL) supplements (randomized order using double-blind procedures) for the first and

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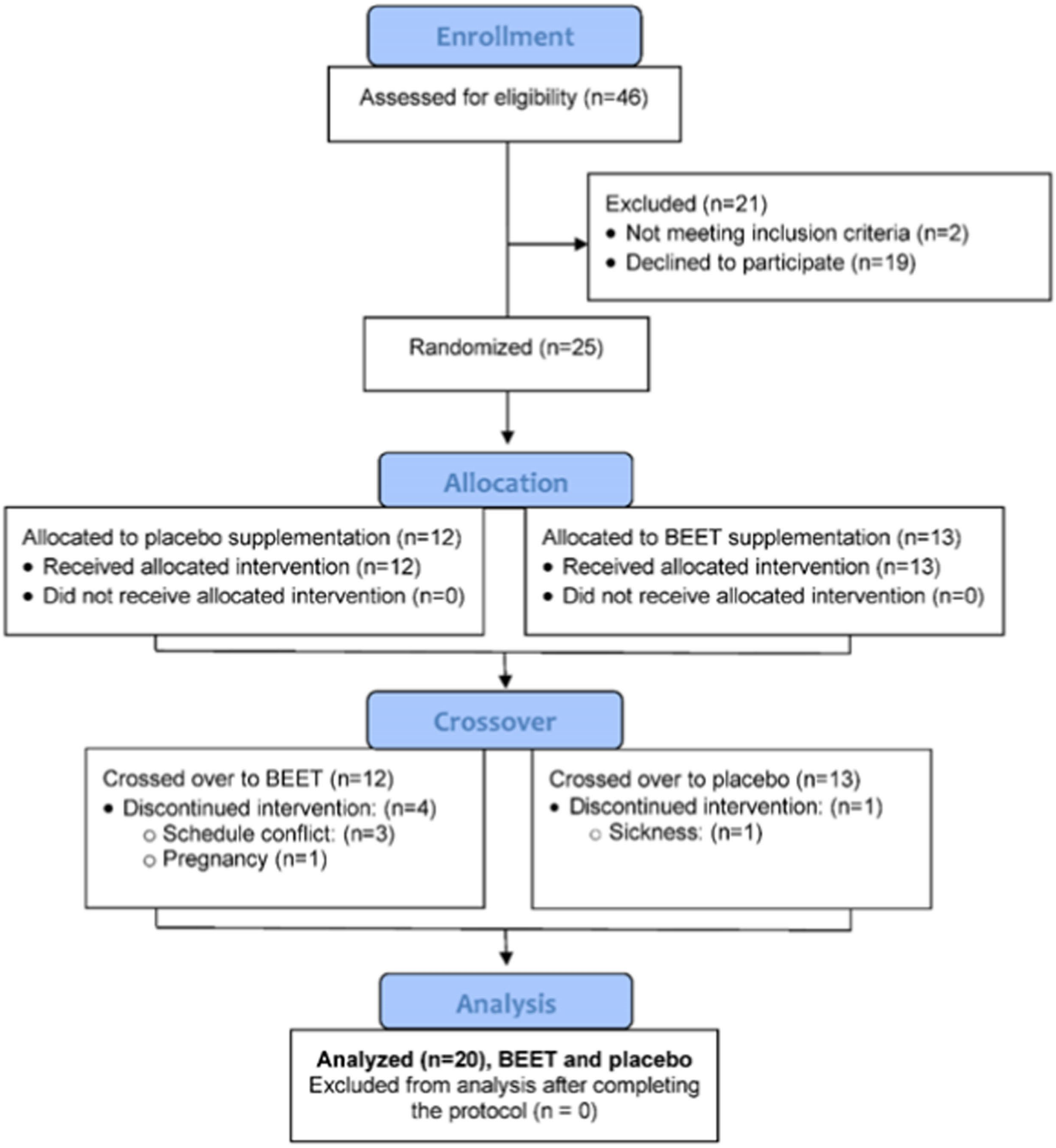

FIGURE 1
Study participant flow diagram.

second 2-week supplementation periods were supplied in coded packets. To facilitate compliance to the supplementation protocol, study participants were contacted via email on a regular basis and also returned the coded packets at the end of the supplementation period. Supplements ingested daily (two doses, early morning and mid-day, each mixed in 250 mL cold water) for 2-weeks prior to participation in the first 2.25 h cycling session. After a 2-week washout period, participants repeated all procedures using the counterbalanced supplement. The BEET and PL supplements were supplied by the sponsor (Standard Process, Palmyra, Wisconsin, USA). Each BEET packet (14 grams) included powder from beets and fermented beets to provide nitrates (106 mg of nitrates per serving), green tea extract with caffeine (100 mg), Camu Camu to provide vitamin C (22 mg), quinoa sprouts to provide B-vitamins (20% of the Daily Value for thiamin, riboflavin, niacin, and vitamin B6), and a mushroom blend of three common mushrooms (1.5 g of caterpillar fungus, lion's mane, chaga). The PL supplement included excipients with none of the active ingredients. PL ingredients included a carbohydrate blend (monosaccharides, disaccharides, maltodextrin, corn starch), monk fruit extract, dietary fibers including cellulose and rice bran, rice silica, malic acid, beet flavor, cranberry flavor, and colorants. Participants reported no adverse events from ingesting the supplements over the 2-week periods and were 100% compliant with the supplementation regimen. After the study was completed, study participants responded to a questionnaire regarding "what supplement do you think you were taking during each of the 2-week supplementation periods?" A total of 22.5 and 60% correctly identified the use of PL and BEET supplements, respectively (Chi-square = 12.95, p = 0.0015). Study participants reported a red color in their urine and stool when using the BEET supplement.

After the 2-week supplementation period, study participants reported to the Human Performance Lab in an overnight fasted state, provided a blood sample, ingested one 14-gram packet of the BEET or PL supplement with 250 mL water, and then cycled for 2.25 h at high intensity (70% VO_{2max}) while ingesting water alone (3 mL/kg every 15 min). Participants cycled on their own bicycles fitted to Saris H3 direct drive smart trainers (Madison, WI, USA) with monitoring by the Zwift online training platform (Long Beach, CA, USA). Heart rate, cycling speed, cadence, distance, power, breathing rate, ventilation, and oxygen intake were measured after 15 min and then every 30 min during the cycling session. To ensure performance consistency between trials and to focus on the effect of the BEET supplement on exercise-induced inflammation, performance data from the first trial were used to ensure a similar power and metabolic output during the second trial. Blood samples were collected at 0 h, 1.5 h, 3 h, and 24 h post-exercise. After each blood collection, participants provided a DOMS rating. Immediately after the 1.5 h post-exercise blood sample, all subjects consumed 7 kilocalories per kilogram of body weight of a fortified nutrient beverage (Boost, Nestlé S.A., Vevey, Switzerland). Blood samples were aliquoted and stored at-80°C prior to analysis for the outcome measures.

Sample analysis

Serum creatine kinase, myoglobin, and coritsol (from serum separator tubes), and complete blood counts (CBCs) with a white blood cell differential count (EDTA tubes) were analyzed using Labcorp services (Burlington, NC). Plasma aliquots were prepared from EDTA blood collection tubes and stored in a-80°C freezer until analysis for nitrate, nitrite, proteomics, and oxylipins after the study was completed.

Plasma nitrate and nitrite

The nitrate/nitrite fluorometric assay kit (item # 780051) from the Cayman Chemical Company (Ann Arbor, MI) was used to measure plasma concentrations of nitrate (NO_3^-) and nitrite (NO_2^-). This assay was performed in accordance with the instructions provided by the manufacturer with the plates read using the SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices, LLC San Jose, CA, USA). Data were processed using BioTek Gen5 2.0 software (BioTek, Agilent, Santa Clara, CA, USA). Samples were analyzed in duplicate (mean coefficient of variation or CV of 9.8%). The sum of both NO_3^- and nitrite NO_2^- was used as an index of total nitric oxide production as recommended by Cayman (lower limit of detection 0.70 µM, intra-assay CV 2.7%). The process includes 2 steps: conversion of nitrate to nitrite using a nitrate reductase, followed by addition of an acidic solution (DAN) which isolates nitrate and yields the product 1(H)-naphthotriazole. Sodium hydroxide (NaOH) is then added to enhance fluorescence of the product. Sample concentrations were plotted along a standard curve, which was made using fresh standards provided in the assay kits.

Plasma oxylipins

Plasma arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and oxylipins were analyzed using a liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM-MS) method as fully described elsewhere (31). Resultant data files were processed with Skyline, and the auto-integrated peaks were inspected manually. Concentrations of each oxylipin were determined from calibration curves of each analyte, which were constructed by normalizing to the selected deuterated internal standards followed by linear regression with 1/x weighting (Supplement Data Sheet 1). The coefficient of variation for the quality control standards was <15% as reported in the method development paper (31). A total of 41 of 67 oxylipins detected increased significantly post-exercise, and these were grouped for statistical analysis. Seven oxylipins generated from arachidonic acid and cytochrome P-450 (ARA-CYP) were grouped and these included 5,6-, 8,9-, 11,12-, and 14,15-dihydroxy-eicosatetraenoic acid (diHETrEs), 16-, 17-hydroxy-eicosatetraenoic acids (HETEs), and the 20-HETE metabolite 20-carboxy-arachidonic acid (20-coohAA). Four abundant oxylipins generated from linoleic acid (LA) with CYP and lipoxygenease (LOX) were also grouped, including 9,10- dihydroxy-9Z-octadecenoic acid (DiHOME), 12,13-DiHOME, 9- hydroxy-octadecadienoic acid (HODE), and 13-HODE (LA-DiHOMES + HODES).

Plasma proteome and statistical procedures

Untargeted proteomics were conducted using methods previously described (22, 32). Briefly, after sample preparation, 200 ng peptides from the plasma samples were loaded onto disposable EvoTip trap-columns (EV-2003, EvoSep, Denmark) and separated on an EvoSep One ™ LC system (EV-1000, EvoSep, Denmark) using a 21 min gradient with 1 µL/min flow rate. Effluents were analyzed on a high resolution Orbitrap Exploris 240 (Thermo) mass spectrometer using the data independent acquisition (DIA) method. The plasma protein library was generated using the gas-phase fractionation DIA method from peptide samples with and without depletion of the top 14 high-abundance plasma proteins (14,120 precursors, 960 proteins). The obtained LC–MS/MS dataset was searched for protein identification and quantitation using DIA-NN (33). Data were normalized by referencing to the protein levels of the first time point from the same individual subject to effectively correct for inter-individual variations (34) (Supplementary Data Sheet 2). The normalized values were statistically analyzed using the ANOVA test with two trials and six timepoints. To consider the protein as significantly changing between or within effects, the *p*-value was set to less than 0.05. Maximum likelihood-based hierarchical clustering analysis was used to cluster proteins with similar level patterns, and the results were visualized as a heatmap with the averaged value of each time-point after normalization by z-score. The list of significantly changed proteins in the enriched clusters were functionally enriched using STRING (Ver.11.5).[1] The top enriched biological processes from STRING analysis were selected to represent the functions of the proteins.

Additional statistical procedures

The data are expressed as mean ± SE and were analyzed using the generalized linear model (GLM), repeated measures ANOVA module in SPSS (IBM SPSS Statistics, Version 28.0, IBM Corp, Armonk, NY, USA). The statistical model utilized the within-subjects approach: 2 (trials) x 6 (time points) repeated measures ANOVA and provided time (i.e., the collective effect of the cycling exercise bout) and

1 https://string-db.org/

interaction effects (i.e., whether the data pattern over time differed between trials). If the interaction effect was significant ($p \leq 0.05$), then post-hoc analyses were conducted using paired *t*-tests comparing time point contrasts between trials. An alpha level of $p \leq 0.01$ was used after Bonferroni correction for 5 multiple tests. The positive false discovery rate (FDR or "q-value") was calculated for multiple testing correction of the plasma oxylipin and plasma proteomics data. Cohen's d effect size was calculated for the oxylipin data as the mean difference between the trials at the immediate post-exercise time point divided by the pooled standard deviation.

Results

Table 1 summarizes characteristics for the $n = 20$ study participants ($n = 14$ males, $n = 6$ females) who completed the study protocol. Age, percent body fat, aerobic capacity (VO2max), and training volume were similar between the male and female cyclists. The pattern of change over time did not differ between the male and female cyclists for a key outcome measurement (total plasma oxylipins, supplement x time x sex interaction effect, p-value =0.835). Thus, outcome measures for this randomized, crossover study are presented for all participants combined.

Performance data for each trial are summarized in Table 2. As designed, the two trials were similar in all cycling performance measures including total distance cycled, and percent of maximal heart rates, watts, and oxygen consumption rates.

Data for delayed onset of muscle soreness (DOMS), muscle damage biomarkers, the neutrophil/lymphocyte ratio, and serum cortisol are summarized in Table 3. Time effects for each of these variables were significant but the patterns of increase did not differ significantly between the BEET and PL trials (interaction effects, p-values >0.05).

BEET compared to placebo intake was associated with a significant increase in the sum of plasma nitrate and nitrite (NA + NI) (time and interaction effects, both $p < 0.001$) (Figure 2). The increase in NA + NI was significantly different between the BEET and PL trials after 2-weeks supplementation and throughout at least 3 h of recovery from the cycling bout. Trial differences for NA + NI at 24 h post-exercise tended to be different ($p = 0.089$).

Plasma arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) increased significantly post-exercise (time effects, $p < 0.001$) with no significant interaction effects (all $p > 0.50$) (Supplementary Data Sheet 1). A total of 67 oxylipins were detected in study samples. Of these, 41 oxylipins increased significantly post-exercise (Supplementary Data Sheet 1). These 41 oxylipins were summed for a composite variable and analysis showed significant post-exercise increases without trial differences between BEET and PL (interaction effect, $p = 0.166$) (Cohen's $d = 0.219$, immediately post-exercise) (Table 4). Three other composite variables were calculated including seven oxylipins generated from arachidonic acid and cytochrome P-450 (ARA-CYP), two abundant oxylipins generated from linoleic acid and CYP (9,10-DiHOME, 12,13-DiHOME), and two abundant oxylipins generated from lipoxygenase (LOX) (9-HODE, 13-HODE) (Table 4). Significant time effects were shown for each of these composite oxylipin variables but without significant

TABLE 1 Subject characteristics ($n = 20$) for male ($n = 14$) and female ($n = 6$) cyclists.

	Sex	Mean	SE
Age (years)	M = male	46.5	2.6
	F = female	51.3	4.3
Body mass (kg)	M	80.5*	3.5
	F	60.2	2.6
Height (cm)	M	179*	1.6
	F	167	1.6
Body mass index (BMI) (kg/m^2)	M	25.0*	0.9
	F	21.7	1.0
Body fat (%)	M	19.5	1.8
	F	25.0	3.7
Maximum oxygen consumption (VO_{2max}) (mL·kg.$^{-1}$ min^{-1})	M	41.2	1.6
	F	40.9	2.9
Maximum heart rate (beats/min)	M	170	4.0
	F	160	3.8
Maximum watts	M	282*	12.4
	F	217	20.1
Cycling training distance (km/wk)	M	124	18.2
	F	139	31.1

*$p \leq 0.05$.

TABLE 2 Average 2.25 h cycling performance outcomes for $n = 20$ cyclists (mean ± SE).

Performance measurement		Mean ± SE
Cycling power (watts)	PL	161 ± 6.9 (61.9 ± 1.4% max)
	BEET	164 ± 6.8 (63.0 ± 1.3% max)
Heart rate (beats/min)	PL	131 ± 2.6 (78.9 ± 1.7% max)
	BEET	132 ± 2.8 (80.2 ± 1.8% max)
Oxygen consumption rate (VO_2) (ml·kg^{-1} min^{-1})	PL	30.1 ± 1.1 (73.6 ± 2.1% max)
	BEET	31.7 ± 1.1 (77.5 ± 2.2% max)
Respiratory exchange ratio (RER)	PL	0.834 ± 0.001
	BEET	0.817 ± 0.007
Distance cycled (km)	PL	64.9 ± 1.6
	BEET	65.7 ± 1.7
Elevation gain (m)	PL	314 ± 33.0
	BEET	282 ± 24.2
Speed (km/h)	PL	27.7 ± 1.1
	BEET	29.1 ± 0.6
Cycling cadence (pedal revolutions/min)	PL	71.4 ± 2.9
	BEET	74.0 ± 1.8
Weight change (kg)	PL	1.13 ± 0.11
	BEET	1.08 ± 0.13

No significant placebo (PL) vs. BEET trial differences in performance measurements were found as designed.

TABLE 3 Trial comparisons for $n = 20$ participants across all time points for muscle soreness* and damage markers (serum creatine kinase and myoglobin), the neutrophil/lymphocyte blood count ratio, and serum cortisol.

Variable	Trial	Pre- Study	2-Wks Suppl.	0 h Post-Ex	1.5 h Post-Ex	3 h Post-Ex	24 h Post-Ex	*p*- value
DOMS (1–10 scale)	PL	1.7 ± 0.3	1.7 ± 0.2	4.1 ± 0.4	3.6 ± 0.4	2.7 ± 0.3	1.9 ± 0.2	<0.001; 0.069
	BEET	2.1 ± 0.4	1.6 ± 0.2	4.2 ± 0.4	3.3 ± 0.4	2.5 ± 0.3	2.1 ± 0.2	
Creatine kinase (U/L)	PL	143 ± 14.4	141 ± 17.1	177 ± 24.3	166 ± 21.4	172 ± 22.9	158 ± 22.3	0.008; 0.974
	BEET	174 ± 21.4	174 ± 23.7	279 ± 83.1	266 ± 81.9	266 ± 77.8	229 ± 50.5	
Myoglobin (ng/ml)	PL	35.5 ± 2.9	32.4 ± 1.9	47.4 ± 3.9	58.1 ± 10.0	52.0 ± 9.2	36.7 ± 3.1	0.002; 0.595
	BEET	40.1 ± 3.5	34.1 ± 2.1	57.0 ± 6.6	67.0 ± 6.8	63.7 ± 9.3	38.0 ± 3.6	
Neutrophil/ lymphocyte	PL	1.6 ± 0.1	1.5 ± 0.1	4.0 ± 0.4	5.2 ± 0.5	4.9 ± 0.5	1.7 ± 0.2	<0.001; 0.163
	BEET	1.6 ± 0.2	1.6 ± 0.2	4.2 ± 0.5	6.1 ± 0.7	5.5 ± 0.6	1.8 ± 0.2	
Cortisol (μg/dl)	PL	14.0 ± 0.8	15.4 ± 0.8	16.9 ± 1.2	11.8 ± 1.0	9.7 ± 0.6	14.3 ± 0.9	<0.001; 0.682
	BEET	13.8 ± 0.8	14.8 ± 0.8	18.3 ± 1.4	13.3 ± 1.1	11.0 ± 0.8	14.7 ± 0.7	

P-values represent time (first value) and trial x time interaction effects. *Ex, exercise; Suppl, supplement; PL, placebo; DOMS, delayed onset of muscle soreness.

interaction effects (Table 4) (Cohen's $d = 0.102$, 0.221, 0.283, respectively, immediately post-exercise). Two anti-inflammatory oxylipins were significantly higher in the BEET versus PL trials (Figures 3A,B). These included the EPA-derived oxylipin 18-hydroxyeicosapentaenoic acid (18-HEPE) (time effect, $p < 0.001$, interaction effect, $p = 0.016$) and the DHA-derived oxylipin 4-hydroxydocosahexaenoic acid (4-HDoHE) (time effect $p < 0.001$, interaction effect, $p = 0.010$).

A total of 616 plasma proteins were identified with no missing data for 458 proteins across all time points (Supplementary Data Sheet 2). Two-way ANOVA analysis of the normalized data showed that 45 proteins were lower and 21 proteins were higher in the BEET versus PL trials (all $p < 0.05$). The heatmap and line graphs of the clustered proteins are shown in Figure 4, and the identity of the clustered proteins is summarized in Table 5. Functional enrichment and protein–protein interaction networks are depicted in Figure 5 and supported a linkage of BEET supplementation with reduced complement activation and inflammatory responses, metabolic process and negative regulation of biological processes, and responses to stimulus, and an increased regulation of insulin-like growth factors (IGF) and uptake of IGF binding proteins, and symbiotic interactions.

Discussion

This study employed a strong research design to investigate the effects of 2-weeks intake of a mixed beet-based supplement (212 mg nitrates per day) on inflammation induced by a 2.25 h cycling bout with 20 cyclists. A multi-omics approach was used, and this included a comprehensive targeted oxylipins panel and the measurement of several hundred proteins using untargeted proteomics. BEET supplementation significantly increased plasma $NO_3^- + NO_2^-$ 1.6-fold after the 2-week supplementation period and 2.6-fold above placebo levels immediately after the cycling bout. The 2.25 h cycling bout caused significant inflammation with a composite variable of 41 oxylipins rising 3.4-fold immediately post-exercise above baseline plasma levels. Plasma oxylipins were still elevated 1.5-fold at the 3 h-post-exercise time point before falling to near baseline levels after 24 h recovery. BEET supplementation did not reduce post-exercise plasma concentrations of pro-inflammatory oxylipins but did have a modest effect in elevating two anti-inflammatory EPA- and DHA-derived oxylipins (18-HEPE and 4-HDoHE). Two-way ANOVA revealed 66 proteins that were increased or decreased with BEET supplementation, and functional enrichment supported significant BEET-related reductions in inflammation-related proteins including proteins related to complement activation, the acute phase response, and immune cell adhesion, migration, and differentiation.

This is the first exercise-based human clinical trial to investigate the influence of BEET supplementation on inflammation using a human systems biology approach. Other similar studies used a few targeted outcomes, had widely disparate research designs, and generally reported null or minor effects of increased plasma $NO_3^- + NO_2^-$ from nitrate supplementation on post-exercise inflammation biomarkers including cytokines (8, 17–19). In one study with 34 runners, beetroot juice versus placebo supplementation for 3 days following a marathon race had no effect on muscle soreness, creatine kinase, interleukin (IL)-6, IL-8, tumor necrosis factor-α (TNFα), blood leukocyte cell counts, or C-reactive protein (CRP) (19). The major limitation was that beetroot juice supplementation was restricted to the 3-day period after running the marathon race when most of the inflammation biomarkers were similar to pre-marathon levels.

Nearly all inflammation regulatory oxylipins generated in response to exercise stress come from PUFA substrates that are released from cell membranes and subsequently oxidized from CYP, LOX, and COX enzyme systems (20). Oxylipin production during recovery from prolonged and intensive cycling is sensitive to nutritional interventions including carbohydrate and blueberry supplementation (20–24). Underlying mechanisms are still being explored but may include the influence of glucose and polyphenol metabolites on CYP, LOX, and COX enzyme activity and the subsequent generation of specific oxylipin subgroups (21–24). Nitric oxide in cell culture and rodent-based studies inhibits P450 and LOX enzymes through multiple pathways, and can inhibit or stimulate COX enzymes depending on the physiological context (35, 36). Nitric oxide and oxylipins are viewed as interdependent signaling systems that help regulate inflammation, immune, and metabolic responses

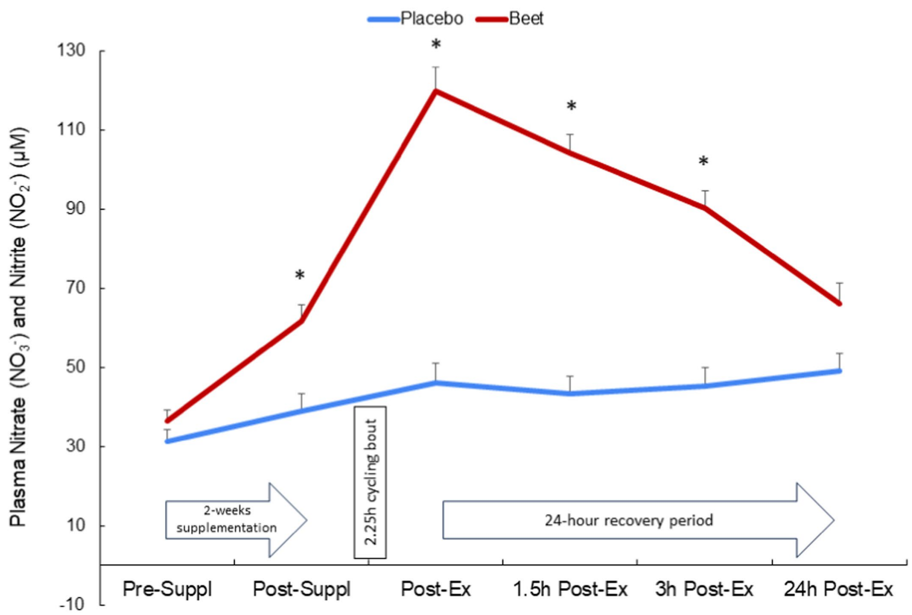

FIGURE 2
Change in the sum of plasma nitrate and nitrite after 2-weeks BEET and placebo supplementation and during 24 h recovery from 2.25 h cycling in $n = 20$ cyclists. Time and interaction effects, both $p < 0.001$.

TABLE 4 Trial comparisons across all time points for total plasma oxylipins and oxylipin subgroups.

Variable (ng/mL)	Trial	Pre-Suppl.	2-Wks Suppl.	0 h Post-Ex	1.5 h Post-Ex	3 h Post-Ex	24 h Post-Ex	*p*- value
Oxylipins, total (*n* = 41 oxylipins)	PL	21.3 ± 1.7	24.1 ± 2.9	67.3 ± 6.2	35.0 ± 3.3	32.5 ± 8.3	19.3 ± 1.2	<0.001; 0.166
	BEET	21.2 ± 1.7	20.5 ± 1.4	76.4 ± 11.6	37.7 ± 4.4	31.9 ± 8.7	20.3 ± 1.3	
DiHOMES (9,10 + 12,13)	PL	3.2 ± 0.5	3.8 ± 0.7	10.4 ± 1.0	3.9 ± 0.6	5.9 ± 2.7	2.5 ± 0.2	<0.001; 0.255
	BEET	3.1 ± 0.5	2.6 ± 0.3	11.6 ± 1.4	3.6 ± 0.6	6.6 ± 3.4	2.7 ± 0.3	
HODES (9 + 13)	PL	5.7 ± 0.9	7.6 ± 1.5	25.7 ± 2.6	8.9 ± 1.2	9.3 ± 4.9	4.7 ± 0.4	0.002; 0.094
	BEET	6.0 ± 1.0	5.2 ± 0.4	31.8 ± 6.3	9.1 ± 1.9	7.9 ± 4.4	5.0 ± 0.5	
ARA-CYP* (*n* = 7 oxylipins)	PL	4.5 ± 0.3	4.6 ± 0.3	8.4 ± 0.8	10.6 ± 1.0	8.8 ± 0.9	4.9 ± 0.3	<0.001; 0.163
	BEET	4.5 ± 0.4	4.7 ± 0.4	9.3 ± 1.0	13.6 ± 1.9	10.3 ± 1.4	4.9 ± 0.4	

p-values represent time (first value) and trial x time interaction effects. *ARA-CYP = 7 oxylipins generated from arachidonic acid and cytochrome P-450 (ARA-CYP) were grouped and these included 5,6-, 8,9-, 11,12-, and 14,15-dihydroxy-eicosatetraenoic acid (diHETrEs), 16-, 17-hydroxy-eicosatetraenoic acids (HETEs), and the 20-HETE metabolite 20-carboxy-arachidonic acid (20-coohAA).

(37). Human clinical trials focused on nitrate supplementation and inflammation regulation from nitric oxide and oxylipins, however, are lacking.

In our study, BEET supplementation had a modest effect in increasing post-exercise EPA-COX derived HEPE-18 and DHA-LOX derived 4-HDoHE but had no significant effects on the generation of pro-inflammatory linoleic acid (LA)-CYP, LA-LOX, or arachidonic (ARA)-CYP oxylipins. These results suggest that pro-inflammatory oxylipin generation after 2.25 h intensive cycling is relatively unaffected by increases in plasma $NO_3^- + NO_2^-$ with 2-weeks BEET intake. Whether or not higher and/or longer duration BEET or nitrate dosing may alter these results remains to be determined. In two previous studies, we showed that 14–18 days supplementation with 1 cup equivalent of blueberries had significant effects on post-exercise plasma oxylipin concentrations (21, 23). In one of these studies, blueberry ingestion was linked to a sustained elevation in DHA- and EPA-derived anti-inflammatory oxylipins including 18-HEPE in response to a 90-min bout of unaccustomed exercise by untrained adults (21). 18-HEPE is a precursor for resolvins that are specialized pro-resolving lipid mediators (SPM) with well-defined roles in inflammation resolution (38). 4-HDoHE is a beneficial 5-LOX oxidation product from DHA. Limited data indicate that this oxylipin in cell culture exerts anti-oxidative and anti-inflammatory effects on brain and blood vessel tissues through nuclear factor erythroid 2–related factor 2 (Nrf2) activation (39, 40).

Previous studies using untargeted proteomics methods indicate that acute prolonged and intensive exercise bouts have a widespread effect on plasma concentrations of proteins involved in immune system and inflammation responses (22, 25–27, 41, 42). The use of untargeted proteomics in studies with a sports nutrition focus is an emerging research design approach (22, 43). In a recent untargeted proteomics-based study, our research group

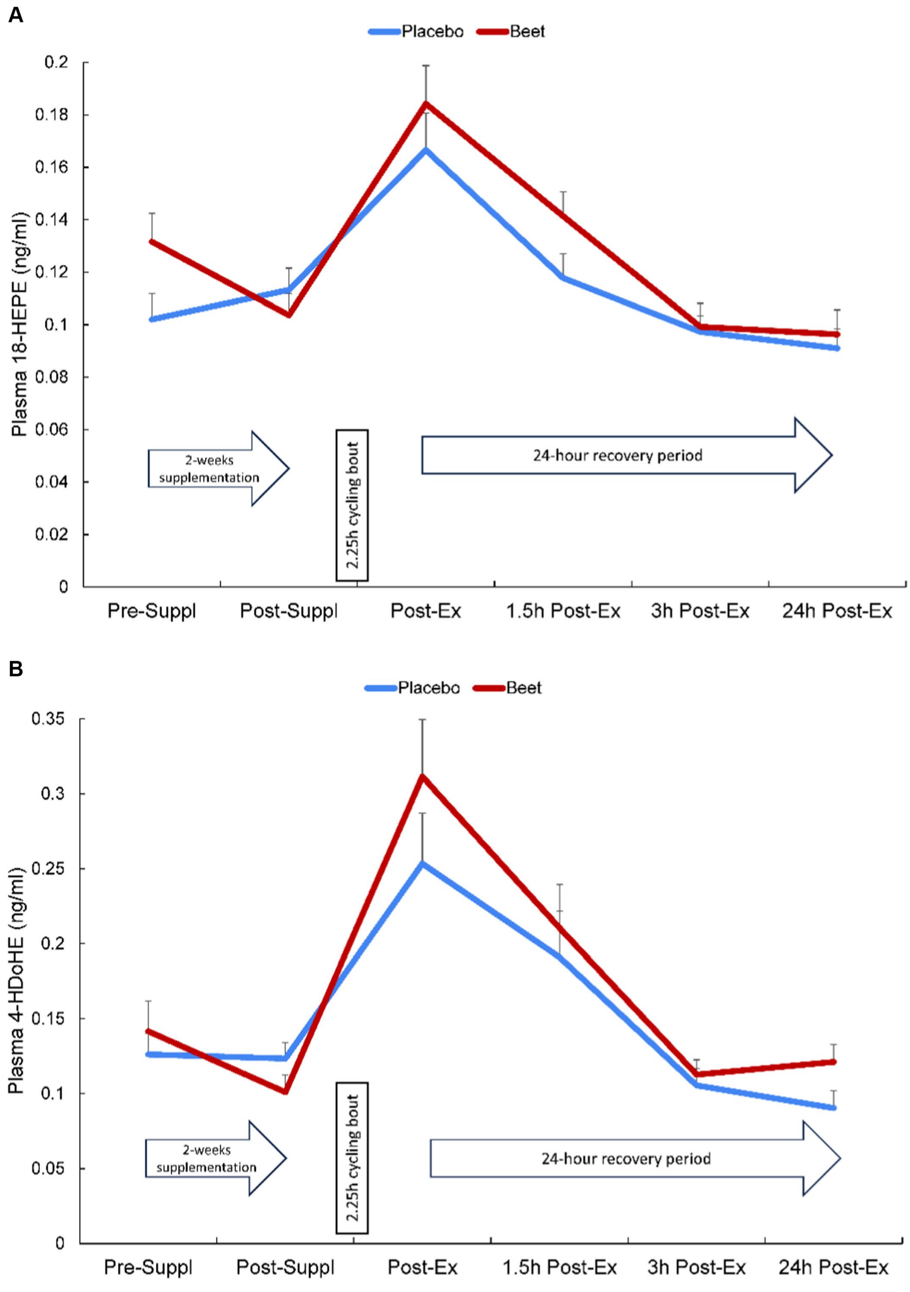

FIGURE 3
Change in **(A)** plasma HEPE-18 and **(B)** 4-HDoHE in BEET and placebo trials during 24 h recovery from 2.25 h cycling in $n = 20$ cyclists. Interaction effect, $p = 0.016$ and 0.010, respectively.

showed that 4-weeks supplementation with the keto-carotenoid astaxanthin (8 mg/day) countered post-exercise decreases in humoral immunity and plasma immunoglobulins in athletes engaging in a vigorous 2.25 h running bout (22). In the current study, untargeted proteomics revealed an extensive effect of BEET versus placebo supplementation on 66 proteins, with lower plasma levels for a cluster of 45 proteins and higher levels for a cluster of 21 proteins. Functional enrichment supported a significant reduction in exercise-induced complement activation and inflammatory and stimulus responses. Key inflammation-related proteins that were lower with BEET supplementation included complements C3, C5, and C9, properdin (a positive regulator of the alternate pathway of complement), ficolin-3 (innate immune activator through the lectin complement pathway), serum amyloids A1 and P (acute phase proteins), alpha-1-microglobulin (an inflammation biomarker with acute phase proteins), clusterin (diverse inflammation roles), fibulin-1 and fermitin family homolog 3 (both involved with cell adhesion, migration, and differentiation), hypoxia up-regulated protein 1 (heat shock protein), coronin-1C (plays an important role in immune cell motility and vesicle trafficking), peroxiredoxin-1 (anti-oxidant that can promote inflammation), peroxisome proliferator-activated receptor-delta (regulates cellular metabolic functions and can promote inflammation), reelin (pro-inflammatory and

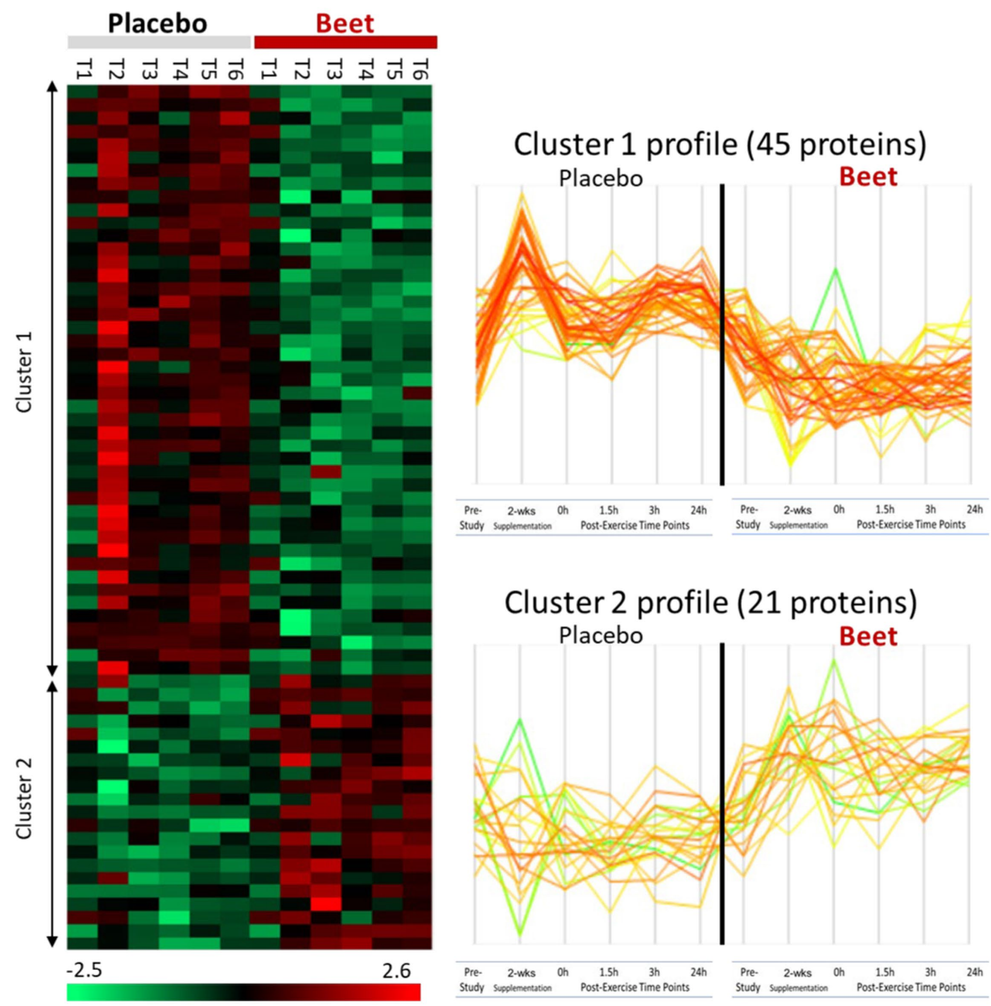

FIGURE 4
Heatmap and line graphs of clustered proteins in the BEET and placebo trials. T1, pre-study; T2, 2-weeks supplementation, pre-exercise; T3, immediately post-exercise (2.25 h cycling bout); T4, 1.5 h post-exercise; T5, 3 h post-exercise; T6, 24 h post-exercise.

pro-thrombotic factor), and dipeptidyl peptidase 4 membrane form (regulator of T-cell proliferation and natural killer cell kappa B activation). The liver is the source of most complement and acute phase proteins, and complement activation after prolonged and intensive exercise is an acute phase response to physiological stress (44). Nitric oxide is a pleiotropic and pervasive signaling molecule and influences inflammation in multiple ways including the suppression of immune cell growth, cytokine production, and platelet activation, and regulation of NF-κB activation (45).

Table 5 lists 14 immunoglobulin proteins that were lower with BEET versus placebo supplementation. Functional enrichment through STRING does not include immunoglobulins. Although human data are limited, emerging results from animal-based studies indicate an inverse relationship between nitric oxide and immunoglobulin production (46–48). Elevated nitric oxide may have multiple negative influences on B cell function. Nitric oxide generated from inflammatory monocytes, for example, can decrease B cell survival and hence antibody production (47). Vaccine adjuvants are being developed to counter the negative effect of nitric oxide on antibody responses (48). Our finding that BEET intake was associated with lower immunoglobulin proteins in cyclists during recovery from vigorous exercise was unexpected and needs to be confirmed in future studies and then evaluated for clinical significance.

BEET supplementation was linked to an increase in proteins involved with regulation of insulin like growth factors (IGFs) and symbiotic interactions. The IGF-axis mediates many of the physiological effects of growth hormone and is involved in the regulation of cell growth, proliferation, and survival (49). IGF binding protein 2 (IGFBP2) modifies IGF-1 functions and this protein was elevated with BEET supplementation. Nitric oxide mediates some of the effects of IGF-1 on vasodilation, increased blood flow, and lowered blood pressure (50). Other proteins with symbiotic interactions were elevated with BEET supplementation including neural cell adhesion molecule 1 (NCAM1 or CD56), heat shock cognate 71 kDa protein (HSPA8), and annexin A5. These proteins contribute to many biological processes including cell growth and differentiation, signaling, protein homeostasis, and apoptosis (51–53). Alpha-2-HS-glycoprotein or fetuin-A is a multifunctional protein, has a complex involvement with inflammation depending on the physiological context, and can function as an anti-inflammatory acute phase protein (54). Intercellular adhesion molecule 1 (ICAM-1) is an adhesion receptor and was also

TABLE 5 List of 45 proteins that were lower and 21 proteins that were higher with BEET compared to placebo supplementation.

Protein ID	Gene	Protein description
Lower in BEET trial		
Q9BXT5; Q9Y4L1	HYOU1	Hypoxia up-regulated protein 1
Q9ULV4	CORO1C	Coronin-1C
Q96KN2	CNDP1	Beta-Ala-His dipeptidase
Q86UX7	FERMT3	Fermitin family homolog 3
Q6UXB8	PI16	Peptidase inhibitor 16
Q06830	PRDX1	Peroxiredoxin-1
Q03181	PPARD	Peroxisome proliferator-activated receptor delta
P80748	IGLV3-21	Immunoglobulin lambda variable 3–21
P78509	RELN	Reelin
P52566	ARHGDIB	Rho GDP-dissociation inhibitor 2
P40197	GP5	Platelet glycoprotein V
P35916	FLT4	Vascular endothelial growth factor receptor 3
P30086	PEBP1	Phosphatidylethanolamine-binding protein 1
P27918	CFP	Properdin
P27487	DPP4	Dipeptidyl peptidase 4
P23142	FBLN1	Fibulin-1
P19021	PAM	Peptidyl-glycine alpha-amidating monooxygenase
P15531; P22392; O60361	NME1;NME2	Nucleoside diphosphate kinase A
P13727	PRG2	Bone marrow proteoglycan
P13647	KRT5	Keratin, type II cytoskeletal 5
P11597	CETP	Cholesteryl ester transfer protein
P10909	CLU	Clusterin
P10586	PTPRF	Receptor-type tyrosine-protein phosphatase F
P0DJI8; P0DJI9	SAA1	Serum amyloid A-1 protein
P08581	MET	Hepatocyte growth factor receptor
P06312	IGKV4-1	Immunoglobulin kappa variable 4–1
P05062	ALDOB	Fructose-bisphosphate aldolase B
P04406	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
P04075	ALDOA	Fructose-bisphosphate aldolase A
P02760	AMBP	Alpha-1-microglobulin
P02748	C9	Complement component C9
P02743	APCS	Serum amyloid P-component
P01857; P01859; P0DOX5	IGHG2	Immunoglobulin heavy constant gamma 2
P01857; P01860; P01861; P0DOX5	IGHG1	Immunoglobulin heavy constant gamma 1
P01834; P0DOX7	IGKC	Immunoglobulin kappa constant
P01766	IGHV3-13	Immunoglobulin heavy variable 3–13

(Continued)

TABLE 5 (Continued)

Protein ID	Gene	Protein description
P01742	IGHV1-69	Immunoglobulin heavy variable 1–69
P01714	IGLV3-19	Immunoglobulin lambda variable 3–19
O75636	FCN3	Ficolin-3
P0CG04; P0DOY2; P0DOY3; A0M8Q6; P0CF74; B9A064; P0DOX8	IGLL5	Immunoglobulin lambda-like polypeptide 5
P01599; A0A0C4DH72	IGKV1-6	Immunoglobulin kappa variable 1–6
P01814; A0A0C4DH43	IGHV2-70D	Immunoglobulin heavy variable 2-70D
A0A0B4J1X8	IGHV3-43	Immunoglobulin heavy variable 3–43
P01615; A0A075B6P5	IGKV2-28; IGKV2D-28	Immunoglobulin kappa variable 2–28
A0A0B4J1V2	IGHV2-26	Immunoglobulin heavy variable 2–26
Higher in BEET trial		
Q8NBJ4	GOLM1	Golgi membrane protein 1
Q7Z7M0	MEGF8	Multiple epidermal growth factor-like domains protein 8
Q7Z7G0	ABI3BP	Target of Nesh-SH3
Q15084	PDIA6	Protein disulfide-isomerase A6
Q13103	SPP2	Secreted phosphoprotein 24
Q05682	CALD1	Caldesmon
P54289	CACNA2D1	Voltage-dependent calcium channel subunit alpha-2/delta-1
P23528; Q9Y281	CFL1	Cofilin-1
P18065	IGFBP2	Insulin-like growth factor-binding protein 2
P16930	FAH	Fumarylacetoacetase
P13645	KRT10	Keratin, type I cytoskeletal 10
P13591	NCAM1	Neural cell adhesion molecule 1
P0DMV8; P0DMV9; P11142; P17066; P34931; P54652; P48741	HSPA8	Heat shock cognate 71 kDa protein
P08758	ANXA5	Annexin A5
P05362	ICAM1	Intercellular adhesion molecule 1
P04070	PROC	Vitamin K-dependent protein C
P02765	AHSG	Alpha-2-HS-glycoprotein
P02753	RBP4	Retinol-binding protein 4
P02656	APOC3	Apolipoprotein C-III
P01614; A0A087WW87	IGKV2-40; IGKV2D-40	Immunoglobulin kappa variable 2–40
O15031	PLXNB2	Plexin-B2

elevated with BEET supplementation. ICAM-1 regulates inflammatory responses by controlling leukocyte recruitment from the blood compartment to sites of inflammation and also is involved in epithelial injury-resolution responses (55).

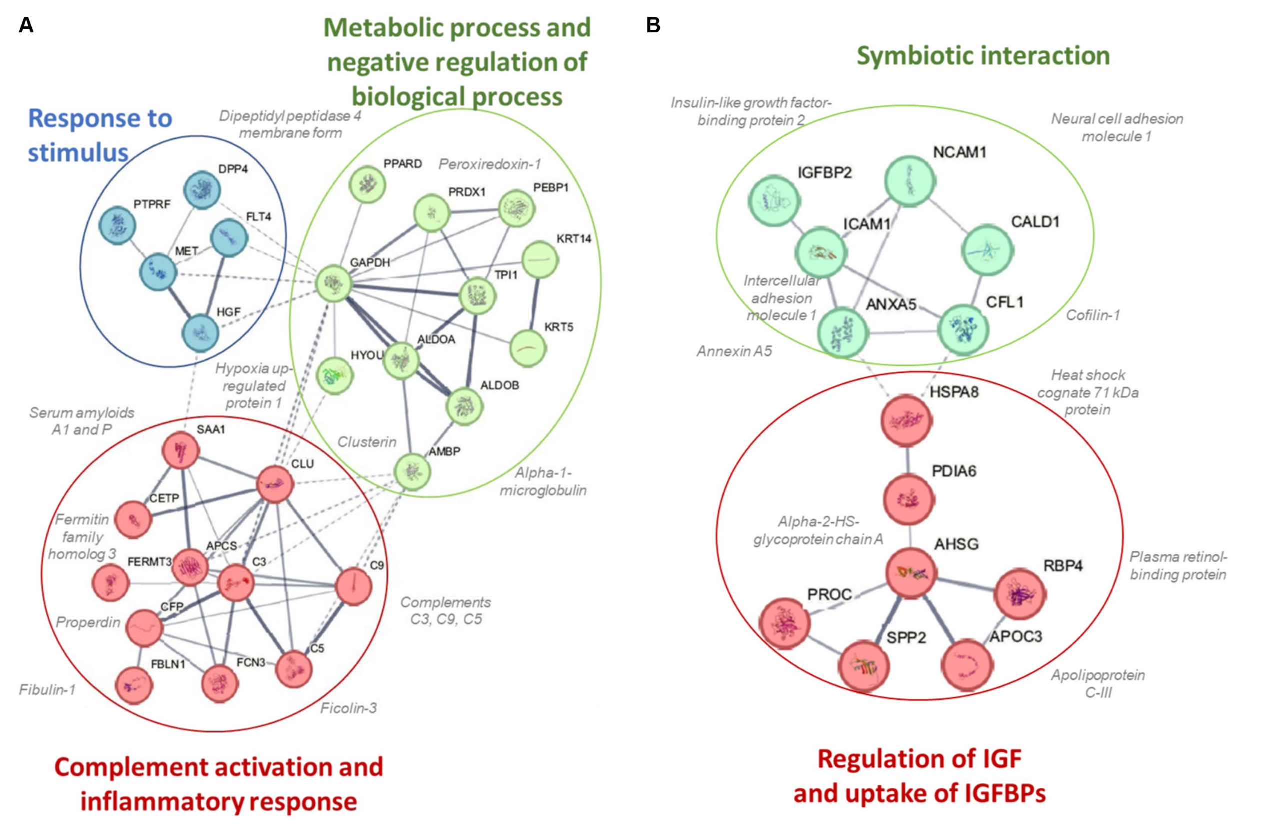

FIGURE 5
Protein–protein interaction networks and functional enrichments of **(A)** cluster 1 (45 proteins) that were lower and **(B)** cluster 2 (21 proteins) that were higher in the BEET compared to placebo trials.

The strength of this study was the combination of a strong research design and multi-omics outcomes to determine if exercise-induced inflammation could be moderated with BEET supplementation. There are several research design features and limitations that should be considered when interpreting study results. This study included non-elite male and female cyclists who were generally middle-aged and cycled for 2 h 15 min at about 75% VO_{2max}. Thus, the results of this study may not apply to younger elite endurance athletes who exercise at higher intensities. The research design included a 2-week supplementation period of BEET or placebo (two doses per day) with intake continued the day of the exercise challenge. Thus, the changes in protein biomarkers reported in this study could be related to both acute and chronic BEET intake. This research design element is consistent with the practice of many athletes who consume supplements both during training and on the day of competition. Plant-based supplements undergo a complex metabolic process involving the oral and gut microbiomes, metabolite formation from substrates, and subsequent biochemical effects on various enzyme systems including those related to inflammation. Our blood samples were collected in an overnight fasted state (pre- and post-supplementation). On the exercise challenge day, the blood collection was followed by the normal BEET dose and then the cycling bout. Thus, subjects had not consumed the BEET or placebo supplement since the prior day (midday) and most likely explains the low pre-exercise plasma $NO_3^- + NO_2^-$ levels. The primary purpose of this study was to investigate the effects of BEET supplementation on exercise-induced inflammation. For this reason, participants exercised in an overnight fasted state to avoid the interactive effects of carbohydrate intake on inflammation. The BEET supplement included a moderate amount of nitrate from beets and fermented beets with caffeine, vitamin C, and other food components that may minimize risks from N-nitroso compounds. Beetroot powder also contains other phytochemicals that may have bioactive effects. We cannot rule out the potential synergistic effects of these other ingredients with beetroot on inflammation.

Conclusion

This randomized crossover study used a 2.25 h cycling bout to induce inflammation. Using double blind, placebo-controlled methods, 2-weeks intake of a BEET-based supplement with a moderate amount of nitrate (212 mg/d) increased plasma levels of $NO_3^- + NO_2^-$. BEET supplementation did not counter exercise-induced increases in pro-inflammatory oxylipins but did increase post-exercise levels of two anti-inflammatory EPA- and DHA-derived oxylipins. The strongest effect of BEET supplementation was related to a significant decrease in a cluster of proteins involved in complement activation and inflammation. These data support that 2-weeks intake of a mixed beet-based supplement with morning and midday doses moderates protein biomarkers of exercise-induced inflammation in athletes. Carbohydrate and blueberry ingestion also mitigate post-exercise inflammation, an effect that requires more investigation for biological and clinical significance, but is generally interpreted as beneficial to the athlete over the long term (21, 23, 24, 29). This study did not focus on testing different formulations or dosing approaches. Future research can establish whether higher beet-nitrate intake with various types of adjuvants over a longer time period can amplify the anti-inflammatory effects, especially on pro-inflammatory oxylipins.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by Appalachian State University Institutional Review Board, NIH. 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

DN: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. CS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. JW: Formal analysis, Investigation, Methodology, Writing – review & editing. FM: Data curation, Formal analysis, Methodology, Writing – review & editing. PS: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. AO: Data curation, Formal analysis, Investigation, Writing – review & editing. QZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

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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

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References

1. Babateen AM, Fornelli G, Donini LM, Mathers JC, Siervo M. Assessment of dietary nitrate intake in humans: a systematic review. *Am J Clin Nutr*. (2018) 108:878–88. doi: 10.1093/ajcn/nqy108

2. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov*. (2008) 7:156–67. doi: 10.1038/nrd2466

3. Kapil V, Khambata RS, Jones DA, Rathod K, Primus C, Massimo G, et al. The noncanonical pathway for in vivo nitric oxide generation: the nitrate-nitrite-nitric oxide pathway. *Pharmacol Rev*. (2020) 72:692–766. doi: 10.1124/pr.120.019240

4. Raubenheimer K, Bondonno C, Blekkenhorst L, Wagner K-H, Peake JM, Neubauer O. Effects of dietary nitrate on inflammation and immune function, and implications for cardiovascular health. *Nutr Rev*. (2019) 77:584–99. doi: 10.1093/nutrit/nuz025

5. Lorenzo Calvo J, Alorda-Capo F, Pareja-Galeano H, Jiménez SL. Influence of nitrate supplementation on endurance cyclic sports performance: a systematic review. *Nutrients*. (2020) 12:1796. doi: 10.3390/nu12061796

6. McMahon NF, Leveritt MD, Pavey TG. The effect of dietary nitrate supplementation on endurance exercise performance in healthy adults: a systematic review and meta-analysis. *Sports Med*. (2017) 47:735–56. doi: 10.1007/s40279-016-0617-7

7. Senefeld JW, Wiggins CC, Regimbal RJ, Dominelli PB, Baker SE, Joyner MJ. Ergogenic effect of nitrate supplementation: a systematic review and meta-analysis. *Med Sci Sports Exerc*. (2020) 52:2250–61. doi: 10.1249/MSS.0000000000002363

8. Gamonales JM, Rojas-Valverde D, Muñoz-Jiménez J, Serrano-Moreno W, Ibáñez SJ. Effectiveness of nitrate intake on recovery from exercise-related fatigue: a systematic review. *Int J Environ Res Public Health*. (2022) 19:12021. doi: 10.3390/ijerph191912021

9. Hogwood AC, Anderson KC, Ortiz de Zevallos J, Paterson C, Weltman A, Allen JD. Limited effects of inorganic nitrate supplementation on exercise training responses: a systematic review and meta-analysis. *Sports Med Open*. (2023) 9:84. doi: 10.1186/s40798-023-00632-1

10. Berends JE, van den Berg LMM, Guggeis MA, Henckens NFT, Hossein IJ, de Joode MEJR, et al. Consumption of nitrate-rich beetroot juice with or without vitamin C supplementation increases the excretion of urinary nitrate, nitrite, and n-nitroso compounds in humans. *Int J Mol Sci*. (2019) 20:2277. doi: 10.3390/ijms20092277

11. Said Abasse K, Essien EE, Abbas M, Yu X, Xie W, Sun J, et al. Association between dietary nitrate, nitrite intake, and site-specific cancer risk: a systematic review and meta-analysis. *Nutrients*. (2022) 14:666. doi: 10.3390/nu14030666

12. Poortmans JR, Gualano B, Carpentier A. Nitrate supplementation and human exercise performance: too much of a good thing? *Curr Opin Clin Nutr Metab Care*. (2015) 18:1–604. doi: 10.1097/MCO.0000000000000222

13. Zamani H, de Joode MEJR, Hossein IJ, Henckens NFT, Guggeis MA, Berends JE, et al. The benefits and risks of beetroot juice consumption: a systematic review. *Crit Rev Food Sci Nutr*. (2021) 61:788–804. doi: 10.1080/10408398.2020.1746629

14. Inchem. Nitrate (JECFA food additives series 50). (2003). Available at: https://inchem.org/documents/jecfa/jecmono/v50je06.htm (Accessed March 25, 2024).

15. Pinaffi-Langley ACDC, Dajani RM, Prater MC, HVM N, Vrancken K, Hays FA, et al. Dietary nitrate from plant foods: a conditionally essential nutrient for cardiovascular health. *Adv Nutr*. (2024) 15:100158. doi: 10.1016/j.advnut.2023.100158

16. Ferrada-Contreras E, Bonomini-Gnutzmann R, Jorquera-Aguilera C, MacmiIlan Kuthe N, Peña-Jorquera H, Rodríguez-Rodríguez F. Does co-supplementation with beetroot juice and other nutritional supplements positively impact sports performance?: a systematic review. *Nutrients*. (2023) 15:4838. doi: 10.3390/nu15224838

17. Jones L, Bailey SJ, Rowland SN, Alsharif N, Shannon OM, Clifford T. The effect of nitrate-rich beetroot juice on markers of exercise-induced muscle damage: a systematic review and meta-analysis of human intervention trials. *J Diet Suppl*. (2022) 19:749–71. doi: 10.1080/19390211.2021.1939472

18. Rojano-Ortega D, Peña Amaro J, Berral-Aguilar AJ, Berral-de la Rosa FJ. Effects of beetroot supplementation on recovery after exercise-induced muscle damage: a systematic review. Sports. *Health*. (2022) 14:556–65. doi: 10.1177/19417381211036412

19. Clifford T, Allerton DM, Brown MA, Harper L, Horsburgh S, Keane KM, et al. Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Appl Physiol Nutr Metab*. (2017) 42:263–70. doi: 10.1139/apnm-2016-0525

20. Signini ÉF, Nieman DC, Silva CD, Sakaguchi CA, Catai AM. Oxylipin response to acute and chronic exercise: a systematic review. *Meta*. (2020) 10:E264. doi: 10.3390/metabo10060264

21. Nieman DC, Sakaguchi CA, Omar AM, Davis KL, Shaffner CE, Strauch RC, et al. Blueberry intake elevates post-exercise anti-inflammatory oxylipins: a randomized trial. *Sci Rep*. (2023) 13:11976. doi: 10.1038/s41598-023-39269-1

22. Nieman DC, Woo J, Sakaguchi CA, Omar AM, Tang Y, Davis K, et al. Astaxanthin supplementation counters exercise-induced decreases in immune-related plasma proteins. *Front Nutr*. (2023) 10:1143385. doi: 10.3389/fnut.2023.1143385

23. Nieman DC, Gillitt ND, Chen G-Y, Zhang Q, Sha W, Kay CD, et al. Blueberry and/or banana consumption mitigate arachidonic, cytochrome P450 oxylipin generation during recovery from 75-km cycling: a randomized trial. *Front Nutr*. (2020) 7:121. doi: 10.3389/fnut.2020.00121

24. Nieman DC, Gillitt ND, Chen G-Y, Zhang Q, Sakaguchi CA, Stephan EH. Carbohydrate intake attenuates post-exercise plasma levels of cytochrome P450-generated oxylipins. *PLoS One*. (2019) 14:e0213676. doi: 10.1371/journal.pone.0213676

25. Nieman DC, Groen AJ, Pugachev A, Vacca G. Detection of functional overreaching in endurance athletes using proteomics. *Proteomes*. (2018) 6:33. doi: 10.3390/proteomes6030033

26. Nieman DC, Groen AJ, Pugachev A, Simonson AJ, Polley K, James K, et al. Proteomics-based detection of immune dysfunction in an elite adventure athlete trekking across the Antarctica. *Proteomes*. (2020) 8:4. doi: 10.3390/proteomes8010004

27. Merritt EK, Nieman DC, Toone BR, Groen A, Pugachev A. Proteomic markers of non-functional overreaching during the race across America (RAAM): a case study. *Front Physiol*. (2019) 10:1410. doi: 10.3389/fphys.2019.01410

28. Nieman DC, Sakaguchi CA, Pelleigrini M, Thompson MJ, Sumner S, Zhang Q. Healthy lifestyle linked to innate immunity and lipoprotein metabolism: a cross-sectional comparison using untargeted proteomics. *Sci Rep*. (2023) 13:16728. doi: 10.1038/s41598-023-44068-9

29. Nieman DC, Lila MA, Gillitt ND. Immunometabolism: a multi-omics approach to interpreting the influence of exercise and diet on the immune system. *Annu Rev Food Sci Technol*. (2019) 10:341–63. doi: 10.1146/annurev-food-032818-121316

30. Smith LL, Brunetz MH, Chenier TC, McCammon MR, Houmard JA, Franklin ME, et al. The effects of static and ballistic stretching on delayed onset muscle soreness and creatine kinase. *Res Q Exerc Sport*. (1993) 64:103–7. doi: 10.1080/02701367.1993.10608784

31. Chen G-Y, Zhang Q. Comprehensive analysis of oxylipins in human plasma using reversed-phase liquid chromatography-triple quadrupole mass spectrometry with heatmap-assisted selection of transitions. *Anal Bioanal Chem*. (2019) 411:367–85. doi: 10.1007/s00216-018-1446-3

32. Woo J, Zhang Q. A streamlined high-throughput plasma proteomics platform for clinical proteomics with improved proteome coverage, reproducibility, and robustness. *J Am Soc Mass Spectrom*. (2023) 34:754–62. doi: 10.1021/jasms.3c00022

33. Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat Methods*. (2020) 17:41–4. doi: 10.1038/s41592-019-0638-x

34. Geyer PE, Arend FM, Doll S, Louiset M-L, Virreira Winter S, Müller-Reif JB, et al. High-resolution serum proteome trajectories in COVID-19 reveal patient-specific seroconversion. *EMBO Mol Med*. (2021) 13:e14167. doi: 10.15252/emmm.202114167

35. Morgan ET, Skubic C, Lee C-M, Cokan KB, Rozman D. Regulation of cytochrome P450 enzyme activity and expression by nitric oxide in the context of inflammatory disease. *Drug Metab Rev*. (2020) 52:455–71. doi: 10.1080/03602532.2020.1817061

36. Salvemini D, Kim SF, Mollace V. Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications. *Am J Physiol Regul Integr Comp Physiol*. (2013) 304:R473–87. doi: 10.1152/ajpregu.00355.2012

37. Baker PRS, Schopfer FJ, O'Donnell VB, Freeman BA. Convergence of nitric oxide and lipid signaling: anti-inflammatory nitro-fatty acids. *Free Radic Biol Med*. (2009) 46:989–1003. doi: 10.1016/j.freeradbiomed.2008.11.021

38. Weylandt KH, Chiu C-Y, Gomolka B, Waechter SF, Wiedenmann B. Omega-3 fatty acids and their lipid mediators: towards an understanding of resolvin and protectin formation. *Prostaglandins Other Lipid Mediat*. (2012) 97:73–82. doi: 10.1016/j.prostaglandins.2012.01.005

39. Ishikado A, Morino K, Nishio Y, Nakagawa F, Mukose A, Sono Y, et al. 4-Hydroxy hexenal derived from docosahexaenoic acid protects endothelial cells via Nrf2 activation. *PLoS One*. (2013) 8:e69415. doi: 10.1371/journal.pone.0069415

40. Yang B, Li R, Michael Greenlief C, Fritsche KL, Gu Z, Cui J, et al. Unveiling anti-oxidative and anti-inflammatory effects of docosahexaenoic acid and its lipid peroxidation product on lipopolysaccharide-stimulated BV-2 microglial cells. *J Neuroinflammation*. (2018) 15:202. doi: 10.1186/s12974-018-1232-3

41. Dalle Carbonare L, Manfredi M, Caviglia G, Conte E, Robotti E, Marengo E, et al. Can half-marathon affect overall health? The yin-yang of sport. *J Proteomics*. (2018) 170:80–7. doi: 10.1016/j.jprot.2017.09.004

42. Balfoussia E, Skenderi K, Tsironi M, Anagnostopoulos AK, Parthimos N, Vougas K, et al. A proteomic study of plasma protein changes under extreme physical stress. *J Proteome*. (2014) 98:1–14. doi: 10.1016/j.jprot.2013.12.004

43. Nieman DC. Multiomics approach to precision sports nutrition: limits, challenges, and possibilities. *Front Nutr*. (2021) 8:796360. doi: 10.3389/fnut.2021.796360

44. Rothschild-Rodriguez D, Causer AJ, Brown FF, Collier-Bain HD, Moore S, Murray J, et al. The effects of exercise on complement system proteins in humans: a systematic scoping review. *Exerc Immunol Rev*. (2022) 28:1–35.

45. Xiao S, Yuan Z, Huang Y. The potential role of nitric oxide as a therapeutic agent against SARS-CoV-2 infection. *Int J Mol Sci*. (2023) 24:17162. doi: 10.3390/ijms242417162

46. Darling RJ, Senapati S, Kelly SM, Kohut ML, Narasimhan B, Wannemuehler MJ. STING pathway stimulation results in a differentially activated innate immune phenotype associated with low nitric oxide and enhanced antibody titers in young and aged mice. *Vaccine*. (2019) 37:2721–30. doi: 10.1016/j.vaccine.2019.04.004

47. Sala E, Kuka M. The suppressive attitude of inflammatory monocytes in antiviral antibody responses. *Viral Immunol*. (2020) 33:327–33. doi: 10.1089/vim.2019.0132

48. Senapati S, Darling RJ, Loh D, Schneider IC, Wannemuehler MJ, Narasimhan B, et al. Pentablock copolymer micelle nanoadjuvants enhance cytosolic delivery of antigen and improve vaccine efficacy while inducing low inflammation. *ACS Biomater Sci Eng*. (2019) 5:1332–42. doi: 10.1021/acsbiomaterials.8b01591

49. Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GYF, Kaplan RC, Muzumdar R, et al. The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. *Diabetes Metab Res Rev*. (2009) 25:3–12. doi: 10.1002/dmrr.919

50. Muniyappa R, Walsh MF, Rangi JS, Zayas RM, Standley PR, Ram JL, et al. Insulin like growth factor 1 increases vascular smooth muscle nitric oxide production. *Life Sci*. (1997) 61:925–31. doi: 10.1016/s0024-3205(97)00594-8

51. Rutishauser U, Acheson A, Hall AK, Mann DM, Sunshine J. The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. *Science*. (1988) 240:53–7. doi: 10.1126/science.3281256

52. Dores-Silva PR, Cauvi DM, Coto ALS, Silva NSM, Borges JC, De Maio A. Human heat shock cognate protein (HSC70/HSPA8) interacts with negatively charged phospholipids by a different mechanism than other HSP70s and brings HSP90 into membranes. *Cell Stress Chaperones*. (2021) 26:671–84. doi: 10.1007/s12192-021-01210-8

53. Jing J. The relevance, predictability, and utility of annexin A5 for human physiopathology. *Int J Mol Sci*. (2024) 25:2865. doi: 10.3390/ijms25052865

54. Dogru T, Kirik A, Gurel H, Rizvi AA, Rizzo M, Sonmez A. The evolving role of fetuin-a in nonalcoholic fatty liver disease: an overview from liver to the heart. *Int J Mol Sci*. (2021) 22:6627. doi: 10.3390/ijms22126627

55. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: a master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. (2020) 108:787–99. doi: 10.1002/JLB.2MR0220-549R

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Can molecular hydrogen supplementation enhance physical performance in healthy adults? A systematic review and meta-analysis

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Background: Physical exertion during exercise often leads to increased oxidative stress and inflammatory responses, significantly affecting physical performance. Current strategies to mitigate these effects are limited by their effectiveness and potential side effects. Molecular hydrogen (H_2) has gained attention for its antioxidant and anti-inflammatory properties. Studies have suggested that H_2 supplementation contributes to antioxidant potential and anti-fatigue during exercise, but the variance in the observations and study protocols is presented across those studies.

Objective: This systematic review and meta-analysis aimed to comprehensively characterize the effects of H_2 supplementation on physical performance (i.e., endurance, muscular strength, and explosive power), providing knowledge that can inform strategies using H_2 for enhancing physical performance.

Methods: We conducted a literature search of six databases (PubMed, Web of Science, Medline, Sport-Discus, Embase, and PsycINFO) according to the PRISMA guidelines. The data were extracted from the included studies and converted into the standardized mean difference (SMD). After that, we performed random-effects meta-analyses and used the I^2 statistic to evaluate heterogeneity. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) was used to assess the quality of the evidence obtained from this meta-analysis.

Results: In total, 27 publications consisting of 597 participants were included. The search finally included aerobic endurance, anaerobic endurance, muscular strength, lower limb explosive power, rating of perceived exertion (RPE), blood lactate (BLA), and average heart rate (HR_{avg}) in the effect size (ES) synthesis. The ES of H_2 on aerobic endurance, including VO_{2max} (SMD = 0.09, $p = 0.394$; $I^2 = 0\%$) and aerobic endurance exercise (SMD = 0.04, $p = 0.687$; $I^2 = 0\%$), were not significant and trivial; the ES of H_2 on 30 s maximal anaerobic endurance (SMD = 0.19, $p = 0.239$; $I^2 = 0\%$) was not significant and trivial; the ES of H_2 on muscular strength (SMD = 0.19, $p = 0.265$; $I^2 = 0\%$) was not significant and trivial; but the ES of H_2 on lower limb explosive power (SMD = 0.30, $p = 0.018$; $I^2 = 0\%$) was significant and small. In addition, H_2 reduces RPE (SMD = −0.37, $p = 0.009$;

I^2 = 58.0%) and BLA (SMD = −0.37, p = 0.001; I^2 = 22.0%) during exercise, but not HR_{avg} (SMD = −0.27, p = 0.094; I^2 = 0%).

Conclusion: These findings suggest that H_2 supplementation is favorable in healthy adults to improve lower limb explosive power, alleviate fatigue, and boost BLA clearance, but may not be effectively improving aerobic and anaerobic endurance and muscular strength. Future studies with more rigorous designs are thus needed to examine and confirm the effects of H_2 on these important functionalities in humans.

Systematic review registration: http://www.crd.york.ac.uk/PROSPERO.

KEYWORDS

molecular hydrogen, physical performance, aerobic endurance, maximum oxygen uptake, maximal anaerobic test, muscle strength, countermovement jump

1 Introduction

Physical performance, including endurance, muscle strength, and explosive power, is the cornerstone of achievement in sports for non-athletic populations or athletes (1, 2). It not only contributes to improving athletes' competitive performance on the field but also provides motivation for healthy adults to participate in sports (3–5). Oxidative stress occurs when the oxygen metabolism is produced and accumulated, eventually going beyond oxidation-resist ability (6, 7). Studies have shown that physical activity of various intensities alters the levels of various oxidative biomarkers (8, 9). However, physical exercise, especially with moderate- to high-intensity exertion, could lead to excessive oxidative stress, which may negatively impact redox homeostasis, worsen fatigue, and ultimately reduce physical performance (10–13). Therefore, efforts have been put into exploring potential antioxidant approaches, which can thus help develop appropriate strategies to enhance physical performance (13–15).

Molecular hydrogen (H_2) is a promising antioxidant that selectively reduces hydroxyl radicals (·OH) and peroxynitrite (ONOO-) in cells without leading to a reduction of other reactive substances such as superoxide (O_2-), hydrogen peroxide (H_2O_2), and nitric oxide (NO) (16–18). Studies have shown that H_2 molecular, which can be delivered via different forms (i.e., H_2 gas and water, and intravenous H_2-saline), can penetrate cell membranes and diffuse rapidly into organelles (e.g., mitochondria) (19), thus enhancing mitochondria functional performance (e.g., respiration and enzyme activity) and promoting ATP production or lactate oxidation (20, 21). More recently, human studies have emerged to explore the potential benefits of using H_2 for physical performance in healthy adults and showed great promise of the H_2-based intervention to improve physical performance (22–25). However, the observations and protocol design across these studies on the effects of H_2 on physical performance were inconsistent. For example, some studies have observed that H_2-rich water (HRW) supplementation before exercise could effectively increase maximal oxygen uptake (VO_{2max}), anaerobic endurance, muscle strength, and lower limb explosive power in healthy adults (26–28), but other studies have shown contradictory findings (29–31). These inconsistencies may arise from the variance in participant characteristics, the protocol of H_2 administration, and types of exercise across studies. Only one previous review by Kawamura et al. (32) summarized the observations from only six studies and suggested that the validity of the observations from that literature should be examined and confirmed due to the very small number of included studies. Since then, many new studies have been performed to examine the effects of H_2 on endurance, muscle strength, and explosive power (24–26, 33, 34). Therefore, it is urgently demanded to more comprehensively characterize and explicitly examine the effects of H_2 on physical performance in healthy adults by summarizing the results of the most up-to-date publications.

We have thus conducted a systematic review and meta-analysis based on the available peer-reviewed publications. Only studies with randomized controlled or crossover designs are included, and several subgroup analyses are performed with the goal of providing critical knowledge of the appropriate design of H_2-based intervention design for the improvement of physical performance.

2 Methods

This systematic review and meta-analysis were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guideline (35) and registered with PROSPERO (ID CRD42022351559).

2.1 Data sources and search strategies

Two authors (K.Z. and Z.S.) independently searched PubMed, Web of Science, Medline, Sport-Discus, Embase, and PsycINFO databases from inception to 10 May 2024. The keywords of the search were as follows: "molecular hydrogen," "hydrogen rich water," "hydrogen-rich water," "hydrogen rich saline," "hydrogen-rich saline," "hydrogen gas," "hydrogen inhalation," "hydrogen bathing," "hydrogen-rich calcium powder," "physical performance," "athletic performance," "exercise performance," "physical exercise," "aerobic performance," "aerobic capacity," "anaerobic performance," "intermittent exercise," "sprint," "strength training," and "resistance training" (The detailed search strategy is shown in Supplementary Table S1). In addition, a manual search was performed based on the reference lists of selected articles. The search was limited to English only, and no date restrictions were applied.

2.2 Selection criteria

To be included in this systematic review, previous studies must meet the following eligibility criteria in accordance with PICOS.

1. Participants: the participants were healthy adults with a mean age of ≥18 years and were free from any dietary supplements or medications while the experiment lasted;
2. Intervention: the intervention was the supplementation of H_2 by the participants. The source of H_2 was not limited;
3. Comparator/Control: the control group used placebos that were identical in appearance, texture, and flavor to H_2 products (e.g., drinking water, air, and capsules);
4. Outcomes: the outcomes include at least one of the measures related to physical performance (e.g., aerobic and anaerobic endurance, muscular strength, lower limb explosive power, subjective fatigue, blood lactate (BLA), and heart rate);
5. Study design: the design of the study was a randomized crossover or randomized controlled trial.

Articles were excluded if they fulfilled the following criteria: 1) animal trials; 2) written in a language other than English or unable to obtain outcome data; 3) review papers and conference articles; and 4) repeated publications.

2.3 Data extraction and outcomes

According to the Cochrane Collaboration Handbook, the data extraction process was conducted independently by two authors (C.Y. and Z.S.) (36). The extracted information from the publications included the following: the study (authors and year), sample size, participants (age, height, weight, sex, and training status), methods of H_2 administration, exercise protocol, and outcome measures. Any outcome measures on which the two authors disagreed were discussed with the other two authors (J.Z. and D.B.) until a consensus was achieved.

The mean and standard deviation of each outcome in post-tests were extracted for each included study. If the post-test values were not available, they were calculated using the following formulas, where the correlation coefficient (Corr) was set at 0.5 (36, 37).

$$\text{Meanpost} = \text{Meanpre} + \text{Meanchange}$$

$$\text{SDpost} = \frac{2 \times \text{Corr} \times \text{SDpre} + \sqrt{4 \times \text{Corr2} \times \text{SDpre2} - 4 \times (\text{SDpre2} - \text{SDchange2})}}{2}$$

If relevant data were missing, we emailed the corresponding author or other authors to request it (36). We extracted relevant data using WebPlotDigitizer (version 4.6) for studies when the data could not be obtained by contacting the authors (38).

Based on the included studies, aerobic endurance, anaerobic endurance, muscular strength, and lower limb explosive power performance were ultimately incorporated into the data synthesis.

The primary outcome of aerobic endurance performance was maximum oxygen uptake (VO_{2max}) during an incremental load exercise test or peak oxygen uptake (VO_{2peak}) when VO_{2max} was not available (39, 40). The secondary outcome of aerobic endurance performance was aerobic endurance exercise performance, for example, time-to-exhaustion (TTE) or power during incremental load exercise test or fixed-load submaximal test; the time or speed in time trial test (TT).

The primary outcome of anaerobic endurance performance was power output during the 30 s maximal anaerobic test.

The primary outcome of muscle strength was peak torque or force in the maximal voluntary isometric strength test (MVIS) or maximal isokinetic strength test performed pre- or post-high-intensity exercise.

The primary outcome of lower limb explosive power was countermovement jump (CMJ) height, time of short sprint, or peak power output during 10 s maximal effort exercises.

The exploratory outcomes were the rating of perceived exertion (RPE), BLA, and average heart rate (HR_{avg}) during physical performance. The RPE, BLA, and HR_{avg} are widely used and are important metrics to characterize subjective fatigue, intensity, and physiologic stress that are closely associated with physical performance (41–43). By exploring the effects of H_2 on them, it will help more comprehensively characterize the effects of H_2 supplementation on physical performance.

2.4 Quality assessment

Two authors independently evaluated the risk of bias in included studies using the Cochrane Collaboration's tool (44), which contains six items: 1) selection bias; 2) performance bias; 3) detection bias; 4) attrition bias; 5) reporting bias; and 6) other bias. Each item is categorized into three levels: low-risk bias (green), unclear risk bias (yellow), and high-risk bias (red). Studies were defined as having a high-risk bias if ≥1 item had a high-risk bias. The risk of bias is low if all items are assessed as low risk of bias. Others were assessed as moderate risk of bias. Additionally, the quality of evidence for outcomes was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (45, 46). The quality of the GRADE evidence was graded as high, moderate, low, and very low based on the quality of study design, quality of implementation, uncertainty of results, and consistency of results (45).

2.5 Statistical analysis

Standardized mean difference (SMD) with 95% confidence interval (CI) was used to assess the effect size (ES). ES was classified as trivial (< 0.2), small (0.2 ~ 0.49), moderate (0.5 ~ 0.79), or large (> 0.8) (47). Meta-analysis was performed in Stata v15.1 (STATA Corp., College Station, TX) using the inverse-variance method. The I^2 statistic was used to evaluate heterogeneity among the trials with the following criteria: trivial (< 25%), low (25 ~ 50%), moderate (50 ~ 75%), and high (> 75%) (48). A random-effects model was used to estimate pooled effects, as heterogeneity was anticipated across studies due to differences in participants and interventions. Subgroup analysis was used to explore potential sources of heterogeneity (49).

The Funnel plots and Egger tests were used to evaluate publication bias. If potential publication bias was detected, we used the trim and fill method for the sensitivity analysis of the results (50). All the statistical significance was set at a p-value of <0.05.

3 Results

3.1 Study selection

The screening procedure of the included studies is shown in Figure 1. A total of 401 potentially relevant publications were retrieved (PubMed $n=77$, SPORT-Discus $n=65$, Medline $n=71$, Web of Science $n=89$, PsycINFO $n=5$, and Embase = 94). Based on the criteria above, 248 publications were discharged after reviewing the titles and abstracts. After evaluating the full texts, 27 publications (29 studies) were included in the systematic review. Finally, 25 publications consisting of 27 studies (23 randomized crossover designs and 4 randomized controlled trials) were included in the quantitative synthesis (Table 1). One study (28) included two randomized controlled trials, and the other study (23) included a randomized crossover trial and a randomized controlled trial.

3.2 Characteristics of included studies

3.2.1 Participant characteristics

A total of 597 participants, with mean ages ranging from 17.5 to 51.5 years, were included. The training status of these participants was classified according to the included studies as untrained ($n=224$) and trained ($n=373$), with 215 of them being well-trained athletes (e.g., professional soccer players and elite runners).

3.2.2 Methods of H_2 administration

A gold standard regimen for H_2 application does not appear to exist. The included studies implemented four sources of H_2, that is, drinking HRW ($n=18$) (22–26, 28–30, 33, 34, 51–53, 55, 56, 58, 63, 65), HRW bathing ($n=2$) (54, 59), inhalation of H_2-rich gas (HRG) ($n=5$) (27, 57, 60, 62, 64), and oral ingestion of H_2-rich calcium (HRC) powder ($n=2$) (31, 61). H_2 concentrations were found to

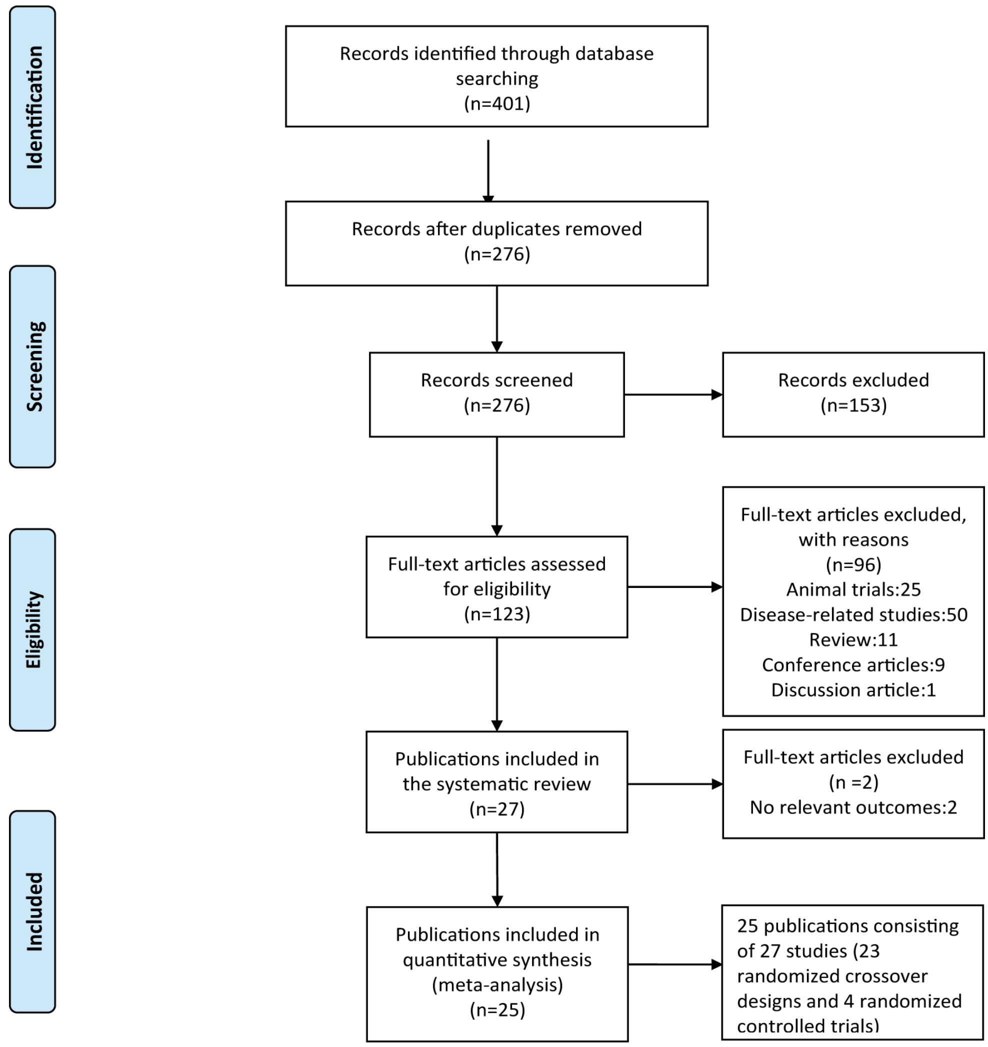

FIGURE 1
Study flowchart.

TABLE 1 Characteristics of the included studies (*n* = 29).

Study	Design	Sample size	Age (yr)	%F	Training status	Methods of H_2 administration	Exercise protocol	Outcome measures
Aoki et al. (51)	RCD	10	20.9 ± 1.3	0	Elite soccer players	HRW (H_2 conc.:0.92 ~ 1.02 ppm) Three 500 mL doses before exercise	Cycling for 30 min at 75% VO_{2max} and maximal isokinetic knee extensions test	Peak torque→; BLA↓; d-ROMs→; BAP→; CK→; MF→; MPF→
Ostojic et al. (52)	RCT	H:26 P:26	25.1 ± 3.4 23.8 ± 4.5	0	Male athletes	HRW (pH:9.3; ORP: −372 mV; EC:12.0 ms/m; DO:6.0 mg/L) 2 L per day for 2 weeks	15 min incremental treadmill running (start at 8 km/h and increase by 2 km/h every 3 min).	Blood pH→; Partial pressure for carbon dioxide→; Serum bicarbonates↑
Drid et al. (53)	RCD	8	21.4 ± 2.2	100	Judo athletes	HRW[#] 300 mL within 30 min before exercise	Special judo fitness test	Fitness performance index→; BLA↓; Blood pH→; Bicarbonate↓; HR_{max}→; $HR_{recovery}$→
Kawamura et al. (54)	RCD	9	25.0 ± 3.0	0	Healthy and active young men	HRW bathing for 20 min before exercise	Downhill running (8% decline at 75%VO_2peak for 30 min)	Running speed→; VO_2→; %VO_{2peak}→, HR_{avg}→; BLA→; VAS→; CK→; Myoglobin; Malondialdehyde→; d-ROMs→; BAP↓; Myeloperoxidase→; Interleukin-6→
Da Ponte et al. (55)	RCD	8	41 ± 7	0	Well-trained cyclists	HRW[#] (pH:9.8; ORP: −180 mV; FH:450 ppb; TDS:180 mg/L) 2 L per day for 2 weeks before exercise	30 min intermittent(10x3min) cycling	Pm for 30min[a]→; BLA→; Fatigue index→; RPE→; VO_2→; RER→; HR_{avg}→; Blood pH→; Bicarbonate [HCO_3-] →; Base excess→; pO_2→; pCO_2→; Hemoglobin→; Hemoglobin Sat→; Glucose→
LeBaron et al. (22)	RCD	19	25.0 ± 8.9	21	Untrained healthy participants	HRW[#] (TDS:13.1 mg/L) 500 mL intake the day before and on the day of exercise	Incremental treadmill running test to exhaustion	VO_{2peak}→; HR_{avg}↓; RER→; RR→
Botek et al. (56)	RCD	12	27.1 ± 4.9	0	Recreationally trained sports science students	HRW (pH:7.4; ORP: -400 mV; Temp: 22°C; H_2 conc.:0.5 ppm) 600 mL within 30 min before exercise	Incremental cycling test to exhaustion	BLA↓; RPE↓; VE→; VO2→; VE/VO_2↑; HR_{max}→; RQ→
Javorac et al. (57)	RCD	20	22.9 ± 1.5	50	Untrained physically active participants	HRG[#] (%4 H_2) 20 min once-per-day inhalation for 7 days	Maximal voluntary isometric strength of leg, YMCA bench press test, and incremental treadmill running test to exhaustion	TTE→; Maximum running speed↑; VO_{2max}→; MVIS→; YMCA endurance→; Resting BLA→; Blood pressure→; Resting HR→; MRS↑; Insulin→; Ghrelin→; IGF-1↑; CK→; Myoglobin→; C-reactive protein↑; Ferritin↑; ESR→
Ooi et al. (29)	RCD	14	34 ± 4	0	Well-trained runners/triathletes	HRW (H_2 conc.: 2.60 ppm) 2 doses of 290 mL within 5 ~ 10 min before exercise	Incremental treadmill running test to exhaustion	TTE→; Speed at OBLA→; VO_{2max}→; BLA→; RPE→; HR_{max}→; RE→; VE_{max}→; RER→; Blood Glucose→; Blood HCO3-→; Blood pH→

(Continued)

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TABLE 1 (Continued)

Study	Design	Sample size	Age (yr)	%F	Training status	Methods of H_2 administration	Exercise protocol	Outcome measures
Mikami et al. (Exp.1) (28)	RCT	H:52	51.2 ± 6.9	55.8	Untrained physically active participants	HRW (H_2 conc.:0.8 ppm) 500 mL within 30 min before exercise	Incremental cycling test to 75% HRmax	VO_{2max}→; Resting RPE↓; VAS↓; HR_{avg} [a]↓
		P:47	51.5 ± 7.9	57.4				
Mikami et al. (Exp.2) (28)	RCT	H:30	43.6 ± 13.3	50	Fitness trainers	HRW (H_2 conc.:1.0 ppm) 500 mL within 10 min before exercise	Incremental cycling test to HRmax	VO_{2max}↑; RPE↓
		P:30	43.2 ± 14.4	50				
Dobashi et al. (30)	RCD	8	19.4 ± 0.85	0	Untrained physically active participants	HRW (Temp: 4°C; H_2 conc.:5.14 ppm) 500 mL within 5 min before and after the exercise for 3 days	Three sets of 10-s repeated sprint cycling over 6 min, CMJ, and MVIS of knee extensions test	BLA→; MVIS→; CMJ→; Pmax for 10 s →; Pm for 10 s→; d-ROMs→; BAP→
Botek et al. (58)	RCD	16	31.6 ± 8.6	0	Well-trained runners	HRW (pH:7.8; H_2 conc.: 0.9 ppm) 420 mL doses at 24 h, 3 h, 2 h, and 40 min before exercise	4.2-km up-hill race	Race time→; RPE→; HR_{avg}→
Shibayama et al. (27)	RCD	8	20.9 ± 0.3	0	Untrained physically active participants	HRG[#] (68% H_2) 60 min after exercise	30 min treadmill running (75%VO_{2max}), CMJ, 10 s sprint cycling, 30s sprint cycling, and MVIS of knee extensions test	Pm for 30s→; MVIS→; CMJ↑; Pmax for 10s→; d-ROMs→; BAP→; U8ER↓; CKa→; LDa→; White blood cells→
Todorovic et al. (59)	RCD	6	24 ± 4	0	Healthy and active young men	HRW bathing (dissolve magnesium malate effervescent tablets in 200 L of tap water in a bathtub) immerse the whole body for 30 min immediately after exercise.	Five sets x 10 reps of eccentric leg presses (120% 1RM) followed by two sets x 10 reps of eccentric leg presses (100% 1RM), with 3 min between sets.	VAS↓; CK↓; Lactate dehydrogenase→; Aldolase→; Aspartate transaminase→; Troponin I→; Myoglobin→; White blood cells→; C-reactive protein→
Hori et al. (60)	RCD	12	21.8 ± 5.8	0	Untrained healthy participants	HRG (1% H_2) inhalation of H_2 gas 10 min before and 20 min during exercise	Cycling for 30 min at 60% VO_{2peak}	VCO_2↑; VE↑; HRavg [a]→; Resting HR → Vacetone↑; VO_2 rest→; VCO_2 rest→; VE rest→; Vacetone rest→; d-ROMs→; BAP→
Hori et al. (Exp.1) (23)	RCD	9	19.9 ± 1.2	33.3	Untrained university students	HRW (H_2 conc.:4.3 ppm) 500 mL doses at 35 min before exercise	Incremental cycling test to exhaustion	VO_{2peak}↑; Peak load→; BLA→; RPE→; HRmax→; Resting HR→; CDO→; RER→; VE→; d-ROMs→; BAP→
Hori et al. (Exp.2) (23)	RCT	H:10	20.3 ± 1.3	0	Untrained university students	HRW (H_2 conc.:5.9 ppm) 500 mL on all weekdays for 2 weeks	Incremental cycling test to exhaustion	VO_{2peak}→; BLA [a]→; Peak load→; RPE→; CDO→; RER→; VE→; HR_{max}→; Resting HR→; d-ROMs↑; BAP↑
		P:10	20.4 ± 4.7					
Botek et al. (34)	RCD	12	23.8 ± 1.9	0	Resistance trainers	HRW (Temp: 22°C; pH:7.8; ORP: −652 mV; H_2 conc.: 0.9 ppm) 210 mL at 30 min and at 1 min before training, 210 mL in the middle of the exercise session, then another 210 mL immediately after the end of the exercise session, and 420 mL of HRW at 30 min of recovery	A half squat, knee flexion, and extension exercises with the load set at 70%1RM for 3 sets (10 reps/set) + Lunges were performed with a load of 30% of body mass for 3 sets (20 reps/set)	RPE→; BLA↓; VAS↓; CK→; CMJ→; HRV→; Time of lunges↑

(Continued)

TABLE 1 (Continued)

Study	Design	Sample size	Age (yr)	%F	Training status	Methods of H_2 administration	Exercise protocol	Outcome measures
Timon et al. (26)	RCD	27	25.9 ± 5.6	Un	Recreationally trained cyclists (n = 12) and untrained participants (n = 15)	HRW (pH: 7.5; H_2 conc.:1.9 ppm; ORP: -600 mV) 1920 and 2,240 mL per day for 7 days	Incremental cycling test to exhaustion and 30 s maximal anaerobic test	VO_{2max}↑; TTE↑; Pm of maximal anaerobic test↑; BLA→; Pmax of maximal cycling test↑; Fatigue index↓; RPE→; HR_{max}→; VT2%VO_{2max}↑
Alharbi et al. (31)	RCD	18	21 ± 1	0	Recreationally trained participants	HRC[#] (0.636 μg/capsule) 2.544 μg/day for 3 days	Incremental cycling test to exhaustion	VO_{2peak}→; TTE→; BLA→; P_{max}→; HR_{max}→; Electrolytes (Na + →; K + →; Ca2 + →; Cl − →; AGap↑; AGapK→); VE↑; VO_2↑; VCO_2↑; Blood gas (pH↑; PO_2→; PCO_2→; HCO_3-↑); TR-NIRS in the RF/VL (Total [Hb + Mb] →; Deoxy [Hb + Mb] ↑; StO_2↑)
Dong et al. (24)	RCT	H:9	23.22 ± 1.09	33	Dragon boat athletes	HRW[#] (FH:1600 ppb) 1,000 mL per day for 8 days	30 s maximal dynamometer rowing test	Predicted time of rowing 500 m→; Pm→; Pmax↑; HRmax↓; $HR_{recovery}$↓; Resting HR→;
		P:9	22.67 ± 0.87					
Botek et al. (25)	RCD	16	18.8 ± 1.2	0	Professional soccer players	HRW (pH:7.9 ORP: −652 mV; Temp: 20°C; H_2 conc.:0.9 ppm) 420 mL at 120 min, 60 min and 210 mL at 15 min, and 5 min before exercise	Repeated sprints (15 × 30 m track sprints with recovery 20 s)	15th 30-meter sprint time↓; BLA → RPE→
Valenta et al. (33)	RCD	24	17.5 ± 1.8	0	Trained track and field runners	HRW (pH:7.8; ORP: -600 mV; H2 conc.:0.9 ppm) 420 mL was applied 120 min and 60 min before exercise, and 210 mL was applied 30 min and 10 min before exercise	Individual maximal aerobic speed until exhaustion (the time to exhaustion)	TTE→; DTE→; BLA→; HR_{max}→; BF→; VE→; VO2→; VCO2→; VE/VO2→; RQ→
Alharbi et al. (61)	RCD	10	20.0 ± 1.0	0	Trained track and field runners	HRC (0.636 μg/capsule) 2.544 μg supplements 1 h before exercise	Repeated cycling (Six repetitions of the 7 s all-out pedaling at 7.5% body weight separated by 40 s intervals)	Pmax↑; Muscle deoxygenation↑; Tissue O_2 saturation↑; HR_{max}[a]→; $HR_{recovery}$[a]→; Blood pH↑
Hong et al. (62)	RCD	24	21.3 ± 2.7	0	Physical education students	HRG (The ratio of oxygen to hydrogen in the H_2 gas is 2:1); Inhaled H_2 gas for 20 min before exercise	Constant workload cycling exercise; MVIS of knee extensions test	RPE↓; HR↓; PFC↑; MVIS→
Jebabli et al. (63)	RCD	22	21 ± 1	0	Amateur middle-distance runners	HRW (Temp: 12°C; pH:7.4; H_2 conc.: 0.55–0.65 mmol) 500 mL before exercise	Vameval test and race with maximal aerobic speed until voluntary exhaustion	Speed of the Vameval test↑; TTE↑; SJ→, CMJ→; 5JT→; RPE↑; HR_{max}↑

(Continued)

TABLE 1 (Continued)

Study	Design	Sample size	Age (yr)	%F	Training status	Methods of H_2 administration	Exercise protocol	Outcome measures
Dong et al. (64)	RCD	24	21.3 ± 2.7	0	Healthy adult men	HRG (The ratio of oxygen to hydrogen in the H_2 gas is 2:1); Inhaled H_2 gas for 60 min before exercise	Ride T_{max} at 80% W_{max} on cycle ergometers	RPE↓[a]; VAS↓; CMJ→; BLA↓; OH-↑; GSH-PX→
Sládečková et al. (65)	RCD	12	f:21.5 ± 5.0 m:18.9 ± 1.3	66.6	Elite swimmers	HRW (Temp: 20/20°C; pH 7.9/7.7; OPR: −652/+170 mV) 2,520 mL (1,260 mL/day) 3 days before the sessions and 2,520 mL on the experimental day	Morning session:4 × 50 m x 3 sets; Afternoon session:400 m dash	VAS↓; CK↓; CMJ↑

[a]Outcome data were not available by contacting the corresponding author and other authors on the publication; AGap, anion gap; AGapK, anion gap potassium; BLA, blood lactate; BF, breathing frequency; BAP, biological antioxidant potential; CK, creatine kinase; CKa, creatine kinase activity, CMJ, countermovement jump; CDO, carbon dioxide output; d-ROMs, diacron-reactive oxygen metabolites; DO, dissolved oxygen; DTE, distance to exhaustion; Exp., experiment; EC, electric conductivity; ESR, erythrocyte sedimentation rate; f, female; FH, free hydrogen; GSH-PX, glutathione peroxidase activity; H, H_2; H_2 conc., H_2 concentration; HRW, hydrogen-rich water; HRG, hydrogen-rich gas; HRC, hydrogen-rich calcium powder; HRG: hydrogen-rich gas; HR, heart rate; HRV, HRmax, maximal heart rate; HRavg, average heart rate; HR recovery, recovery heart rate; heart rate variability; LDa, lactate dehydrogenase activity; m, male; MF, median frequency; MPF, mean power frequency; MVIS, maximal voluntary isometric strength; MRS, maximal running speed; ORP, oxidation reduction potential; OH·-, the ability to inhibit hydroxyl radicals; OBLA, onset of blood lactate accumulation at 4 mmol•L-1; P, placebo; Pm, mean power; Pmax, maximum power; PFC, prefrontal cortex activation; RCD, randomized crossover design; RCT, randomized controlled trial; RE, running economy; rep., repetitions; RER, respiratory exchange ratio; RR, respiratory rate; RQ, respiratory quotient; RPE, rating of perceived exertion; RF, rectus femoris muscle; SCKa, serum creatine kinas activity; SLDa, serum lactate dehydrogenase activity; Temp, temperature; TTE, time-to-exhaustion; TDSs, total dissolved solids; TR-NIRS, time-resolved near-infrared spectroscopy; T_{max}, maximum cycling time; U8ER, urinary 8-hydroxydeoxyguanosine excretion rate; Un, unreported; VO_{2max}, maximum oxygen uptake; VO_{2peak}, peak oxygen uptake; VO_2, oxygen uptake; VT2 %VO_{2max}, percentage of maximal oxygen uptake in the ventilatory anaerobic threshold; VE, ventilation volume; VASs, visual analogue scales; VL, vastus lateralis muscle; W_{max}, maximum cycling power; %F, %female; 5JT, five jump test; f, female; m, male; SJ, squat jump test; ↓, H_2 significantly ($p < 0.05$) reduced the outcome compared to placebo; ↑, H_2 significantly ($p < 0.05$) improved the outcome compared to placebo; →, no significant difference ($p > 0.05$) between H_2 and placebo; [#], unreported molecular hydrogen concentration.

be highly variable (e.g., HRW:0.5 ~ 5.9 ppm; HRG:1 to 68%) among the various products examined. Nine studies did not report the concentration of H_2 (22, 24, 27, 31, 53–55, 57, 59). Single ($n = 9$) or multiple doses (ranging from 3 to 4 doses) of H_2 supplementation prior to exercise is a common intervention protocol. In total, 14 studies examined the effects of H_2 intake within 24 h before exercise (23, 25, 28, 29, 33, 51, 53, 54, 56, 58, 61–64). Nine studies implemented the protocol of repeated intake of H_2 from 2 to 14 days before exercise (22–24, 26, 30, 31, 52, 55, 57). One study (60) used 30 min inhalation of HRG during exercise. Another study (65) examined the effects of multiple doses of H_2 supplementation before and during exercise. Two studies examined the effects of a single intake of H_2 after exercise (27, 59). One study (34) used 210 mL at 30 min and 1 min before exercise, 210 mL during mid-exercise, another 210 mL immediately after exercise, and 420 mL of HRW 30 min after recovery. The physicochemical properties of HRW, HRW bathing, HRG, and HRC are shown in Table 1. Placebos were identical in appearance, texture, and taste to H_2 products, such as drinking water, air, and capsules.

3.2.3 Exercise protocol and outcome measurements

The included studies highlighted the effects of H_2 supplementation on aerobic endurance, anaerobic endurance, muscular strength, and lower extremity explosive strength in participants. In these studies, continuous incremental load and fixed-load subliminal exercise were the most commonly used aerobic endurance intervention or testing protocols. VO_{2max}, VO_{2peak}, TTE, race time, and power were metrics used to measure aerobic endurance performance (24, 26, 28, 29, 33, 55, 57, 61, 63). The 30 s maximal anaerobic power test (i.e., pedaling bicycle or rowing dynamometer) was used to assess the anaerobic endurance (i.e., mean or maximal power) (24, 26, 27). One study (57) used the MVIS to assess the force of knee extension prior to high-intensity aerobic exercise; four studies (27, 30, 51, 62) were conducted to evaluate the magnitude of knee extensor force or peak torque in the MVIC after vigorous exercise. Eight studies (25, 27, 30, 34, 61, 63–65) evaluated alterations in lower limb explosive power (i.e., CMJ height and peak power output during 10 s or 30 m sprint) during or after vigorous exercise in participants. One study (53) used the special fitness test to assess the effects of HRW intake on athletic performance in judo athletes. Additionally, the included studies focused on assessing the effects of H_2 administration on various physiological parameters during exercise, such as RPE, BLA, HR, pH, respiratory function, antioxidant levels, muscle oxygenation, and endocrine system. The outcomes of each study are summarized in Table 1.

3.3 Quality assessment

The risk of bias in the 27 publications (29 studies) was assessed, and a consensus was reached after discussion. The overall result is shown in Figure 2. Two studies (23, 28) did not adequately report on participant randomization and concealment methods. Five studies (23, 24, 30, 55, 60) did not adequately describe participant, staff, or evaluator blinding. No studies had incomplete results due to

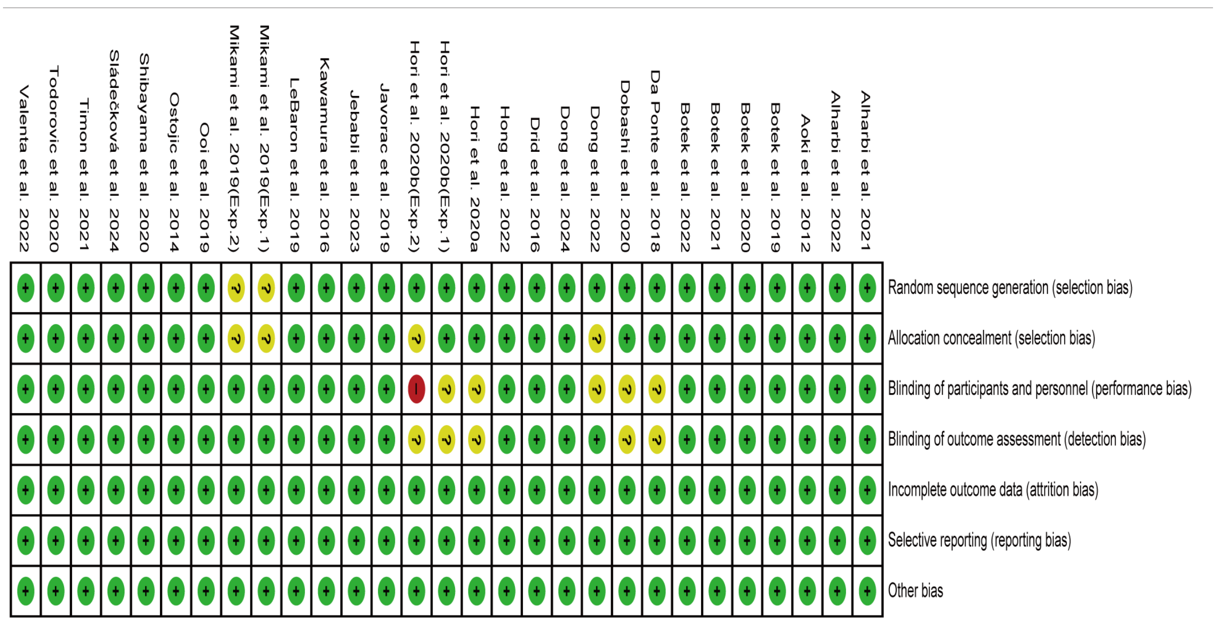

FIGURE 2
Risk of bias in the included studies.

participants' withdrawal. All studies reported experimental procedures and conducted the experiments as planned. According to the possibility of bias, the study was assessed as being low risk, moderate risk, or high risk. One study (23) was evaluated as having a high-risk bias, five studies (24, 28, 30, 55, 60) had a moderate risk bias, and others were assessed as having a low-risk bias. The quality of evidence for outcomes was evaluated as moderate to high, and details for the evaluation of the GRADE framework are presented in Supplementary Table S2.

3.4 Meta-analysis

A subgroup analysis was performed on aerobic endurance, anaerobic endurance, muscle strength, lower limb explosive power, RPE, and BLA, considering potential sources of heterogeneity, including exercise types and H_2 sources. Additionally, we used a subgroup analysis to explore the effects of H_2 supplementation on muscle performance before or after vigorous exercise (Table 2).

3.4.1 Effects of H_2 on aerobic endurance

3.4.1.1 VO_{2max} (VO_{2peak})

Three studies (23, 26, 28) showed that H_2 can significantly improve VO_{2max} or VO_{2peak} as compared to the placebo; while another five publications (six studies) (22, 28, 29, 31, 57) showed opposite results: H_2 cannot significantly improve VO_{2max} or VO_{2peak} (Table 1).

The pooled ES of VO_{2max} and VO_{2peak} was not significant and trivial (SMD = 0.09, 95% CI −0.11 to 0.28, p = 0.394, Figure 3) and without heterogeneity (I^2 = 0%, p = 0.996). The funnel plot (Supplementary Figure S1A) and Egger's test (t = −0.30, p = 0.776) indicated that there was no publication bias. Subgroup analyses showed non-significant trivial ESs for HRG (SMD = -0.06, 95% CI −0.68 to 0.56, p = 0.861), HRC (SMD = −0.04, 95% CI −0.70 to 0.61, p = 0.895), and HRW (SMD = 0.12, 95% CI −0.10 to 0.34, p = 0.290) on VO_{2max} (VO_{2peak}).

3.4.1.2 Aerobic endurance exercise performance

Two studies (26, 63) showed that H_2 can significantly improve aerobic exercise performance as compared to the placebo, while another eight publications (nine studies) (23, 24, 29, 31, 33, 54, 57, 58) showed that H_2 cannot (Table 1).

The pooled ES of aerobic exercise performance was not significant and trivial (SMD = 0.04, 95% CI -0.17 to 0.25, p = 0.687, Figure 4) and without heterogeneity (I^2 = 0%, p = 0.991). The funnel plot (Supplementary Figure S1B) and Egger's test (t = 0.75, p = 0.474) indicated that there was no publication bias on these results. Subgroup analyses showed non-significant trivial ESs for HRG (SMD = 0.002, 95% CI −0.618 to −0.622, p = 0.994), HRC (SMD = −0.02, 95% CI −0.68 to 0.63, p = 0.941), and HRW (SMD = 0.06, 95% CI −0.18 to 0.29, p = 0.632) on aerobic exercise performance.

3.4.2 Effects of H_2 on anaerobic endurance

One study (26) showed that H_2 can significantly improve mean and peak power output during the 30 s maximal anaerobic test as compared to the placebo. One study (24) showed that H_2 can significantly improve peak power output in a 30 s maximal anaerobic test compared to placebo but cannot significantly improve mean power, while another study (27) showed the opposite result that H_2 cannot significantly improve mean power output during the 30 s maximal anaerobic test (Table 1).

TABLE 2 Subgroup analysis results regarding the effects of H_2 on RPE and BLA.

Outcomes	Variables	No. of studies	SMD (95% CI)	*p*-value	Test of heterogeneity		
					χ^2	*p*-value	I^2 (%)
RPE	Exercise types						
	Strength training	1	−1.41 (−2.32, −0.50)	0.002	0	—	—
	Repeated sprints	1	−0.96 (−1.70, −0.22)	0.011	0	—	—
	Aerobic endurance exercise	10	−0.33 (−0.59, −0.07)	0.013	15.29	0.083	41.2
	Anaerobic endurance exercise	1	0.29 (−0.25, 0.83)	0.290	0	—	—
	Hydrogen source						
	HRW	12	−0.32 (−0.60, −0.03)	0.029	25.13	0.009	56.2
	HRG	1	−0.91 (−1.51, −0.31)	0.003	0	—	—
BLA	Exercise types						
	Strength training	1	−0.53 (−1.34, 0.29)	0.206	0	—	—
	Repeated sprints	2	−0.20 (−0.77, 0.37)	0.496	0.99	0.320	0
	Aerobic endurance exercise	9	−0.38 (−0.67, −0.08)	0.013	12.08	0.148	33.8
	Anaerobic endurance exercise	2	−0.67 (−1.72, 0.38)	0.213	3.01	0.083	66.8
	Hydrogen source						
	HRW	12	−0.37 (−0.59, −0.16)	0.001	15.37	0.166	28.4
	HRG	1	−0.47 (−1.04, 0.11)	0.111	0	—	—
	HRC	1	0.00 (−0.65, 0.65)	0.999	0	—	—

RPE, Rating of perceived exertion; BLA, blood lactate; HRW, hydrogen-rich water; HRG, hydrogen-rich gas; HRC, hydrogen-rich calcium powder.

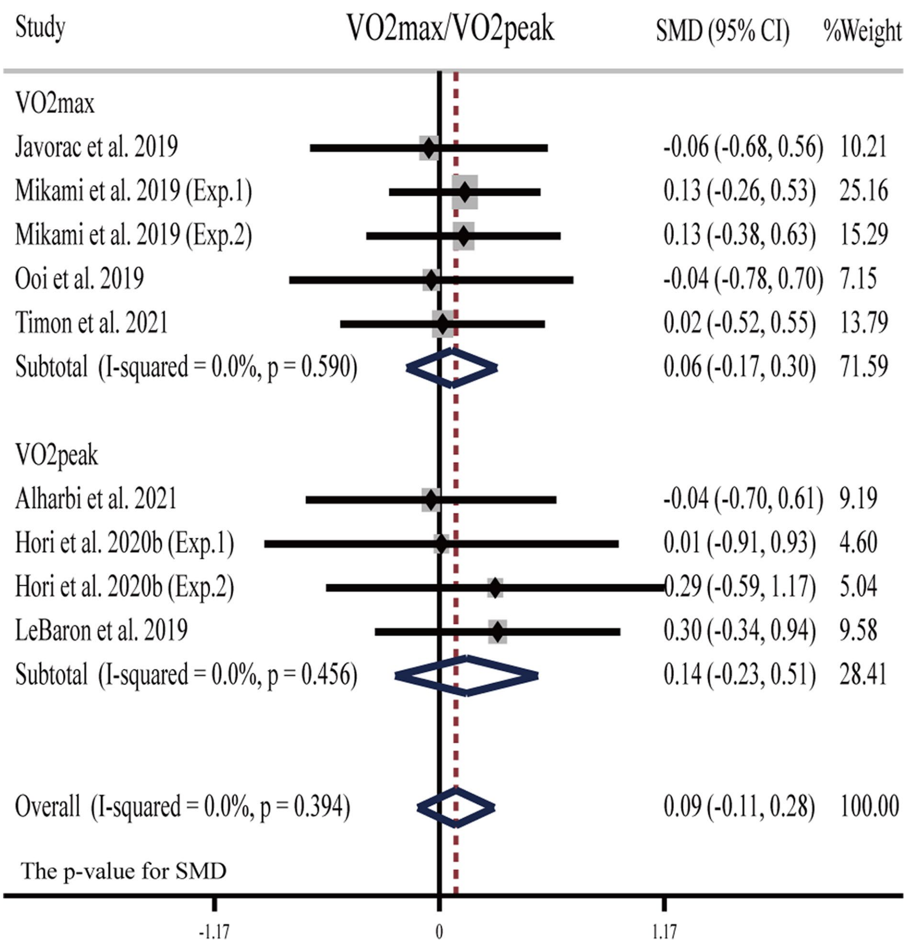

FIGURE 3
Forest plot of the effects of H_2 supplementation on VO_{2max}/VO_{2peak}. Exp.1, Experiment 1; Exp.2, Experiment 2.

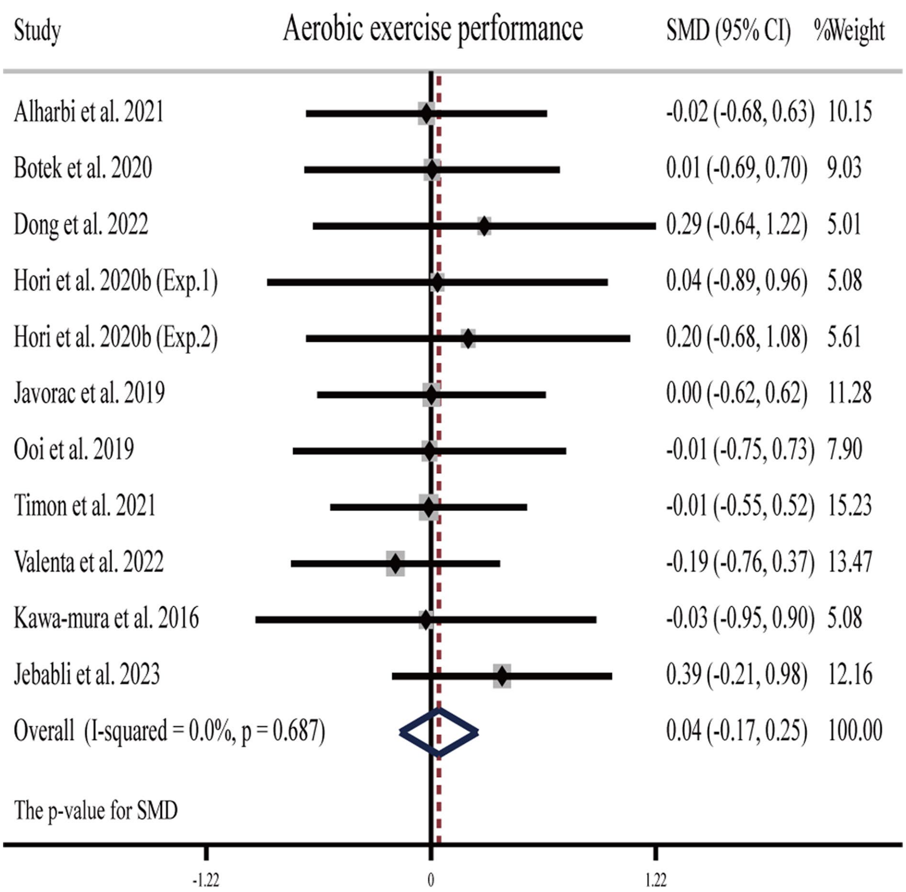

FIGURE 4
Forest plot of the effects of H_2 supplementation on aerobic exercise performance. Exp.1, Experiment 1; Exp.2, Experiment 2.

The pooled ES of anaerobic exercise performance was not significant and close to small (SMD = 0.19, 95% CI −0.12 to 0.50, p = 0.239, Figure 5) with low heterogeneity (I^2 = 0%, p = 0.929). The funnel plot (Supplementary Figure S1C) and Egger's test (t = 0.58, p = 0.586) indicated that there was no publication bias. With regard to the source of H_2, the ES was trivial for HRG (SMD = −0.09, 95% CI −1.07 to 0.89, p = 0.853), while it was small (SMD = 0.22, 95% CI −0.11 to 0.55, p = 0.192) for HRW.

3.4.3 Effects of H_2 on muscle strength

Five studies (27, 30, 51, 57, 62) showed that H_2 cannot significantly improve maximum strength compared to the placebo (Table 1).

The pooled ES of muscle strength was not significant and close to small (SMD = 0.19, 95% CI -0.14 to 0.52, p = 0.265, Figure 6) and with low heterogeneity (I^2 = 0%, p = 0.770). The funnel plot (Supplementary Figure S1D) and Egger's test (t = 2.67, p = 0.076) indicated no publication bias.

Subgroup analyses showed that the ES was trivial (SMD = 0.10, 95% CI −0.52 to 0.72, p = 0.741) for muscle strength assessed before vigorous exercise, and it was small (SMD = 0.22, 95% CI −0.17 to 0.62, p = 0.266) for H_2 on muscle strength assessed after vigorous exercise. With regards to the source of H_2, the ES was not significantly trivial for HRG (SMD = 0.13, 95% CI −0.26 to 0.51, p = 0.520), while it was small (SMD = 0.38, 95% CI −0.29 to 1.05, p = 0.265) for HRW.

3.4.4 Effects of H_2 on lower limb explosive power

Three studies (25, 61, 65) showed that H_2 can significantly improve lower limb explosive power as compared to the placebo, while another five studies (27, 30, 34, 63, 64) showed opposite results that H_2 cannot improve lower limb explosive power (Table 1).

The pooled ES of lower limb explosive power was significant and small (SMD = 0.30, 95% CI 0.05 to 0.55, p = 0.018, Figure 7) and without heterogeneity (I^2 = 0%, p = 0.949). The funnel plot (Supplementary Figure S1E) and Egger's test (t = 0.49, p = 0.636) indicated no publication bias. Subgroup analyses showed that the ES of HRG on lower limb explosive power was significant and moderate (SMD = 0.52, 95% CI 0.07 to 0.97, p = 0.023), while HRC was not significant and small (SMD = 0.20, 95% CI −0.68 to 1.08, p = 0.655), and HRW was not significant and small (SMD = 0.20, 95% CI −0.11 to 0.52, p = 0.206).

3.4.5 Effects of H_2 on the exploratory outcomes

3.4.5.1 RPE

Four studies (28, 56, 62, 64) showed that H_2 can significantly reduce RPE score as compared to the placebo, while another eight publications (nine studies) (23, 25, 26, 29, 34, 55, 58, 63) showed that H_2 cannot significantly reduce RPE score (Table 1). The pooled ES of the RPE score was small and significant (SMD = −0.37, 95% CI −0.65

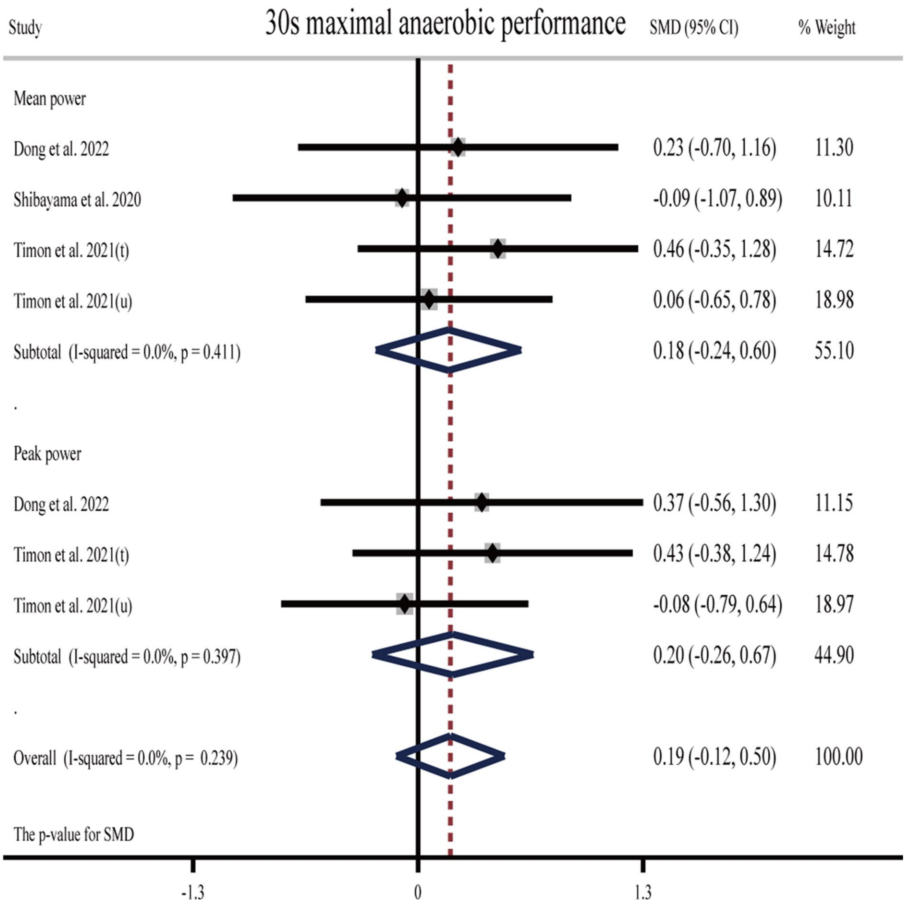

FIGURE 5
Forest plot of the effects of H_2 supplementation on anaerobic exercise performance. t, trained participants; u, untrained participants.

to −0.09, $p = 0.009$, Supplementary Figure S2), with moderate heterogeneity ($I^2 = 58.0\%$, $p = 0.005$). The funnel plot (Supplementary Figure S1F) and Egger's test ($t = -0.06$, $p = 0.955$) indicated no publication bias.

The results of subgroup analyses revealed that strength training and repeated sprints yielded significant and large ESs (SMD = −1.41, 95% CI −2.32 to −0.50, $p = 0.002$ and SMD = −0.96, 95% CI −1.70 to −0.22, $p = 0.011$, respectively), while aerobic endurance exercise produced a significant and small ES (SMD = −0.33, 95% CI −0.59 to −0.07, $p = 0.013$). Conversely, anaerobic endurance exercise elicited small and non-significant ES (SMD = 0.29, 95% CI −0.25 to 0.83, $p = 0.290$). With regards to the source of H_2, the ES was significantly large for HRG (SMD = −0.91, 95% CI −1.51 to −0.31, $p = 0.003$), while it was relatively small (SMD = −0.32, 95% CI −0.60 to −0.03, $p = 0.029$) for HRW.

3.4.5.2 BLA

Five studies (34, 51, 53, 56, 64) showed that H_2 can significantly improve BLA as compared to the placebo, while another eight publications (nine studies) (23, 25, 26, 29–31, 33, 55) showed opposite results that H_2 cannot significantly improve BLA (Table 1). The pooled ES of BLA was small and significant (SMD = −0.37, 95% CI −0.60 to −0.15, $p = 0.001$, Supplementary Figure S3), with low heterogeneity ($I^2 = 22.0\%$, $p = 0.215$). The funnel plot (Supplementary Figure S1G) and Egger's test ($t = -3.44$, $p = 0.005$) indicated that there was a potential risk of publication bias on these results, but the trim and fill method for sensitive analysis showed that the pooled ES (fixed: SMD = −0.349, $p < 0.001$; Random: SMD = −0.375, $p = 0.001$) was robust after filled meta-analysis.

The results of subgroup analyses revealed that aerobic endurance exercise yielded a significant and small ES (SMD = −0.38, 95% CI −0.67 to −0.08, $p = 0.013$), while anaerobic endurance exercise produced a non-significant and small ES (SMD = −0.67, 95% CI −1.72 to 0.38, $p = 0.213$); strength training elicited moderate and non-significant ES (SMD = −0.53, 95% CI −1.34 to 0.29, $p = 0.206$). Repeated sprints yielded a non-significant and small ES (SMD = −0.20, 95% CI −0.77 to 0.37, $p = 0.496$). With regards to the source of H_2, the ES was significantly small for HRW (SMD = −0.42, 95% CI −0.68 to −0.15, $p = 0.002$), and the ES of HRG was small and not significant (SMD = −0.47, 95% CI −1.04 to 0.11, $p = 0.111$), while it was trivial (SMD = 0.00, 95% CI −0.65 to 0.65, $p = 0.999$) for HRC.

3.4.5.3 HR_{avg}

Two studies (22, 62) showed that H_2 can significantly improve HR_{avg} during exercise as compared to the placebo, while another three studies (54, 55, 58) showed the opposite result that H_2 cannot significantly improve HR_{avg} (Table 1). The pooled ES of HR_{avg} was not significant and small (SMD = −0.27, 95% CI −0.60 to 0.05, $p = 0.094$, Supplementary Figure S4) and without heterogeneity ($I^2 = 0\%$,

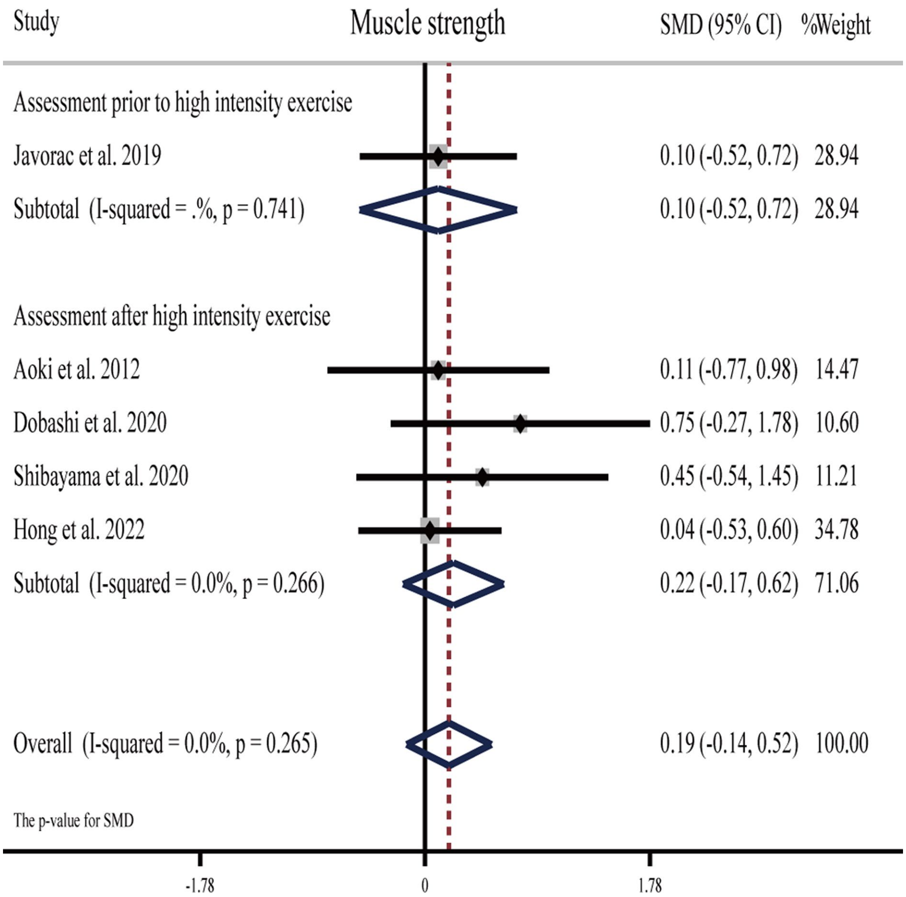

FIGURE 6
Forest plot of the effects of H_2 supplementation on muscle strength.

$p = 0.557$). The funnel plot (Supplementary Figure S1H) and Egger's test ($t = 1.26$, $p = 0.296$) indicated that there was no publication bias.

4 Discussion

To our knowledge, this is the first systematic review and meta-analysis exploring the effects of H_2 supplementation on physical performance in healthy adults. The results suggest that H_2 supplementation is promising for improving lower limb explosive power and reducing RPE and BLA clearance during vigorous exercise. However, it does not enhance endurance performance and muscle strength or decrease HR_{avg}.

This meta-analysis suggests that administering H_2 before or after exercise may serve as a potential strategy to effectively enhance lower limb explosive power in healthy adults. One potential mechanism underlying the effects of H_2 on explosive power is that H_2 can directly react with strong oxidants *in vivo* [e.g., hydroxyl radicals (•OH)] to modulate Ca^{2+} or mitochondrial ATP-dependent K^+ channels, thus facilitating mitochondrial ATP production (20, 66–69). Additionally, H_2 could reduce intracellular reactive oxygen species (ROS) levels and thus enhance muscle contractile function (27, 70). For example, a study conducted on soccer players demonstrated that administering three successive doses of 500 mL of HRW prior to high-intensity aerobic exercise increased the mean power frequency of skeletal muscles during subsequent strength tests (51). However, this finding that H_2 promotes lower limb explosive power may be influenced by a small sample size ($n = 92$) or movement pattern. One example is that H_2 significantly improved participants' sprint performance compared to their vertical jump performance (25, 63). Therefore, more research is still needed to confirm this finding in the future. The result showed that H_2 did not significantly improve muscle strength after aerobic endurance exercise. One possible reason is that intense aerobic exercise leads to a consumption of H_2 in the body that does not continually provide benefits for subsequent muscle strength performance. One study (34) shows that 1,260 mL of HRW intake can increase the movement velocity of multiple lunges during resistance training. Therefore, more studies are needed in the future to clarify the effects of H_2 supplementation on muscular strength performance in isolated resistance training. It has been observed that H_2 supplementation cannot significantly improve aerobic and anaerobic endurance performance. Endurance performance depends on the multiple factors of human respiratory function, oxygen transport, and local muscle oxygen utilization during exercise (71, 72). Studies have shown that using H_2 failed to significantly improve these critical factors (e.g., VO_{2max} and running economy) of endurance performance (23, 29, 31, 56, 57), thus leading to the insignificant benefits of H_2 on this important function.

While H_2 supplementation does not appear to enhance endurance performance or increase muscle strength, it does demonstrate favorable

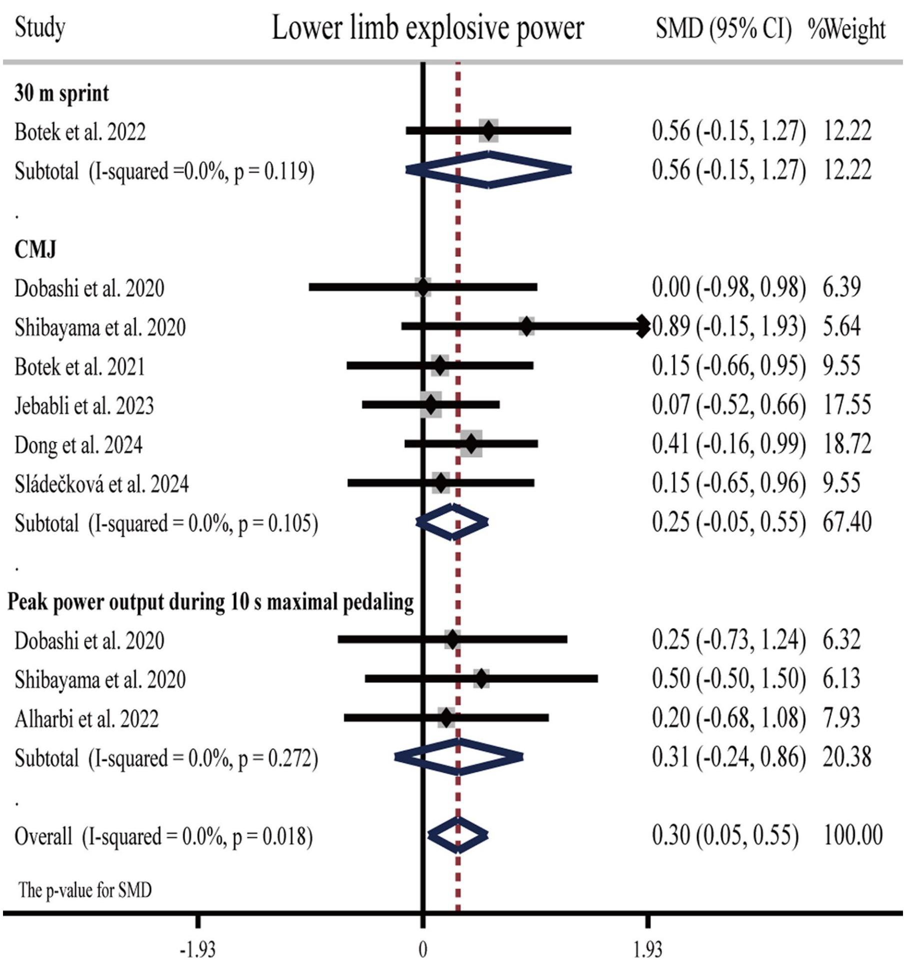

FIGURE 7
Forest plot of the effects of H_2 supplementation on lower limb explosive power.

effects in reducing RPE, BLA levels, and HR_{avg} among individuals engaged in high-intensity exercise. H_2 appears to be a neuroprotective agent that facilitates the restoration of neuronal oxidative damage by reducing oxidative stress and neuroinflammation (16, 73–75). H_2 intake has also been reported to induce positive effects on exercise acidosis (56), thus modulating intracellular and extracellular buffering capacity during vigorous exercise (76). The decrease in BLA during exercise may be attributed to the fact that molecular H_2 accelerates the transport of BLA to the liver for storage and oxidation, as well as increasing the utilization of lactate as a fuel by the muscles (56, 77). Subgroup analysis reveals that H_2 supplementation reduces BLA concentration in aerobic endurance exercise, which is superior to other exercise types. The reduction in BLA response during aerobic endurance exercise may indicate that H_2 supplementation enhances oxidative energy metabolism (28). Indeed, this finding may be unreliable due to the small number of studies on other exercise types. Therefore, future research should focus more on the effects of H_2 supplementation on anaerobic endurance, muscular strength, and repeated sprint performance. Subgroup analyses reveal two important factors that likely contribute to the effects of H_2 supplementation on RPE. First, we observed that the effects were greater in strength training and repeated sprints as compared to endurance exercise. The observed variations in RPE could be attributed to the disparities in the energy supply mechanisms across different types of exercises. It is plausible that H_2 gas may exhibit a higher affinity toward the phosphagen system when compared to the oxidative and glycolytic systems (66). Second, inhalation of H_2 gas (HRG) is superior to the ingestion of HRW in mitigating RPE. The observed discrepancy can be attributed to the fact that the respiratory absorption of molecular H_2 is significantly more efficient and comprehensive in comparison to its digestive absorption in HRW. Nonetheless, given the restricted sample size, it is imperative to ensure further validation of the outcomes of the subgroup analysis.

5 Limitations

Five included studies with a small number of participants ($n \leq 10$) (23, 27, 30, 52, 53) may lead to potential bias. Most studies to date focus on only younger and middle-aged men, and future studies are highly demanded to examine the benefits of H_2 for women and those with older age. The current studies only investigated the effects of H_2 supplementation for 1–14 days and future studies need to focus on the effects of longer supplementation periods. Some studies did not report or detect H_2 concentrations, and the H_2 dosing regimen was highly variable. The dose–response relationship between H_2 and physical performance has not been established, which should be explored in the future to determine the most appropriate dosage and intervention protocol for H_2 for enhancing physical performance.

6 Conclusion

In summary, this systematic review and meta-analysis suggest that short-term (<14 days) H_2 supplementation protocols contribute to improved lower limb explosive power, fatigue relief, and BLA clearance but may not significantly improve aerobic endurance, anaerobic endurance, or muscular strength. Inhaling H_2 shows promise as the optimal method for improving physical performance (i.e., lower limb explosive power) in healthy adults. Future studies with rigorous designs are needed to help obtain more definitive conclusions on the effects of H_2 on lower limb explosive power and muscle strength in healthy adults.

Data availability statement

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

Author contributions

KZ: Data curation, Formal analysis, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. ZS: Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. CY: Data curation, Formal analysis, Methodology, Writing – original draft. ZG: Conceptualization, Writing – original draft. YW: Formal analysis, Methodology, Software, Writing – original draft. DB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. JZ: Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1387657/full#supplementary-material

References

1. Thiele D, Prieske O, Chaabene H, Granacher U. Effects of strength training on physical fitness and Sport-specific performance in recreational, sub-elite, and elite rowers: A systematic review with Meta-analysis. *J Sports Sci*. (2020) 38:1186–95. doi: 10.1080/02640414.2020.1745502

2. Pescatello LS, Arena R, Riebe D, Thompson PD. Acsm's guidelines for exercise testing and prescription. *Physiotherapy*. (2006) 77:311. doi: 10.1016/S0031-9406(10)61772-5

3. Viribay A, Fernández-Landa J, Castañeda-Babarro A, Collado PS, Fernández-Lázaro D, Mielgo-Ayuso J. Effects of Citrulline supplementation on different aerobic exercise performance outcomes: A systematic review and Meta-analysis. *Nutrients*. (2022) 14:3479. doi: 10.3390/nu14173479

4. Williams J, Abt G, Kilding AE. Effects of Creatine monohydrate supplementation on simulated soccer performance. *Int J Sports Physiol Perform*. (2014) 9:503–10. doi: 10.1123/ijspp.2013-0407

5. Medrano-Ureña MDR, Ortega-Ruiz R, Benítez-Sillero JD. Physical fitness, exercise self-efficacy, and quality of life in adulthood: A systematic review. *Int J Env Res Public Health*. (2020) 17:6343. doi: 10.3390/ijerph17176343

6. Lu Z, Xu Y, Song Y, Bíró I, Gu Y. A mixed comparisons of different intensities and types of physical exercise in patients with diseases related to oxidative stress: A systematic review and network Meta-analysis. *Front Physiol*. (2021) 12:700055. doi: 10.3389/fphys.2021.700055

7. Powers SK, Deminice R, Ozdemir M, Yoshihara T, Bomkamp MP, Hyatt H. Exercise-induced oxidative stress: friend or foe? *J Sport Health Sci*. (2020) 9:415–25. doi: 10.1016/j.jshs.2020.04.001

8. El Abed K, Ammar A, Boukhris O, Trabelsi K, Masmoudi L, Bailey SJ, et al. Independent and combined effects of all-out Sprint and low-intensity continuous exercise on plasma oxidative stress biomarkers in trained judokas. *Front Physiol*. (2019) 10:842. doi: 10.3389/fphys.2019.00842

9. Ammar A, Trabelsi K, Boukhris O, Glenn JM, Bott N, Masmoudi L, et al. Effects of aerobic-, anaerobic- and combined-based exercises on plasma oxidative stress biomarkers in healthy untrained young adults. *Int J Env Res Public Health*. (2020) 17:2601. doi: 10.3390/ijerph17072601

10. Powers SK, Nelson WB, Hudson MB. Exercise-induced oxidative stress in humans: cause and consequences. *Free Radic Biol Med*. (2011) 51:942–50. doi: 10.1016/j.freeradbiomed.2010.12.009

11. Huang WC, Wei CC, Huang CC, Chen WL, Huang HY. The beneficial effects of *Lactobacillus Plantarum* Ps128 on high-intensity, exercise-induced oxidative stress, inflammation, and performance in triathletes. *Nutrients*. (2019) 11:353. doi: 10.3390/nu11020353

12. Rubio-Arias J, Ávila-Gandía V, López-Román FJ, Soto-Méndez F, Alcaraz PE, Ramos-Campo DJ. Muscle damage and inflammation biomarkers after two ultra-Endurance Mountain races of different distances: 54 km vs 111 km. *Physiol Behav*. (2019) 205:51–7. doi: 10.1016/j.physbeh.2018.10.002

13. Pingitore A, Lima GP, Mastorci F, Quinones A, Iervasi G, Vassalle C. Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports. *Nutrition*. (2015) 31:916–22. doi: 10.1016/j.nut.2015.02.005

14. Fernández-Lázaro D, Fernandez-Lazaro CI, Mielgo-Ayuso J, Navascués LJ, Córdova Martínez A, Seco-Calvo J. The role of selenium mineral trace element in

exercise: antioxidant defense system, muscle performance, hormone response, and athletic performance. A systematic review. *Nutrients*. (2020) 12:1790. doi: 10.3390/nu12061790

15. Arazi H, Eghbali E, Suzuki K. Creatine supplementation, physical exercise and oxidative stress markers: A review of the mechanisms and effectiveness. *Nutrients*. (2021) 13:869. doi: 10.3390/nu13030869

16. Iketani M, Ohsawa I. Molecular hydrogen as a neuroprotective agent. *Curr Neuropharmacol*. (2017) 15:324–31. doi: 10.2174/1570159x14666160607205417

17. Hong Y, Chen S, Zhang JM. Hydrogen as a selective antioxidant: A review of clinical and experimental studies. *J Int Med Res*. (2010) 38:1893–903. doi: 10.1177/147323001003800602

18. Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med*. (2007) 13:688–94. doi: 10.1038/nm1577

19. Ohta S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. *Pharmacol Ther*. (2014) 144:1–11. doi: 10.1016/j.pharmthera.2014.04.006

20. Gvozdjáková A, Kucharská J, Kura B, Vančová O, Rausová Z, Sumbalová Z, et al. A new insight into the molecular hydrogen effect on coenzyme Q and mitochondrial function of rats. *Can J Physiol Pharmacol*. (2020) 98:29–34. doi: 10.1139/cjpp-2019-0281

21. Ostojic SM. Hydrogen gas as an exotic performance-enhancing agent: challenges and opportunities. *Curr Pharm Des*. (2021) 27:723–30. doi: 10.2174/1381612826666200922155242

22. LeBaron TW, Larson AJ, Ohta S, Mikami T, Barlow J, Bulloch J, et al. Acute supplementation with molecular hydrogen benefits submaximal exercise indices. Randomized, double-blinded, placebo-controlled crossover pilot study. *J Lifestyle Med*. (2019) 9:36–43. doi: 10.15280/jlm.2019.9.1.36

23. Hori A, Sobue S, Kurokawa R, Hirano SI, Ichihara M, Hotta N. Two-week continuous supplementation of Hydrogenrich water increases peak oxygen uptake during an incremental cycling exercise test in healthy humans: A randomized, single-blinded, placebo-controlled study. *Med Gas Res*. (2020) 10:163–9. doi: 10.4103/2045-9912.304223

24. Dong G, Fu J, Bao D, Zhou J. Short-term consumption of hydrogen-rich water enhances power performance and heart rate recovery in dragon boat athletes: evidence from a pilot study. *Int J Env Res Public Health*. (2022) 19:5413. doi: 10.3390/ijerph19095413

25. Botek M, Khanna D, Krejčí J, Valenta M, McKune A, Sládečková B, et al. Molecular hydrogen mitigates performance decrement during repeated sprints in professional soccer players. *Nutrients*. (2022) 14:508. doi: 10.3390/nu14030508

26. Timon R, Olcina G, Gonzalez-Custodio A, Camacho-Cardenosa M, Camacho-Cardenosa A, Martinez GI. Effects of 7-day intake of hydrogen-rich water on physical performance of trained and untrained subjects. *Biol Sport*. (2021) 38:269–75. doi: 10.5114/biolsport.2020.98625

27. Shibayama Y, Dobashi S, Arisawa T, Fukuoka T, Koyama K. Impact of hydrogen-Rich gas mixture inhalation through nasal cannula during post-exercise recovery period on subsequent oxidative stress, muscle damage, and exercise performances in men. *Med Gas Res*. (2020) 10:155–62. doi: 10.4103/2045-9912.304222

28. Mikami T, Tano K, Lee H, Lee H, Ohta S. Drinking hydrogen water enhances endurance and relieves psychometric fatigue: randomized, double-blind, placebo-controlled study. *Can J Physiol Pharmacol*. (2019) 97:857–62. doi: 10.1139/cjpp-2019-0059

29. Ooi CH, Ng SK, Omar EA. Acute ingestion of hydrogen-rich water does not improve incremental treadmill running performance in endurance-trained athletes. *Appl Physiol Nutr Metab*. (2019) 45:513–9. doi: 10.1139/apnm-2019-0553

30. Dobashi S, Takeuchi K, Koyama K. Hydrogen-rich water suppresses the reduction in blood Total antioxidant capacity induced by 3 consecutive days of severe exercise in physically active males. *Med Gas Res*. (2020) 10:21–6. doi: 10.4103/2045-9912.279979

31. Alharbi AAD, Ebine N, Nakae S, Hojo T, Fukuoka Y. Application of molecular hydrogen as an antioxidant in responses to Ventilatory and ergogenic adjustments during incremental exercise in humans. *Nutrients*. (2021) 13:459. doi: 10.3390/nu13020459

32. Kawamura T, Higashida K, Muraoka I. Application of molecular hydrogen as a novel antioxidant in sports science. *Oxidative Med Cell Longev*. (2020) 2020:1–7. doi: 10.1155/2020/2328768

33. Valenta M, Botek M, Krejčí J, McKune A, Sládečková B, Neuls F, et al. Acute pre-exercise hydrogen rich water intake does not improve running performance at maximal aerobic speed in trained track and field runners: A randomized, double-blind, placebo-controlled crossover study. *PLoS One*. (2022) 17:e0279307. doi: 10.1371/journal.pone.0279307

34. Botek M, Krejčí J, McKune A, Valenta M, Sládečková B. Hydrogen rich water consumption positively affects muscle performance, lactate response, and alleviates delayed onset of muscle soreness after resistance training. *J Strength Cond Res*. (2021) 36:2792–9. doi: 10.1519/jsc.0000000000003979

35. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The Prisma 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

36. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. *Cochrane handbook for systematic reviews of interventions*. *2rd* ed. Hoboken, NJ: John Wiley & Sons (2019).

37. Xue Y, Yang Y, Huang T. Effects of chronic exercise interventions on executive function among children and adolescents: A systematic review with Meta-analysis. *Br J Sports Med*. (2019) 53:1397–404. doi: 10.1136/bjsports-2018-099825

38. Petzold A, Steeb T, Wessely A, Koch EAT, Vera J, Berking C, et al. Is Tebentafusp superior to combined immune checkpoint blockade and other systemic treatments in metastatic uveal melanoma? A comparative efficacy analysis with population adjustment. *Cancer Treat Rev*. (2023) 115:102543. doi: 10.1016/j.ctrv.2023.102543

39. Baumgart JK, Brurok B, Sandbakk Ø. Comparison of peak oxygen uptake between upper-body exercise modes: A systematic literature review and Meta-analysis. *Front Physiol*. (2020) 11:412. doi: 10.3389/fphys.2020.00412

40. Wallace A, Pietrusz A, Dewar E, Dudziec M, Jones K, Hennis P, et al. Community exercise is feasible for neuromuscular diseases and can improve aerobic capacity. *Neurology*. (2019) 92:e1773–85. doi: 10.1212/wnl.0000000000007265

41. Deshayes TA, Pancrate T, Goulet EDB. Impact of dehydration on perceived exertion during endurance exercise: A systematic review with Meta-analysis. *J Exerc Sci Fit*. (2022) 20:224–35. doi: 10.1016/j.jesf.2022.03.006

42. Engel FA, Ackermann A, Chtourou H, Sperlich B. High-intensity interval training performed by young athletes: A systematic review and Meta-analysis. *Front Physiol*. (2018) 9:1012. doi: 10.3389/fphys.2018.01012

43. Delleli S, Ouergui I, Messaoudi H, Trabelsi K, Ammar A, Glenn JM, et al. Acute effects of caffeine supplementation on physical performance, physiological responses, perceived exertion, and technical-tactical skills in combat sports: A systematic review and Meta-analysis. *Nutrients*. (2022) 14:2996. doi: 10.3390/nu14142996

44. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of Bias in randomised trials. *BMJ*. (2011) 343:d5928. doi: 10.1136/bmj.d5928

45. Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, et al. Grade guidelines: 3. Rating the quality of evidence. *J Clin Epidemiol*. (2011) 64:401–6. doi: 10.1016/j.jclinepi.2010.07.015

46. Meader N, King K, Llewellyn A, Norman G, Brown J, Rodgers M, et al. A checklist designed to aid consistency and reproducibility of grade assessments: development and pilot validation. *Syst Rev*. (2014) 3:82. doi: 10.1186/2046-4053-3-82

47. Cohen J. *Statistical power analysis for the behavioral sciences*. Mahwah, NJ: Lawrence Earlbaum Associates (1988).

48. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in Meta-analyses. *BMJ*. (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

49. Bandeira-Guimaraes M, Blanco-Rambo E, Vieira AF, Saez de Asteasu ML, Pinto RS, Izquierdo M, et al. Chronic effects of different intensities of power training on neuromuscular parameters in older people: A systematic review with Meta-analysis. *Sports Med Open*. (2023) 9:98. doi: 10.1186/s40798-023-00646-9

50. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication Bias in Meta-analysis. *Biometrics*. (2000) 56:455–63. doi: 10.1111/j.0006-341x.2000.00455.x

51. Aoki K, Nakao A, Adachi T, Matsui Y, Miyakawa S. Pilot study: effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes. *Med Gas Res*. (2012) 2:12. doi: 10.1186/2045-9912-2-12

52. Ostojic SM, Stojanovic MD. Hydrogen-rich water affected blood alkalinity in physically active men. *Res Sports Med*. (2014) 22:49–60. doi: 10.1080/15438627.2013.852092

53. Drid P, Trivic T, Casals C, Trivic S, Stojanovic M, Ostojic SM. Is molecular hydrogen beneficial to enhance post-exercise recovery in female athletes? *Sci Sports*. (2016) 31:207–13. doi: 10.1016/j.scispo.2016.04.010

54. Kawamura T, Gando Y, Takahashi M, Hara R, Suzuki K, Muraoka I. Effects of hydrogen bathing on exercise-induced oxidative stress and delayed-onset muscle soreness. *Jpn J Phys Fitness Sports Med*. (2016) 65:297–305. doi: 10.7600/jspfsm.65.297

55. Da Ponte A, Giovanelli N, Nigris D, Lazzer S. Effects of hydrogen rich water on prolonged intermittent exercise. *J Sports Med Phys Fitness*. (2018) 58:612–21. doi: 10.23736/s0022-4707.17.06883-9

56. Botek M, Krejčí J, McKune AJ, Sládečková B, Naumovski N. Hydrogen rich water improved Ventilatory, perceptual and lactate responses to exercise. *Int J Sports Med*. (2019) 40:879–85. doi: 10.1055/a-0991-0268

57. Javorac D, Stajer V, Ratgeber L, Betlehem J, Ostojic S. Short-term H(2) inhalation improves running performance and torso strength in healthy adults. *Biol Sport*. (2019) 36:333–9. doi: 10.5114/biolsport.2019.88756

58. Botek M, Krejčí J, McKune AJ, Sládečková B. Hydrogen-rich water supplementation and up-hill running performance: effect of athlete performance level. *Int J Sports Physiol Perform*. (2020) 15:1193–6. doi: 10.1123/ijspp.2019-0507

59. Todorovic N, Javorac D, Stajer V, Ostojic SM. The effects of supersaturated hydrogen-rich water bathing on biomarkers of muscular damage and soreness perception in young men subjected to high-intensity eccentric exercise. *J Sports Med (Hindawi Publ Corp)*. (2020) 2020:8836070–5. doi: 10.1155/2020/8836070

60. Hori A, Ichihara M, Kimura H, Ogata H, Kondo T, Hotta N. Inhalation of molecular hydrogen increases breath acetone excretion during submaximal exercise: A randomized, single-blinded, placebo-controlled study. *Med Gas Res*. (2020) 10:96–102. doi: 10.4103/2045-9912.296038

61. Alharbi AAD, Iwamoto N, Ebine N, Nakae S, Hojo T, Fukuoka Y. The acute effects of a single dose of molecular hydrogen supplements on responses to ergogenic

adjustments during high-intensity intermittent exercise in humans. *Nutrients*. (2022) 14:3974. doi: 10.3390/nu14193974

62. Hong Y, Dong G, Li Q, Wang V, Liu M, Jiang G, et al. Effects of pre-exercise H(2) inhalation on physical fatigue and related prefrontal cortex activation during and after high-intensity exercise. *Front Physiol*. (2022) 13:988028. doi: 10.3389/fphys.2022.988028

63. Jebabli N, Ouerghi N, Abassi W, Yagin FH, Khlifi M, Boujabli M, et al. Acute effect of hydrogen-rich water on physical, perceptual and cardiac responses during aerobic and anaerobic exercises: A randomized, placebo-controlled, double-blinded cross-over trial. *Front Physiol*. (2023) 14:1240871. doi: 10.3389/fphys.2023.1240871

64. Dong G, Wu J, Hong Y, Li Q, Liu M, Jiang G, et al. Inhalation of hydrogen-Rich gas before acute exercise alleviates exercise fatigue. *Int J Sports Med*. (2024). doi: 10.1055/a-2318-1880

65. Sládečková B, Botek M, Krejčí J, Valenta M, McKune A, Neuls F, et al. Hydrogen-rich water supplementation promotes muscle recovery after two strenuous training sessions performed on the same day in elite fin swimmers: randomized, double-blind, placebo-controlled, crossover trial. *Front Physiol*. (2024) 15:1321160. doi: 10.3389/fphys.2024.1321160

66. Ohta S. Direct targets and subsequent pathways for molecular hydrogen to exert multiple functions: focusing on interventions in radical reactions. *Curr Pharm Des*. (2021) 27:595–609. doi: 10.2174/1381612826666200806101137

67. Zheng H, Yu YS. Chronic hydrogen-rich saline treatment attenuates vascular dysfunction in spontaneous hypertensive rats. *Biochem Pharmacol*. (2012) 83:1269–77. doi: 10.1016/j.bcp.2012.01.031

68. Ishibashi T. Therapeutic efficacy of molecular hydrogen: A new mechanistic insight. *Curr Pharm Des*. (2019) 25:946–55. doi: 10.2174/1381612825666190506123038

69. Iuchi K, Imoto A, Kamimura N, Nishimaki K, Ichimiya H, Yokota T, et al. Molecular hydrogen regulates gene expression by modifying the free radical chain reaction-dependent generation of oxidized phospholipid mediators. *Sci Rep*. (2016) 6:18971. doi: 10.1038/srep18971

70. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev*. (2008) 88:1243–76. doi: 10.1152/physrev.00031.2007

71. Bassett DR Jr, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc*. (2000) 32:70–84. doi: 10.1097/00005768-200001000-00012

72. Coyle EF. Integration of the physiological factors determining endurance performance ability. *Exerc Sport Sci Rev*. (1995) 23:25–63. doi: 10.1249/00003677-199500230-00004

73. Liu L, Xie K, Chen H, Dong X, Li Y, Yu Y, et al. Inhalation of hydrogen gas attenuates brain injury in mice with Cecal ligation and puncture via inhibiting Neuroinflammation, oxidative stress and neuronal apoptosis. *Brain Res*. (2014) 1589:78–92. doi: 10.1016/j.brainres.2014.09.030

74. Dohi K, Satoh K, Miyamoto K, Momma S, Fukuda K, Higuchi R, et al. Molecular hydrogen in the treatment of acute and chronic neurological conditions: mechanisms of protection and routes of administration. *J Clin Biochem Nutr*. (2017) 61:1–5. doi: 10.3164/jcbn.16-87

75. Ishii A, Tanaka M, Watanabe Y. Neural mechanisms of mental fatigue. *Rev Neurosci*. (2014) 0:469–79. doi: 10.1515/revneuro-2014-0028

76. Lancha Junior AH, Painelli Vde S, Saunders B, Artioli GG. Nutritional strategies to modulate intracellular and extracellular buffering capacity during high-intensity exercise. *Sports Med*. (2015) 45:71–81. doi: 10.1007/s40279-015-0397-5

77. Brooks GA. The science and translation of lactate shuttle theory. *Cell Metab*. (2018) 27:757–85. doi: 10.1016/j.cmet.2018.03.008

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Moderate-dose caffeine enhances anaerobic performance without altering hydration status

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The effects of direct nutritional supplements on athletic performance are still being investigated and arouse curiosity. Only one study in the literature was found that investigated the kicking speed performance of futsal players following low-dose caffeine supplementation (3 mg/kg); thus, the question of whether caffeine supplementation improves kicking speed as well as essential physical parameters in soccer players is still controversial. Therefore, the aim of this study was to determine the effect of caffeine supplementation on vertical jump (VJ), sprint, reaction time, balance, change of direction (COD), and ball-kicking speed in soccer players. In a double-blind, cross-over design, nine moderately trained male soccer players (21.11 ± 2.02 years, 171.22 ± 6.14 cm, 71.78 ± 10.02 kg) consumed caffeine (6 mg/kg) or a placebo 60 min before completing balance, reaction time, vertical jump, agility, 30 m sprint, and ball-kicking speed tests. Greater VJ height ($p = 0.01$) and power ($p = 0.08$), and faster completion time according to the Illinois Agility Test ($p = 0.08$) were found following caffeine supplementation compared to placebo. Elapsed time ($p = 0.01$), average ($p = 0.01$) time, and the slowest reaction times ($p = 0.016$) were significantly reduced after caffeine consumption compared to placebo supplementation. Caffeine intake significantly improved VJ, agility, and reaction time ($p < 0.05$) but did not affect 30 m sprint, ball-kicking speed, balance, and RPE values in soccer players ($p > 0.05$). Although non-significant, caffeine intake also improved sprint (0.67%) and ball kicking (2.7%) performance percentages. Also, caffeine consumption did not induce dehydration, and the athletes' body hydration levels were normal. These findings support the use of caffeine supplementation as an effective nutritional ergogenic aid to enhance anaerobic performance, at least for vertical jumps, COD speed, and reaction time, in trained male soccer players.

KEYWORDS

sports nutrition, supplements, ergogenic aid, soccer, caffeine

1 Introduction

Soccer performance is characterized by bursts of high-intensity physical activity, which requires players to simultaneously perform intense running, and explosive soccer-specific actions such as kicking, jumping, sprinting, and agility (1). An ergogenic aid is defined as any intervention encompassing training techniques, mechanical devices, nutritional components, pharmacological methods, or psychological strategies that can enhance exercise performance capacity or improve training adaptations (2). Thus, the efficacy of ergogenic aids, such as caffeine, on anaerobic performance is of interest to soccer players, coaches, and sports scientists. Caffeine is the most widely used ergogenic aid by the athletic population (3). Indeed, following the removal of caffeine from the World Anti-Doping Agency's list of banned substances, it has been reported that three of every four professional athletes use caffeine before or during competition (4). As such, in a study by Tallis et al. (5) 35 of the 36 clubs across the English professional soccer leagues reported that caffeine was administered to enhance soccer performance. The rationale behind this phenomenon is the underlying mechanisms by which the ergogenicity of caffeine may benefit anaerobic performance, which are the antagonism of the adenosine receptor at concentrations in the micromolar range in the central nervous system (CNS) (6), increased β-endorphins secretion via activation of the hypothalamic-pituitary-adrenal (HPA) axis (7), and intracellular calcium release from the sarcoplasmic reticulum in muscle cells (8), all of which can benefit performance via improving power, agility, reaction time, and alertness and delaying fatigue (6).

The ergogenic effect of caffeine supplementation has been reported in a variety of soccer-specific performance parameters, such as improved performance in the Loughborough Soccer Passing Test (9), the 20 m sprint test (10), fatigue resistance in 75% of the VO2max to volitional fatigue test (11), jumping performance in the countermovement jump (CMJ) test (11, 12), agility in the arrowhead agility test (10), and reaction time (10, 11, 13). However, direct extrapolation of these findings to the complexity of soccer is complicated, and no study has yet investigated all these parameters in the same test setting with the same participant groups (6). Since the ergogenicity of caffeine may be influenced by various factors, such as genotype (14), training status (15), habituation to caffeine (16), and supplementation regimen, various physical performance responses to caffeine can differ even in the same person (6). Therefore, evaluating the effect of caffeine supplementation on all these soccer-specific skills and/or parameters in the same field test battery is important for improving understanding of the potential of caffeine supplementation to soccer performance.

Kicking is another key skill and/or component of soccer. Indeed, ball-kicking speed has been suggested as a new, efficient performance indicator in youth soccer players (17). Given that the ball-kicking speed is associated with and/or affected by various physical aspects (e.g., technique, power, and balance), it might be anticipated that caffeine supplementation has the potential to influence ball-kicking speed; however, empirical evidence to support this is presently lacking. While one previous study by Lopez-Samanes et al. (18) reported no difference in ball velocity performance in futsal players after low-dose of caffeine (3 mg/kg) supplementation, it remains unknown whether caffeine supplementation improves the ball-kicking speed in soccer players. As such, further research is required to assess the effect of caffeine supplementation on the ball-kicking speed in soccer players. Therefore, the aim of this study was to determine the effect of moderate-dose (6 mg/kg) caffeine supplementation on vertical jump, sprint, reaction time, balance, agility, and ball-kicking speed in the same field test battery in moderately trained male soccer players. In addition, body hydration levels and fluid balance were analyzed to determine the effect of caffeine on body water homeostasis. It was hypothesized that (I) the ingestion of moderate-dose of caffeine would improve vertical jump, sprinting, reaction time, balance, agility, and ball-kicking speed and (II) not induce dehydration and fluid imbalances.

2 Materials and methods

2.1 Participants

Nine healthy, non-smoking, young, moderately trained (19), male soccer players participated in this study (age 21.11 ± 2.02 years, height 171.22 ± 6.14 cm, weight 71.78 ± 10.02, habitual consumption of caffeine 188 ± 83 mg d^{-1}; mean ± SD, Table 1). The required sample size was estimated using G*Power software (Heinrich-Heine-University Düsseldorf, version 3.1.m9.2, Düsseldorf, Germany). A sample size of eight participants was determined sufficient (effect size: 0.50, confidence interval: 1-β 0.95, error: α 0.05, actual power: 0.96). Based on Acar et al. (20) and discussion between the authors, we set the effect size at 0.5. All participants had 9.89 ± 2.57 years of competitive soccer experience at club standard and at least 3 years of experience playing in regional and university-level leagues. Participants completed at least five weekly soccer training sessions (7.5 h a week). All participants declared that they had not used any ergogenic aids that might alter body hydration levels and exercise performance within 3 months from the start of the study. All participants were informed of the experimental procedures before giving their written informed consent.

2.2 Experimental design

On the first visit, a habitual caffeine consumption questionnaire was given to the participants in addition to their anthropometric and body composition assessments. Body mass was obtained in kg with a bioelectric impedance analysis device (BIA, Inbody 120, InBody Co., Ltd. Seoul, Korea), and height was obtained in cm with a portable stadiometer (Seca 213, Hamburg, Germany). Habitual caffeine intake was determined through a validated questionnaire (21). Only participants with a daily caffeine intake of less than 250 mg d^{-1} were included to control individual differences in responsiveness to caffeine

TABLE 1 Descriptive information of subjects (n = 9).

Variables	X	SD
Age (yr)	21,11	2,02
Height (cm)	171,2	6,14
Weight (kg)	71,78	10
BMI (kg/m^2)	24,37	1,9
Training age (yr)	9,89	2,57
Habitual consumption of caffeine (mg/day^{-1})	188	83

from habituation. In addition, participants were selected based on the following inclusion criteria: (1) were aged between 18 and 25 years; (2) had actively participated in soccer training for at least 3 years, with a minimum of three times/week for the last 3 three months; (3) were non-smokers. On the first visit, participants also performed the testing protocol for familiarization at a low intensity that would not make them exert vigorous effort. Following completion of this initial familiarization, participants were assigned to ingest either caffeine or a placebo in a double-blind, cross-over, randomized counterbalanced design. The participants were randomly assigned to the two conditions using the Research Randomizer software (www.randomizer.org; accessed on 10 June 2022). Participants consumed 6 mg/kg of caffeine (Nature's Supreme, Istanbul, Turkey) or placebo supplements (wheat bran) in capsules (same color and form, made up of gelatin hard form) 60 min before the testing protocol (5). A researcher, who had no further involvement in this research, prepared the caffeine dose using electronic laboratory scales with one milligram of sensitivity at room temperature. There were at least 48 h between sessions to ensure that the caffeine had washout and to allow participants to complete recovery. Participants were asked to record their diet 24 h before the first test session and replicate it 24 h before the second test session. For 24 h before and for each of the testing sessions, participants were asked to refrain from ingestion of caffeine, ergogenic aids (e.g., nitrate, sodium bicarbonate), alcohol, and anti-inflammatory drugs; not to engage in strenuous physical activity; and to be strict with their nutrition and rest. Regarding the rhythm (22), the tests and measurements were applied to the participants at the same time of the day (between 1–3 pm), under similar environmental conditions (ambient temperature 22.00 ± 1.41°C, humidity 62.00 ± 4.24%, pressure 1018.00 ± 1.41 mbar; mean ± SD) in the Sinop University indoor sports hall and performance laboratory. Participants were instructed to wear the same clothing and footwear for all the testing sessions. In the second and third sessions, participants' hydration levels were analyzed just before placebo or caffeine ingestion and immediately after tests. Participants completed a 15 min standardized warm-up. *Ad libitum* water consumption (similar amounts) was allowed in both trials. The testing protocol in each experimental session consisted of balance, vertical jump, reaction time, change-of-direction via the Illinois Agility Test, 30 m sprint, and the ball-kicking speed tests, respectively. Vertical jump, reaction time, change of direction, 30 m sprint, and ball-kicking speed tests were performed in the indoor sports hall while balance, hydration levels, and RPE tests were conducted in the performance laboratory. These performance tests were employed because they were the same as soccer players' moves in training and competitions. A 3 min passive rest period was given between the performance tests (except the vertical jump test) to facilitate recovery (23) and participants were allowed two trials with the best score used for subsequent analysis (24). In addition, the Borg CR10 scale with a range of 0–10 was used to measure the rate of perceived exertion (RPE) at the end of the tests (25). A schematic diagram of the experimental protocol is displayed in Figure 1.

2.3 Anthropometric and body composition assessments

Body mass was obtained in kg with a bioelectric impedance analysis device (BIA, Inbody 120, InBody Co., Ltd. Seoul, Korea), and stature was obtained in cm with a portable stadiometer (Seca 213, Hamburg, Germany).

2.4 Balance test

A portable dynamic balance device (Togu Challenge Disc 2.0, Prien am Chiemsee, Rosenheim, Germany) was utilized to assess the balance of the participants. The platform was free to move in all directions (up to a maximum of 12°) and thus provided an unstable ground. The challenge disc recorded the athlete's movements with three-dimensional motion sensors and sent the data in real time to its software on the smartphone or tablet via Bluetooth. Stability index ranges were categorized into 1 to 5 (1—very good, 2—good, 3—normal, 4—weak, 5—very weak), and a lower score (p) indicated a better balance. Initially, the researcher showed the application on the tablet to the athlete at eye level, and the athlete stood barefoot on the platform to eliminate the possible effects of different types of shoes on the results. Later, the athletes were instructed to stand in the middle of the disc and keep their balance for 20 s (after 10 s of preparation, 5 s of which is a countdown) with their arms free to swing. During the test, participants were told to keep the point in the circle as central and stable as possible. The platform provided a safe measurement for athletes with its non-slip surface. The test was performed two times with a 3 minute passive rest, and the best score was used as the dynamic balance test score (26).

2.5 Reaction time test

Participants' reaction times were determined using the Light Trainer (Reaction Development and Exercise System, Istanbul, Turkey). The reaction time test course consisted of four light modules (stuck to the top of 30 cm height traffic training cones) lined up side by side at a distance of 1 m. The test course was designed depending on the facility conditions (indoor sports hall) and the physical characteristics of the experimental group (trial with different numbers of modules and feedback). Athletes stood 1 m from the modules in the middle of the course. Participants got ready for the reaction time test on the "ready" command. The athletes deactivated the light modules, which were lit on the right or left side, with precise movements, touching with their dominant hand to the top of the module. The test started with activating the first light automatically and ended with the athletes' "deactivating" the last light. Athletes were asked to deactivate 30 light modules in total. The test was repeated if the participants hit the modules, dropped them, or deformed the test course. The test was performed two times with a 3 minute passive rest, and the best score was used as the reaction time test score (27).

2.6 Vertical jump and anaerobic power test

A digital vertical jump device (Takei 5,406 Jump-MD Vertical Jumpmeter, Tokyo, Japan) was used to measure the vertical jump scores of the participants. Firstly, the rubber vertical jump plate was placed on a flat surface. In order to eliminate the possible effects of different types of shoes on the results, the participants were instructed to take off their shoes and stand "ready" with bare feet centered on the

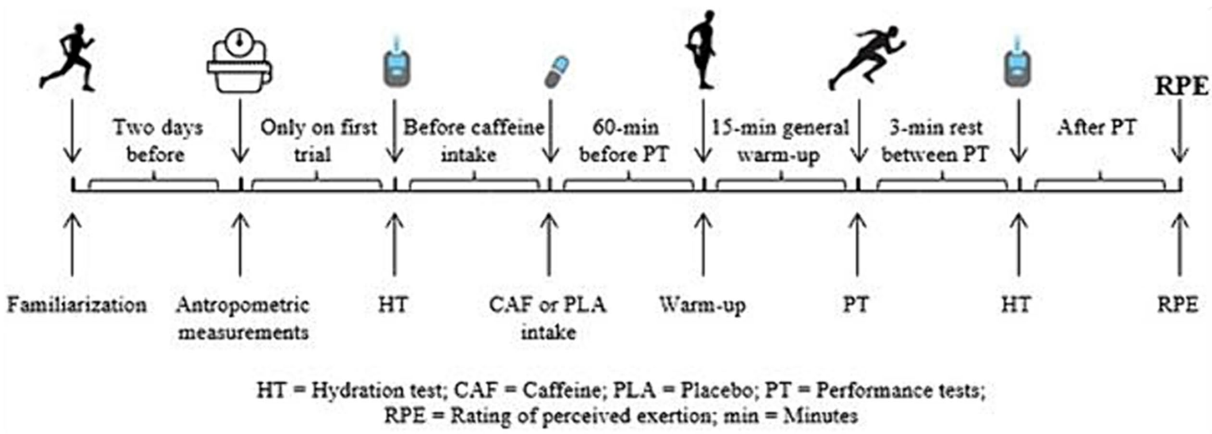

FIGURE 1
Schematic diagram of the experimental design.

plate (10–20 cm from each other). Afterward, the researcher (the same person fastened the digital belt in all trials for test reliability) zeroed the digital belt, wound it tightly around to the waist of the participants, and turned the pulley gently in the direction of the arrow to take the slack out of the rope. Once the athletes were ready, they quickly moved from the upright standing position to a position of 90° flexion of the knees with freely swinging arms and jumped for the maximum height. The test was repeated if participants jumped by stepping forward, the measuring tape was loose, or they did not land on the rubber plate after jumping. Each player performed two trials interspersed with 1 min rest between each vertical jump, and the best (highest) jump was recorded in cm with an accuracy of ±1 (28). Participants' anaerobic power calculations were executed using the Lewis formula: anaerobic power (W) = {$\sqrt{4.9}$ [body weight (kg)] $\sqrt{\text{vertical jump (m)}}$} (29).

2.7 Illinois agility test

The COD was assessed with the Illinois Agility Test by using a photocell (Seven, SE-165 Photocell Stopwatch, Istanbul, Turkey). The agility course is an area of 10 m long and 5 m wide, formed by four 30 cm height traffic training cones lined up in a straight line at 3.3 m intervals from each other in the middle. The test consists of a 40 m straight, and a 20 m slalom run with 180° turns every 10 m. The photocell timing gates were placed at the start and finish lines at a height of approximately 1 m. When they were ready, athletes started the test 30 cm behind the starting point. The participants were asked to run at maximal speed. The test was repeated two times with a 3 minute passive rest between each trial, and the best value was recorded as the Illinois Agility Test time (30).

2.8 30 M sprint test

The linear sprint times of the soccer players were determined with the 30 meter sprint test by using a photocell (±0.01 s precision) device (Seven, SE-165 Photocell Stopwatch, Istanbul, Turkey) in the indoor sports hall. When participants were ready, they started the sprint from a line one meter behind the starting gate with a standing start position. Participants were asked to run at maximum speed and performed the test twice with a 3 minute rest between trials. Test results were recorded in seconds and milliseconds, and the best values were used as 30 m sprint test time (27).

2.9 Ball-kicking speed test

The ball-kicking speed was determined 11 meters (penalty mark) from the goal using a radar gun device (Bushnell Velocity Speed Gun, Overland Park, Kansas, United States), which can measure speed in the range of 16–177 km/h with a sensitivity of ±2 km/h. Initially, the dominant legs of the participants were recorded (subjectively determined); thus, maximal speed was ensured. Then, participants shot the ball with the instep kick technique. Kicks were performed with a ball (size 5 soccer ball for 12 years and older) in compliance with FIFA standards. The researcher (the same person aimed the radar gun in all trials for test reliability) measured the ball-kicking speed behind the goal, directly opposite the penalty spot where the soccer player kicked the ball. Participants were told to kick for accuracy (hitting the target) while attaining the maximum ball-kicking speed. Each player was given two trials to get the best score, and the results were recorded in km/h (31).

2.10 Supplementation protocol

Participants consumed 6 mg/kg of caffeine (Nature's Supreme, Istanbul, Turkey) or placebo supplements (wheat bran) in capsules (same color and form, made up of gelatin hard form) 60 min before the testing protocol (6). A researcher, who had no further involvement in this research, prepared the caffeine dose using electronic laboratory scales with one milligram of sensitivity at room temperature.

2.11 Hydration analysis

The portable hydration test device (MX3 Diagnostics LAB Pro, Melbourne, Australia) was used to assess participants' body hydration levels (32). The device was convenient and easy to use for outdoor measurements and analyzed the hydration level from the saliva taken directly from the tongue with a hydration test strip. The saliva sample was taken from the tongue under the sterilization rules. The saliva sample was collected and analyzed with a hydration test strip attached to the device without waiting or undergoing any other procedure. The values and their assigned hydration status follow as ≤65 = Hydrated, 65–100 = Mildly Dehydrated, 101–150 = Moderately Dehydrated, >150 = Severely Dehydrated.

2.12 Statistical analysis

Data were checked for normality by using the Shapiro–Wilk test. Comparison between groups was analyzed with the paired sample *t*-test to test for differences between the caffeine and placebo supplement in the vertical jump, sprint, reaction time, balance, agility, and ball-kicking speed performances. Cohen's d was utilized in the calculation of effect size (large $d > 0.8$, moderate $d = 0.8$ to 0.5, small $d = 0.5$ to 0.2, and trivial $d < 0.2$) (33). Statistical significance was accepted as $p < 0.05$, and all data were analyzed using SPSS 27.0 (IBM Corp., Armonk, NY) and are presented as mean ± SD.

3 Results

Vertical jump height ($p = 0.001$, $d = 0.83$, Table 2) and power ($p = 0.008$, $d = 0.45$, Table 2) were significantly increased after caffeine compared to placebo supplementation. The Illinois Agility Test completion time was significantly faster after caffeine compared to placebo supplementation ($p = 0.008$, $d = 1.20$, Table 2). There was no difference in the 30 m sprint (caffeine: 4.39 ± 0.14 vs. placebo: 4.42 ± 0.20 s, $p = 0.426$, $d = 0.17$), balance (caffeine: 2.75 ± 0.48 vs. placebo: 2.60 ± 0.76 s, $p = 0.624$, $d = 0.23$) and ball-kicking speed values (caffeine: 93.87 ± 6.87 vs. placebo: 91.37 ± 5.13 km/h, $p = 0.193$, $d = 0.41$) between supplementations (Table 2). RPE values were also similar between caffeine (2.89 ± 0.33 AU) and placebo (2.56 ± 0.72 AU) supplementations ($p = 0.195$, $d = 0.58$) (Figure 2).

Regarding reaction time performance parameters, there was a significantly faster elapsed time ($p = 0.001$, $d = 1.27$, Table 3), average reaction time ($p = 0.001$, $d = 1.37$, Table 3), and the slowest reaction time ($p = 0.016$, $d = 1.52$, Table 3) after caffeine compared to placebo supplementation. However, there was no significant difference in the fastest reaction time (caffeine: 0.71 ± 0.08 vs. placebo: 0.74 ± 0.08 s, $p = 0.238$, $d = 0.37$) and in the last reaction time (caffeine: 0.95 ± 0.27 vs. placebo: 1.13 ± 0.30 s, $p = 0.097$, $d = 0.63$) between supplementations.

Fluid balance was within normal ranges in both groups, and there was no major effect on hydration levels between pre-and post-exercise conditions (placebo: 53.22 ± 16.57 mOsm/L vs. placebo: 54.78 ± 16.64 mOsm/L, $p = 0.863$, $d = 0.09$) (caffeine: 54.44 ± 20.92 mOsm/L vs. caffeine: 57.33 ± 20.79 mOsm/L, $p = 0.715$, $d = 0.13$) (Table 4).

4 Discussion

The aim of this study was to determine the effect of moderate-dose caffeine supplementation on vertical jump, sprint, reaction time, balance, agility, and ball-kicking speed in the same field test battery in moderately trained male soccer players. To the best of our knowledge, the current study is the first to analyze the effect of acute caffeine intake on ball-kicking speed, balance, sprint, agility, vertical jump, reaction time, and hydration status in the same test setting in male soccer players. The main finding was that the ingestion of 6 mg/kg of caffeine significantly enhanced vertical jump, agility, and reaction performance. However, no significant differences between placebo and caffeine were detected in hydration status, ball-kicking speed, balance, sprint performance, and rating of perceived exertion values. These findings are partly in line with our experimental hypothesis and support a moderate dose of caffeine supplementation as an ergogenic aid to enhance anaerobic performance, including vertical jump height, COD speed, and reaction time for male soccer players.

The current study ascertained that 6 mg/kg of caffeine increased vertical jump and change of direction performance, which is parallel to previous studies (9, 34, 35) and is in contrast to the other in which the participants were recreationally active young males who were not habituated to caffeine (36). This lack of significant difference in the abovementioned study may have resulted from the participants' status as non-responders to caffeine. However, a performance improvement was seen with the caffeine for 47% of the participants during the 20-yard shuttle. Our findings significantly extend the observations of those previous studies and support the ergogenic potential of caffeine supplementation on vertical jump and change of direction performance. The positive effect on jumping performance might be related to increased motor unit recruitment (37) and muscle activation (38). Accordingly, caffeine improves performance through two primary mechanisms: antagonism of adenosine receptors (A1, A2A) in the central nervous system, which leads to increases in neurotransmitter release and potentiation of Na^{++}/K^{++} pump activity in skeletal muscle, which may lead to an increase in excitation-contraction coupling (6). This mechanism of caffeine may also have enhanced jumping performance. In the study of futsal players, López-Samanes et al. (18) examined the effects of acute caffeine on physical performance. In light of the data obtained, the researchers suggested that acute caffeine supplementation significantly improved vertical jump performance. Nevertheless, although the researchers also found an increase in ball velocity, they did not find any significance. Ranchordas et al. (39) examined the effects of caffeinated gum on vertical jump, sprint, and recovery levels in soccer players, and they noted that 200 mg of caffeine slightly improved physical performance tests such as jumping and recovery. In line with these results, acute caffeine ingestion of 5 mg/kg 60 min before exercise was found to increase jump height in professional soccer players (12). Notably, the researchers found that a caffeine dose of 3 mg/kg improved performance in the majority of the post-exercise tests (10). These results, along with our findings, support the ergogenic effect of acute caffeine supplementation on jumping performance as it was well established in previous studies (40–42). This effect of caffeine on jumping performance could be attributed to the improvements in force production after caffeine ingestion (6). Evidently, caffeine ingestion has been well-documented to enhance peak power and mean power and reduce the time needed to reach peak power in the Wingate test (43). Also, it has been reported that caffeine decreases contraction time and maximal displacement values, which indicates an increase in muscle contraction performance. More importantly, this study was carried out using tensiomyography, in which an electrical pulse induces muscle contraction independent of the CNS. So, it could be considered

TABLE 2 Changes in mean values of caffeine and placebo groups.

Variables	Placebo	Caffeine	95% CI		*d*	*p*
	X ± SD	X ± SD	LB	UB		
VJ (cm)	56.33 ± 6.87	61.44 ± 5.24	−7.30	−2.92	0.83	0.001*
VJ (watt)	1183.45 ± 105.15	1241.29 ± 146.74	−95.8	−19.87	0.45	0.008*
Balance (s)	2.60 ± 0.76	2.75 ± 0.48	−0.05	0.10	0.23	0.624
30 m sprint (s)	4.42 ± 0.20	4.39 ± 0.14	−0.87	0.55	0.17	0.426
COD (s)	16.43 ± 0.42	15.97 ± 0.34	0.16	0.76	1.20	0.008*
Ball-kicking speed (km/h)	91.37 ± 5.13	93.87 ± 6.87	−6.57	1.56	0.41	0.193

*(p < 0,05); X, mean; SD, standard deviation; *d*, Cohen's *d* effect size; VJ, vertical jump; COD, change of direction (Illionis agility test); 95% CI, confidence interval; LB, lower bound; UB, upper bound.

compelling evidence of caffeine's direct effect on neuromuscular stimulation (44). The improvement in COD time in the present study might be attributed to the "blocking adenosine" mechanism, which increases neurotransmission (45) and motor unit recruitment (46). This change can be elucidated by the fact that caffeine intake increases Ca + and thus improves mobility by facilitating muscle contraction and nerve conduction (47). Similarly, in their study with rugby players, Ranchordas et al. (48) investigated the effects of caffeinated gum on CMJ, Illinois Agility, 6 × 30 m repeated sprint, and Yo-Yo IR2 test performances. Research results indicated that caffeinated gum augmented performance in the Yo-Yo IR2 and the CMJ tests and decreased the fatigue index during repeated sprints. Furthermore, Karayigit et al. (49) reported that low (3 mg/kg) and moderate (6 mg/kg) doses of caffeinated coffee improved repeated sprint performance with increasing epinephrine norepinephrine concentrations in female team sport athletes. In contrast, it was determined that 6 mg/kg acute caffeine consumption did not affect agility and anaerobic power (50). Lastly, it has already been known that chronic exposure to caffeine may result in physiological modifications that lead to tolerance and reduce the ergogenic effects of acute caffeine on high-intensity exercise (16). Because the habitual caffeine intake level of the participants in the current study was not high (188 ± 83 mg/day), the tolerance phenomenon did not appear in this research.

Considering the present study's balance and reaction time performance findings, it was statistically determined that caffeine intake decreased the reaction time, thus increasing the reaction performance. Caffeine has an adenosine-like molecular structure and binds to the adenosine receptor (AA2) in the brain, increasing the concentration of neurotransmitters (51). Also, caffeine is a central nervous system stimulant due to its capacity to block adenosine-specific receptors, which increases the release of several neurotransmitters, including norepinephrine, dopamine, acetylcholine, and serotonin (52). Accordingly, the significant difference in reaction time may have concluded from motivators through hormonal changes. In two similar studies, Impey et al. (53, 54) reported that caffeine consumed 60 min prior to a performance in soccer goalkeepers positively affected reaction time. Similarly, it was shown that both 3 and 6 mg/kg of acute caffeine intake enhanced reaction time in female team sport athletes (55). These decreases in reaction time could be associated with the mechanism of caffeine as an adenosine receptor antagonist (6), which may enhance neuronal excitability that leads to improvements in sport-specific reaction times (56). Moreover, in our study, increase in reaction performance may be responsible for the decrease in change of direction time via the same mechanism that also makes motor unit recruitment easier. Hence, caffeine may play a significant role in individual and team sports where concentration and reaction times influence match/training performance. To the contrary, Bottoms et al. (56) showed that 3 mg/kg caffeine intake had no effect on reaction time in athletes while Balko et al. (57) suggested that a larger amount of caffeine may lead to a decrease in visual and auditory reaction times, in turn, increasing reaction time performance. Interestingly, some results are inconsistent with our findings, most likely because the present study enrolled different levels of soccer players than the studies above. The participants in our study were amateur soccer players with various training modalities and experiences. Therefore, these inconsistencies in findings may be primarily associated with the fact that they are not familiar with the high demand standardized exercises like professional soccer players and thus have a different level of responsiveness to tests. Furthermore, according to previous studies, acute caffeine consumption may improve (58) or diminish (59) the standing balance ability of young adults. This discrepancy may be partially attributable to the variance in caffeine dosage provided, which ranges from 160 to 400 mg. Only Kara et al. (60) gave a caffeine dosage according to body mass (6 mg/kg); this was the only research that found positive results. Given the paucity of published research evaluating the influence of caffeine on balance, it is difficult to contextualize these results in light of prior research. In addition, methodological inconsistencies and changes in the balancing tasks and outcome measures used may possibly account for the inconsistent results. There is a need for a more thorough and extensive evaluation of the effects of caffeine on balance skills, based on the small amount of data and inconsistent results.

The findings of our study indicated that 6 mg/kg caffeine consumption did not induce dehydration, and the athletes' body hydration levels were normal. Reviews have shown that this is a common result, suggesting that no caffeine-induced dehydration or other harmful changes occur in athletes during exercise that negatively affects physical performance (61, 62). Furthermore, Del Coso et al. (63) reported that acute caffeine consumption of 6 mg/kg increased urine flow and sweat electrolyte excretion; however, these effects were not enough to affect dehydration or blood electrolyte levels when exercising for 120 min in a warm environment. These results, consistent with our study's findings, refute the widespread belief that caffeine consumption is accompanied by dehydration and an increased sweat rate in the body. Physiologically, the effects of arginine vasopressin on water retention and the effects of aldosterone on sodium balance seem to be sufficient to tackle the effects of a mild diuretic consumed in a moderate dose (61). Considering RPE values, caffeine is supposed to elicit ergogenic effects on the CNS via the antagonism of adenosine receptors, leading

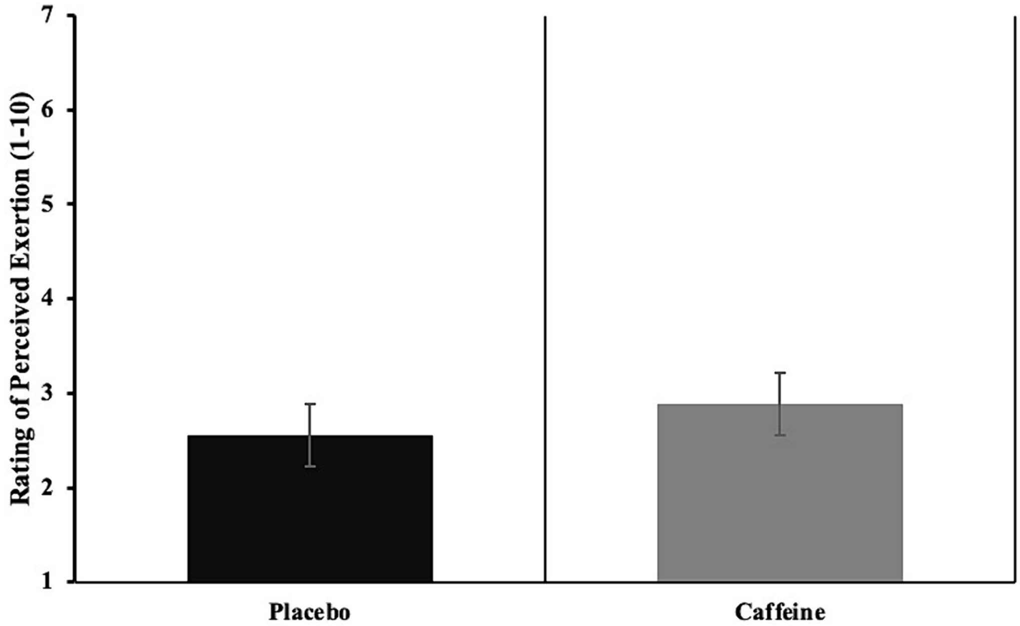

FIGURE 2
Rating of perceived exertion (RPE) (A.U).

TABLE 3 Participants' reaction time performance parameters.

Variables	Placebo	Caffeine	95% CI		*d*	*p*
	X ± SD	X ± SD	LB	UB		
Elapsed time (s)	34.79 ± 1.71	31.92 ± 2.69	1.50	4.24	1.27	0.001*
Average reaction time (s)	1.16 ± 0.05	1.06 ± 0.09	0.06	0.14	1.37	0.001*
Fastest reaction time (s)	0.74 ± 0.08	0.71 ± 0.08	−0.04	0.41	0.37	0.238
Slowest reaction time (s)	1.96 ± 0.31	1.58 ± 0.17	−0.03	0.10	1.52	0.016*
Last reaction time (s)	1.13 ± 0.30	0.95 ± 0.27	0.10	0.65	0.63	0.097

*($p < 0.05$); X, mean; SD, standard deviation; *d*, Cohen's *d* effect size; 95% CI, confidence interval; LB, lower bound; UB, upper bound.

TABLE 4 Hydration levels in both groups between pre- and post-exercise conditions.

Variables	Pre-exercise	Post-exercise	95% CI		*d*	*p*
	X ± SD (mOsm/l)	X ± SD (mOsm/l)	LB	UB		
Placebo	53.22 ± 16.57	54.78 ± 16.64	−1.96	−1.15	0.09	0.863
Caffeine	54.44 ± 20.92	57.33 ± 20.79	−3.60	−2.18	0.13	0.715

*($p < 0.05$); X, mean; SD, standard deviation; *d*, Cohen's *d* effect size; 95% CI, confidence interval; LB, lower bound; UB, upper bound.

to pain suppression, which may attenuate the pain and decrease the RPE (6). Additionally, along with its particular chemical structure, caffeine easily crosses the blood–brain barrier, acts on adenosine receptors (A1, A2), and blocks the receptors that cause pain in the body. This is another factor expected to be effective on RPE values in the present study (64). Intriguingly, we did not observe a significant impact of caffeine on RPE levels, indicating that other variables, such as greater motor neuron activation and a reduced decline in voluntary activation throughout the exercise, may explain caffeine's ergogenic benefits.

The following are some of the limitations of our study. The blinding efficiency was not evaluated by asking individuals to identify which supplement (caffeine vs. placebo) they had ingested. Unknown is whether "caffeine expectancy" may have influenced the outcomes of the present investigation. Although participants were advised to repeat their 24 h meal before each test, macronutrient consumption was not evaluated. In addition, we did not collect samples for determining neurotransmitter concentrations at the baseline and post-exercise, and we did not assess the electromyographic activation of active muscles during performance tests, which would have offered greater insight into the specific processes by which caffeine boosted reaction time, vertical jump, and change of direction performance but not sprint, balance, and ball-kicking performance.

5 Conclusion

The present study found that a 6 mg/kg caffeine intake in soccer players improved physical performance, such as vertical jump height, change of direction speed, and reaction time. Although non-significant, caffeine intake also improved sprint (0.67%) and ball kicking (2.7%) performance percentages. Moreover, it was revealed that caffeine did not impair body hydration levels, so body fluid balance was within normal clinical ranges. Accordingly, further studies conducted with larger sample sizes using a 24 h urine collection method could provide more evidence for the relationship between caffeine consumption and hydration. On the other hand, in our study, caffeine ingestion did not provide any change in balance performance and rating of perceived exertion. Based on these results, it is recommended that coaches and athletes incorporate caffeine into their nutritional strategies as it can improve performance. In future studies, the effects of different doses of caffeine on various components of performance, including small-sided games and ball-kicking speed in soccer and other disciplines, could be investigated to provide new insights. Ultimately, to verify these mechanisms, further studies should be conducted to investigate neuromuscular responses to caffeine supplementation during anaerobic tasks in soccer players.

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 Human Research Ethics Committee at Sinop University (Reference number: E-57452775-050.01.04-104421). 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

AM: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. KA: Data curation, Formal analysis, Writing – review & editing. DA: Funding acquisition, Methodology, Project administration, Software, Supervision, Writing – review & editing. HM: Methodology, Writing – original draft, Writing – review & editing. MA: Project administration, Supervision, Writing – original draft, Writing – review & editing. MM: Data curation, Funding acquisition, Software, Visualization, Writing – review & editing. FK: Data curation, Writing – review & editing. FW: Funding acquisition, Software, Visualization, Writing – review & editing. AY: Formal analysis, Supervision, Writing – review & editing. CA: Data curation, Funding acquisition, Software, Supervision, Visualization, Writing – review & editing.

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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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References

1. Mohr M, Krustrup P, Bangsbo J. Match performance of high-standard soccer players with special reference to development of fatigue. *J Sports Sci.* (2003) 21:519–28. doi: 10.1080/0264041031000071182

2. Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jager R, et al. ISSN exercise and sports nutrition review update: research and recommendations. *J Int Soc Sports Nutr.* (2018) 15:38. doi: 10.1186/s12970-018-0242-y

3. Grgic J, Grgic I, Pickering C, Schoenfeld BJ, Bishop DJ, Pedisic Z. Wake up and smell the coffee: caffeine supplementation and exercise performance-an umbrella review of 21 published meta-analyses. *Br J Sports Med.* (2020) 54:681–8. doi: 10.1136/bjsports-2018-100278

4. Del Coso J, Munoz G, Munoz-Guerra J. Prevalence of caffeine use in elite athletes following its removal from the World Anti-Doping Agency list of banned substances. *Appl Physiol Nutr Metab.* (2011) 36:555–61. doi: 10.1139/h11-052

5. Tallis J, Clarke N, Morris R, Richardson D, Ellis M, Eyre E, et al. The prevalence and practices of caffeine use as an ergogenic aid in English professional soccer. *Biol Sport.* (2021) 38:525–34. doi: 10.5114/biolsport.2021.101125

6. Guest NS, VanDusseldorp TA, Nelson MT, Grgic J, Schoenfeld BJ, Jenkins NDM, et al. International society of sports nutrition position stand: caffeine and exercise performance. *J Int Soc Sports Nutr.* (2021) 18:1. doi: 10.1186/s12970-020-00383-4

7. Laurent D, Schneider KE, Prusaczyk WK, Franklin C, Vogel SM, Krssak M, et al. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. *J Clin Endocrinol Metab.* (2000) 85:2170–5. doi: 10.1210/jc.85.6.2170

8. Graham TE. Caffeine and exercise: metabolism, endurance and performance. *Sports Med.* (2001) 11:785–807. doi: 10.2165/00007256-200131110-00002

9. Foskett A, Ali A, Gant N. Caffeine enhances cognitive function and skill performance during simulated soccer activity. *Int J Sport Nutr Exerc Metab.* (2009) 19:410–23. doi: 10.1123/ijsnem.19.4.410

10. Ellis M, Noon M, Myers T, Clarke N. Low doses of caffeine: enhancement of physical performance in elite adolescent male soccer players. *Int J Sports Physiol Perform.* (2019) 14:569–75. doi: 10.1123/ijspp.2018-0536

11. Apostolidis A, Mougios V, Smilios I, Rodosthenous J, Hadjicharalambous M. Caffeine supplementation: ergogenic in both high and low caffeine responders. *Int J Sports Physiol Perform*. (2019) 14:650–7. doi: 10.1123/ijspp.2018-0238

12. Guerra MA, Caldas LC, De Souza HL, Vitzel KF, Cholewa JM, Duncan MJ, et al. The acute effects of plyometric and sled towing stimuli with and without caffeine ingestion on vertical jump performance in professional soccer players. *J Int Soc Sports Nutr*. (2018) 15:51. doi: 10.1186/s12970-018-0258-3

13. Jordan JB, Korgaokar A, Farley RS, Coons JM, Caputo JL. Caffeine supplementation and reactive agility in elite youth soccer players. *Pediatr Exerc Sci*. (2014) 26:168–76. doi: 10.1123/pes.2013-0134

14. Guest N, Corey P, Vescovi J, El-Sohemy A. Caffeine, CYP1A2 genotype, and endurance performance in athletes. *Med Sci Sports Exerc*. (2018) 50:1570–8. doi: 10.1249/MSS.0000000000001596

15. Boyett JC, Giersch GE, Womack CJ, Saunders MJ, Hughey CA, Daley HM, et al. Time of day and training status both impact the efficacy of caffeine for short duration cycling performance. *Nutrients*. (2016) 8:639. doi: 10.3390/nu8100639

16. Beaumont R, Cordery P, Funnell M, Mears S, James L, Watson P. Chronic ingestion of a low dose of caffeine induces tolerance to the performance benefits of caffeine. *J Sports Sci*. (2017) 35:1920–7. doi: 10.1080/02640414.2016.1241421

17. Rađa A, Kuvačić G, De Giorgio A, Sellami M, Ardigò LP, Bragazzi NL, et al. The ball kicking speed: a new, efficient performance indicator in youth soccer. *PLoS One*. (2019) 14:e0217101. doi: 10.1371/journal.pone.0217101

18. López-Samanes Á, Moreno-Pérez V, Travassos B, Del Coso J. Effects of acute caffeine ingestion on futsal performance in sub-elite players. *Eur J Nutr*. (2021) 60:4531–40. doi: 10.1007/s00394-021-02617-w

19. McKay AKA, Stellingwerff T, Smith ES, Martin DT, Mujika I, Goosey-Tolfrey VL, et al. Defining training and performance caliber: a participant classification framework. *Int J Sports Physiol Perform*. (2022) 17:317–31. doi: 10.1123/ijspp.2021-0451

20. Acar K, Mor A, Mor H, Kargın Z, Alexe DI, Abdioğlu M, et al. Caffeine improves sprint time in simulated freestyle swimming competition but not the vertical jump in female swimmers. *Nutrients*. (2024) 16:1253. doi: 10.3390/nu16091253

21. Bühler E, Lachenmeier DW, Schlegel K, Winkler G. Development of a tool to assess the caffeine intake among teenagers and young adults. *Ernahrungs Umschau*. (2014) 4:58–63. doi: 10.4455/eu.2014.011

22. Mora-Rodriguez R, Garcia Pallares J, Lopez-Samanes A, Ortega JF, Fernandez-Elias VE. Caffeine ingestion reverses the circadian rhythm effects on neuromuscular performance in highly resistance-trained men. *PLoS One*. (2012) 7:e33807. doi: 10.1371/journal.pone.0033807

23. Negra Y, Chaabene H, Fernandez-Fernandez J, Sammoud S, Bouguezzi R, Prieske O, et al. Short-term plyometric jump training improves repeated-sprint ability in prepuberal male soccer players. *J Strength Cond Res*. (2020) 34:3241–9. doi: 10.1519/JSC.0000000000002703

24. Padron-Cabo A, Lorenzo-Martinez M, Perez-Ferreiros A, Costa PB, Rey E. Effects of plyometric training with agility ladder on physical fitness in youth soccer players. *Int J Sports Med*. (2021) 42:896–904. doi: 10.1055/a-1308-3316

25. Borg G. Borg's perceived exertion and pain scales: Human kinetics; (1998)

26. Steidl-Müller L, Hildebrandt C, Müller E, Fink C, Raschner C. Limb symmetry index in competitive alpine ski racers: reference values and injury risk identification according to age-related performance levels. *J Sport Health Sci*. (2018) 7:405–15. doi: 10.1016/j.jshs.2018.09.002

27. Munoz A, Lopez-Samanes Á, Pérez-Lopez A, Aguilar-Navarro M, Moreno-Heredero B, Rivilla-García J, et al. Effects of caffeine ingestion on physical performance in elite women handball players: a randomized, controlled study. *Int J Sports Physiol Perform*. (2020) 15:1406–13. doi: 10.1123/ijspp.2019-0847

28. Erkmen N, Taskin H, Sanioglu A, Kaplan T, Basturk D. Relationships between balance and functional performance in football players. (2010) 2010: 21–29

29. Fox EL, Bowers RW, Foss ML. The physiological basis for exercise and sport. New York: Brown & Benchmark (1993).

30. Daneshjoo A, Mokhtar AH, Rahnama N, Yusof A. Effects of the 11+ and Harmoknee warm-up programs on physical performance measures in professional soccer players. *J Sports Sci Med*. (2013) 10:3001–6. doi: 10.22270/jmpas.v10i3.1148

31. Mor A, Acar K, Yilmaz A, Arsaloglu E. The effects of BCAA and creatine supplementation on anaerobic capacity and ball kicking speed in male football players. *J of Mens Health*. (2022) 1:1–9. doi: 10.31083/jomh.2021.058

32. Faidah N, Soraya GV, Erlichster M, Natzir R, Chana G, Skafidas E, et al. Detection of voluntary dehydration in paediatric populations using non-invasive point-of-care saliva and urine testing. *J Paediatr Child Health*. (2021) 57:813–8. doi: 10.1111/jpc.15325

33. Cohen J. Statistical power analysis for the behavioral sciences Academic press (2013).

34. Berjisian E, Naderi A, Mojtahedi S, Grgic J, Ghahramani MH, Karayigit R, et al. Are Caffeine's effects on resistance exercise and jumping performance moderated by training status? *Nutrients*. (2022) 14:4840. doi: 10.3390/nu14224840

35. Campos Y, Lago-Rodríguez Á, San Juan AF, Moreno-Pérez V, Lopez-Samanes A, Sánchez-Oliver AJ, et al. Caffeine supplementation improves physical performance without affecting fatigue level: a double-blind crossover study. *Biol Sport*. (2022) 39:521–8. doi: 10.5114/biolsport.2022.107479

36. Woolf K, Bidwell WK, Carlson AG. Effect of caffeine as an ergogenic aid during anaerobic exercise performance in caffeine naïve collegiate football players. *J Strength Cond Res*. (2009) 5:1363–9. doi: 10.1519/JSC.0b013e3181b3393b

37. Bazzucchi I, Felici F, Montini M, Figura F, Sacchetti M. Caffeine improves neuromuscular function during maximal dynamic exercise. *Muscle Nerve*. (2011) 43:839–44. doi: 10.1002/mus.21995

38. Behrens M, Mau-Moeller A, Weippert M, Fuhrmann J, Wegner K, Skripitz R, et al. Caffeine-induced increase in voluntary activation and strength of the quadriceps muscle during isometric, concentric and eccentric contractions. *Sci Rep*. (2015) 5:10209. doi: 10.1038/srep10209

39. Ranchordas MK, King G, Russell M, Lynn A, Russell M. Effects of caffeinated gum on a battery of soccer-specific tests in trained university-standard male soccer players. *Int J Sport Nutr Exerc Metab*. (2018) 28:629–34. doi: 10.1123/ijsnem.2017-0405

40. Jiménez SL, Díaz-Lara J, Pareja-Galeano H, Del Coso J. Caffeinated drinks and physical performance in sport: a systematic review. *Nutrients*. (2021) 13:2944. doi: 10.3390/nu13092944

41. Gomez-Bruton A, Marin-Puyalto J, Muñiz-Pardos B, Matute-Llorente A, Del Coso J, Gomez-Cabello A, et al. Does acute caffeine supplementation improve physical performance in female team-sport athletes? Evidence from a systematic review and meta-analysis. *Nutrients*. (2021) 13:3663. doi: 10.3390/nu13103663

42. Mielgo-Ayuso J, Calleja-Gonzalez J, Del Coso J, Urdampilleta A, León-Guereño P, Fernández-Lázaro D. Caffeine supplementation and physical performance, muscle damage and perception of fatigue in soccer players: a systematic review. *Nutrients*. (2019) 11:440. doi: 10.3390/nu11020440

43. San Juan AF, López-Samanes Á, Jodra P, Valenzuela PL, Rueda J, Veiga-Herreros P, et al. Caffeine supplementation improves anaerobic performance and neuromuscular efficiency and fatigue in olympic-level boxers. *Nutrients*. (2019) 11:2120. doi: 10.3390/nu11092120

44. Domaszewski P, Pakosz P, Konieczny M, Bączkowicz D, Sadowska-Krępa E. Caffeine-induced effects on human skeletal muscle contraction time and maximal displacement measured by tensiomyography. *Nutrients*. (2021) 13:815. doi: 10.3390/nu13030815

45. Tarnopolsky C. Effect of caffeine on the neuromuscular system—potential as an ergogenic aid. *Appl Physiol Nutr Metab*. (2008) 33:1284–9. doi: 10.1139/h08-121

46. Tarnopolsky CC. Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. *J Appl Physiol*. (2000) 5:1719–24. doi: 10.1152/jappl.2000.89.5.1719

47. Ferrauti A, Weber K, Strüder HK. Metabolic and ergogenic effects of carbohydrate and caffeine beverages in tennis. *J Sports Med Phys Fitness*. (1997) 4:258–66.

48. Ranchordas MK, Pratt H, Parsons M, Parry A, Boyd C, Lynn A. Effect of caffeinated gum on a battery of rugby-specific tests in trained university-standard male rugby union players. *J Int Soc Sports Nutr*. (2019) 16:17. doi: 10.1186/s12970-019-0286-7

49. Karayigit R, Forbes SC, Osmanov Z, Yilmaz C, Yasli BC, Naderi A, et al. Low and moderate doses of caffeinated coffee improve repeated Sprint performance in female team sport athletes. *Biology*. (2022) 11:1498. doi: 10.3390/biology11101498

50. Lorino AJ, Lloyd LK, Crixell SH, Walker JL. The effects of caffeine on athletic agility. *J Strength Cond Res*. (2006) 20:851–4. doi: 10.1519/r-17445.1

51. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*. (1999) 1:83–133.

52. de Kivit S, Kraneveld AD, Knippels LM, van Kooyk Y, Garssen J, Willemsen LE. Intestinal epithelium-derived galectin-9 is involved in the immunomodulating effects of nondigestible oligosaccharides. *J Innate Immun*. (2013) 5:625–38. doi: 10.1159/000350515

53. Impey J, Bahdur K, Kramer M. The mediating effects of caffeine ingestion and post-activation performance enhancement on reactive dive times in goalkeepers. *Ann Appl Sport Sci*. (2022) 10:979. doi: 10.52547/aassjournal.979

54. Impey J, Bahdur K, Kramer M. Reactive diving and sprinting performances in soccer goalkeepers improved by caffeine consumption and post-activation performance enhancement. *Ger J Exerc Sport Res*. (2022) 1:1–9. doi: 10.1007/s12662-021-00746-2

55. Karayigit R, Naderi A, Akca F, Cruz C, Sarshin A, Yasli BC, et al. Effects of different doses of caffeinated coffee on muscular endurance, cognitive performance, and cardiac autonomic modulation in caffeine naive female athletes. *Nutrients*. (2020) 13:2. doi: 10.3390/nu13010002

56. Bottoms L, Greenhalgh A, Gregory K. The effect of caffeine ingestion on skill maintenance and fatigue in epee fencers. *J Sports Sci*. (2013) 31:1091–9. doi: 10.1080/02640414.2013.764466

57. Balko S, Simonek J, Balko I, Heller J, Chytry V, Balogova K, et al. The influence of different caffeine doses on visual and audial reaction time with different delay from its consumption. *Sci Sports*. (2020) 35:358–63. doi: 10.1016/j.scispo.2019.11.004

58. Naumann T, Kindermann S, Joch M, Munzert J, Reiser M. No transfer between conditions in balance training regimes relying on tasks with different postural demands: specificity effects of two different serious games. *Gait Posture*. (2015) 41:774–9. doi: 10.1016/j.gaitpost.2015.02.003

59. Promsri A, Haid T, Federolf P. Complexity, composition, and control of bipedal balancing movements as the postural control system adapts to unstable support surfaces

or altered feet positions. *Neuroscience*. (2020) 430:113–24. doi: 10.1016/j.neuroscience.2020.01.031

60. Kara M, Patlar S, Stoffregen T, Erkmen N. Effect of caffeine on standing balance during perceptual-cognitive tasks. *MoHE*. (2018) 7:167–75. doi: 10.15282/mohe.v7i2.181

61. Armstrong LE, Casa DJ, Maresh CM, Ganio MS. Caffeine, fluid-electrolyte balance, temperature regulation, and exercise-heat tolerance. *Exerc Sport Sci Rev*. (2007) 35:135–40. doi: 10.1097/jes.0b013e3180a02cc1

62. Goldstein ER, Ziegenfuss T, Kalman D, Kreider R, Campbell B, Wilborn C, et al. International society of sports nutrition position stand: caffeine and performance. *J Int Soc Sports Nutr*. (2010) 7:5. doi: 10.1186/1550-2783-7-5

63. Del Coso J, Estevez E, Mora-Rodriguez R. Caffeine during exercise in the heat: thermoregulation and fluid-electrolyte balance. *Med Sci Sports Exerc*. (2009) 41:164–73. doi: 10.1249/mss.0b013e318184f45e

64. Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. *J Neurochem*. (2008) 105:1067–79. doi: 10.1111/j.1471-4159.2007.05196.x

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A literature review of biomarkers used for diagnosis of relative energy deficiency in sport

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Introduction: The review aims to summarize the markers used in diagnosing relative energy deficiency in sport (REDs) and compare them with the REDs CAT2 score.

Methods: A systematic search was performed in the PubMed, Web of Science, and SPORTDiscus databases during April 2023. The descriptors used were "athlete" AND "REDs," along with respective entry terms. The selection process followed the PRISMA 2020 recommendations, identifying 593 records, from which 13 studies were ultimately selected. Seventy-nine markers were identified and categorized into six groups: bone mineral density (BMD), metabolic resting rate, blood biomarkers, anthropometrics, nutritional intake, and performance parameters. The most frequently utilized biomarkers included BMD, anthropometric parameters (e.g., body mass index, body mass, and fat mass), and the triiodothyronine (T3) concentration.

Results: According to the REDs CAT2 pointed indicators, the biomarkers varied among the studies, while 7 out of the 13 included studies achieved a ≥60% agreement rate with this tool. The prevalence of low energy availability, an etiological factor in the development of REDs, was detected in 4 out of 13 studies, with an average of 39.5%.

Conclusion: In conclusion, this review highlights the most commonly used markers in diagnosing REDs, such as BMD, anthropometric parameters, and T3 hormone concentration. Due to the current inconsistencies, standardizing diagnostic methodologies is crucial for future research. By focusing on widely used markers, this review aids future research planning and result interpretation and points out the ongoing need for methodological consistency in evolving diagnostic tools.

Systematic Review Registration: https://www.crd.york.ac.uk/, PROSPERO (CRD42022320007).

KEYWORDS
REDs, relative energy deficiency in sport, athletes, markers, low energy availability

Introduction

The phenomenon of energy deficiency in sports is a widespread problem among athletes and has emerged as a new syndrome called relative energy deficiency in sport (REDs). In cooperation with the International Olympic Committee (IOC), the concept of REDs and its first official definition were introduced in 2014 (1). REDs is characterized by low energy availability (LEA), causing a profound impact on physiological functions within the organism. It includes, but is not limited to, areas such as abnormalities in metabolic function, menstrual cycle, bone health, immunity, protein synthesis, and cardiovascular health (1). The first symptoms that drew attention to possible disturbances of the athlete's bodily functions were menstrual cycle abnormalities (2). Based on these observations, the female athlete triad (FAT) was created in 1992. The first version of FAT included amenorrhea, osteoporosis, and disordered eating (3). During ongoing research, the definition was updated to include (1) low energy availability with or without disordered eating, (2) low bone mineral density (BMD), and (3) menstrual dysfunction (4). Thus, research had focused primarily on female athletes up to this point. However, it became evident that low energy availability affects many more human health and performance areas. Furthermore, it also affects male athletes (1, 5). Therefore, as mentioned above, the concept of REDs was developed (1). Since 2014, studies have increasingly focused on male athletes, but the number of studies involving female athletes is still noticeably higher.

Although REDs has been widely accepted and respected among the sports science community, there are still numerous limitations in its practical application in monitoring athletes (6, 7).

The etiological factor for REDs is LEA (1, 8); therefore, the diagnosis needs to involve parameters related to LEA. The common practice is to use screening questionnaire tools, which are well applicable to the field and suited for the initial detection of at-risk athletes in large populations (9). Nonetheless, questionnaire tools should be cautiously evaluated due to the frequent design of self-reported questions. It is recommended that questionnaires be used along with objective, practical measurements to provide a more in-depth assessment (10). However, one of the biggest challenges is the unification of the diagnostic methods for REDs (11) and the different methodologies used in studies, which can lead to challenges in assessing and evaluating the research findings (10, 12). Significant progress in this area has been enabled by the latest 2023 IOC Consensus statement and the associated IOC REDs Clinical Assessment Tool-Version 2 (IOC REDs CAT2) (13, 14). This tool has undergone internal expert voting statement validation and external validation through cross-agreement among REDs experts in clinical settings, enabling the identification of a more refined set of markers suitable for diagnosing REDs (14).

Further challenges within the REDs field also involve identifying markers suitable for diagnosis, determining their cutoff values, and fostering more effective collaboration among experts. Despite the great importance of the IOC 2023 Consensus statement (13) and the IOC REDs CAT2 (14), their integration into the diagnostic process and research may require time. Therefore, it is still relevant to highlight the methodological inconsistencies present in current studies.

A comprehensive summary of the markers used to assess REDs in the existing literature is not yet available. Such a review, combined with insights from the REDs CAT2 tool, could assist in selecting a more specific set of markers to increase consistency across studies and facilitate the interpretation and comparison of results. Therefore, this review aims to bridge this gap by providing an overview of practical measurement methods, the frequency of their use in the included studies, and a comparison with the REDs CAT2 tool.

Materials and methods

A systematic review was performed under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, updated in 2020 (15), to answer the research question. The review was registered at PROSPERO with number CRD42022320007.

Eligibility criteria and search strategy

Studies were eligible for inclusion if they met the following criteria: *participants:* athletes of both sexes, all disciplines, and advanced or elite level; *outcomes*: evaluation of the type, variety, number, and frequency of individual markers used in REDs diagnosis, as well as compliance with the IOC recommendation. Studies were ineligible if the outcomes of interest were not measured or the results were not described. Literature reviews, guidelines, letters to the editor, conference abstracts, dissertation thesis, and non-English language articles were also excluded. Considering that REDs was officially defined in 2014, the search was performed with a data range from March 2014 to. The search was conducted on Medline (via PubMed), Web of Science, and SPORTDiscus (via EBSCOhost) in April 2023. The search terms followed the descriptors from categories #1 and #2 related to "athlete" AND "relative energy deficiency" using the entry terms and derivative words (available in the Table 1).

Selection process and data extraction

The articles were imported into Rayyan systematic review software to proceed with the selection process. This process was performed as follows: (1) a researcher (KD) uploaded the articles from each database and then (2) excluded the review articles, letters to the editor, duplicates, and articles in non-English languages (identified by the software); (3) two independent researchers (DP and AW) screened the articles' titles and abstracts, and a third checked those excluded (KD); and (4) finally, two independent researchers (KD and AW) screened the full text of the articles for final inclusion. Any disagreements between reviewers were resolved by a third reviewer (AP). A prior pilot selection, with the first 25 articles, was performed to test the researchers' understanding, demonstrating an agreement of 88% between the two reviewers (DP and AW).

Data related to the sample characteristics (e.g., sex, sport modality, age, and size), the presence of REDs, biomarkers used in REDs diagnosis [e.g., hormones, resting metabolic rate, bone mineral density, blood glucose, body mass index (BMI), and

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TABLE 1 Characteristics of the included studies and categories of used markers (n = 13 studies).

Sample characteristics			BMD	RMR	Blood biomarkers	Anthropometrics parameters	Nutritional intake	Performance
Study	Sex sport modality sample size/age	Control group		✓	✓	✓	✓	✓
Hooper et al. (16)	NCAA Division 1 female distance runners N = 7/22.3 ± 1.5 years	—	—	—	✓	✓	✓	✓
Õnnik et al. (17)	High-level male and female N = 30/28.0 ± 3.75 years and N = 26/28.6 ± 6.34 years	Male and female control groups N = 29/24.1 ± 3.83 years and N = 29/24.97 ± 5.74 years	✓	✓	✓	✓	✓	✓
Torstveit et al. (18)	Well-trained male endurance athletes N = 53/35.3 ± 8.3 years	—	—	✓	✓	—	✓	✓
Keay et al. (19)	Competitive male road cyclists N = 45/36.2 ± 14.3 years	—	✓	✓	✓	✓	✓	✓
Stenqvist et al. (20)	Well-trained male cyclists N = 20/33.3 ± 6.7 years	—	✓	—	✓	✓[a]	—	✓
Keay et al. (21)	Competitive male road cyclists N = 50/35.0 ± 14.2	—	✓[a]	✓	✓	✓	—	✓
Stenqvist et al. (22)	Olympic-level male athletes N = 44/24.7 ± 3.8 years	–	✓	✓	–	✓	✓	✓
Mathisen et al. (23)	Female fitness athletes N = 25/28.1± 5.5 years	Female references N = 26/29.8± 6 years	✓	✓	✓	✓	✓	✓
Civil et al. (24)	Royal Conservatoire of Scotland female ballerinas N = 20/18.1 ± 1.1 years	—	✓	✓	✓	✓	✓	✓
Lee et al. (25)	Male Korean collegiate soccer players N = 10/9.1 ± 0.6 years	—	✓	—	✓[a]	✓	✓	—
Pritchett et al. (26)	National-level para-athletes: males and females N = 9/27 ± 8 years and N = 9/27 ± 7 years	—	✓	—	✓	✓	✓	—
Gibson-Smith et al. (27)	Elite climbers: males and females N = 20/29.1 ± 5.4 and N = 20/31.4 ± 7.7 years	—	—	✓	✓	✓	✓	✓
Kalpana et al. (28)	National-level male Kho-Kho players N = 52/16–31 years	—	✓	—	—	—	—	—

BMD, bone mineral density; T3, triiodothyronine; BMI, body mass index; BM, body mass; FM, fat mass; RMR, resting metabolic rate; EA, energy availability; EI, energy intake; FFM, fat-free mass; IGF-1, insulin-like growth factor 1; EEE, exercise energy expenditure; FTP, functional threshold power; GH, growth hormone, ALP, alkaline phosphatase; LBM, lean body mass; TEE, total energy expenditure; NEAT, non-exercise activity thermogenesis; DIT, dietary induced thermogenesis; TSH, thyroid-stimulating hormone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; WBC, white blood cell; RBC, red blood cell; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; LDL, low density lipoprotein; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; AEE, activity energy expenditure; PR, personal record; IAFF score, International Association of Athletics Federations score.

[a]These markers were evaluated via comparing groups with low vs. adequate energy availability. However, these conditions were only assessed using the questionnaire tools; therefore, these conclusions should be taken with caution.

cholesterol], and any potentially relevant outcomes were extracted from included studies by two researchers (KD and AW).

Methodological quality

The assessment of methodological quality for the articles with a descriptive approach was performed using the STROBE tool (29) and for those with an intervention approach was performed by ROBINS-I (30). Three researchers participated in this phase (KD, AW, and AP). The STROBE checklist assesses the quality of cohort, case–control, and cross-sectional studies. It contains 22 items assessing risk factors for bias. Response options are a score of 0 if the articular checklist item is not fulfilled, 1 if the articular checklist item is fulfilled, and NA if the checklist item does not apply to the specific publication. Based on the sum of the total score and the percentage gain of the possible maximum, the quality of the study is then evaluated as follows: ≥85 = excellent, 70 to <85 = good, 50 to <70 = fair, and <50 = poor, as used previously. The ROBINS-I rating system is based on seven domains, each consisting of a subset of questions focusing on possible areas of systematic error. The domains include confounding, participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported results. In this review, we used only domains 2–7 for evaluation; more details on this process are provided in the Discussion section. The response options are "Yes," "Probably yes," "Probably no," "No," and "No information." Based on the continuous responses, each domain is then evaluated as a whole, and the rating of all the domains is reflected in the labeling of the study as "Low risk," "Moderate risk," "Serious risk," and "Critical risk" of bias.

REDs CAT2 agreement

The biomarkers used in the included studies were compared with the IOC REDs CAT2 (14), an improved version derived from the original IOC REDs Clinical Assessment Tool (CAT) introduced in 2015 (31). The development of the IOC REDs CAT2 involved internal validation through expert voting statements and external validation via clinical cross-agreement assessments by experts. The assessment protocol of IOC REDs CAT2 comprises three sequential steps:

I. Initial screening using population-specific REDs questionnaires or clinical interviews, with individuals deemed at risk moving on.
II. Assessment of various REDs signs/symptoms to uniform the Severity/Risk Assessment Tool and Stratification, with guidelines for sports participation; data obtained from these steps serve as the basis.
III. Physician-led final clinical diagnosis/stratification and associated implementation of a treatment plan, ideally involving a collaboration of a multidisciplinary health team and REDs performance (14).

Based on the scoring outcomes of primary and secondary indicators, the risk is categorized into four-color traffic-light severity/risk classifications, ranging from "none" to "very low," "mild," "moderate to high," to "very high/extreme." Recommendations concerning the monitoring of athletes, participation in training and competitions, and medical interventions complement these classifications. In addition, REDs CAT2 incorporates a set of potential indicators deemed emerging (14).

In the review process, markers identified in the included studies were compared to those outlined in the REDs CAT2. Given the focus on objective measurement methods, subjective markers obtained through interviews or questionnaires were omitted from this comparison. Subsequently, reviewer KD computed agreement rates between each study and the REDs CAT2 tool for scored, potential, and overall indicators. A second independent reviewer (AW) checked this process to ensure reliability.

Results

Study characteristics and methodological quality

In total, 595 articles were found in the databases matching the combination of keywords entered. After excluding articles that were duplicates ($n = 96$) and for other reasons, such as those written in a foreign language (non-English) ($n = 10$) and with no access ($n = 1$), 488 articles were evaluated during the title and abstract screening. Of these articles, 155 were excluded through the review method, and 463 did not meet the eligibility criteria. For 25 articles, the full text was assessed; of these, 12 studies were excluded due to non-compatibility. Therefore, 13 studies were included in the final process (Figure 1).

The main characteristics and the categories of the REDs markers used in the included studies (bone mineral density, resting metabolic rate, blood markers, anthropometric parameters, nutritional intake, and performance) are presented in Table 1. Most of these studies focus on female athletes (7 out of 13); the most investigated disciplines were endurance sports, team sports, ballet, climbing, or a mix of disciplines or para-athletics disciplines. Athletes competed at the performance levels of well-trained, competitive, elite, national, and Olympic levels. Two studies also included a control group.

Among the 13 papers, 12 presented a descriptive study design and 1 presented an intervention design. For the descriptive ones, the methodological quality, assessed by the STROBE tool, demonstrated a range from good to excellent quality. Specifically, six studies were rated as excellent (17, 18, 20, 23–25) and six were rated as good (16, 21, 22, 26–28) (see the Table 2). One paper was designed as an intervention and demonstrated a moderated risk of bias based on the ROBINS-I tool.

Overview of biomarkers used in REDs diagnosis and the frequency of their use

We found 79 biomarkers used to determine the presence of REDs in the 13 included studies. Table 2 presents the complexity and diversity of the biomarkers used within the included studies. The biomarkers were categorized into five groups (bone mineral density biomarkers, resting metabolic rate biomarkers, blood biomarkers, anthropometric parameters, nutritional intake parameters, and performance parameters). All 13 studies used at least two (or more) categories of markers to determine the presence of REDs.

Table 3 presents the quantification of biomarkers used to assess REDs and complements Table 2. It shows a comprehensive overview of the frequency of their use in the included studies. The biomarkers most often used were BMD, BMI, BM (body mass), FM (fat mass), and T3 (triiodothyronine) blood concentrations, which were involved in 10 of the 13 studies (76.9%). Nine studies (69.2%) used the measurement of RMR (resting metabolic rate), while 8 studies (61.5%) used total testosterone level and EI (energy intake). Seven studies (53.9%) used nutritional parameters such as carbohydrate, protein, and fat intake EA (energy availability) was used in six studies (46.2%).

Discussion

This review systematically compiles a list of methods utilized in diagnosing REDs. Our analysis of included studies revealed that the most frequently used biomarkers in current studies are BMD, BMI,

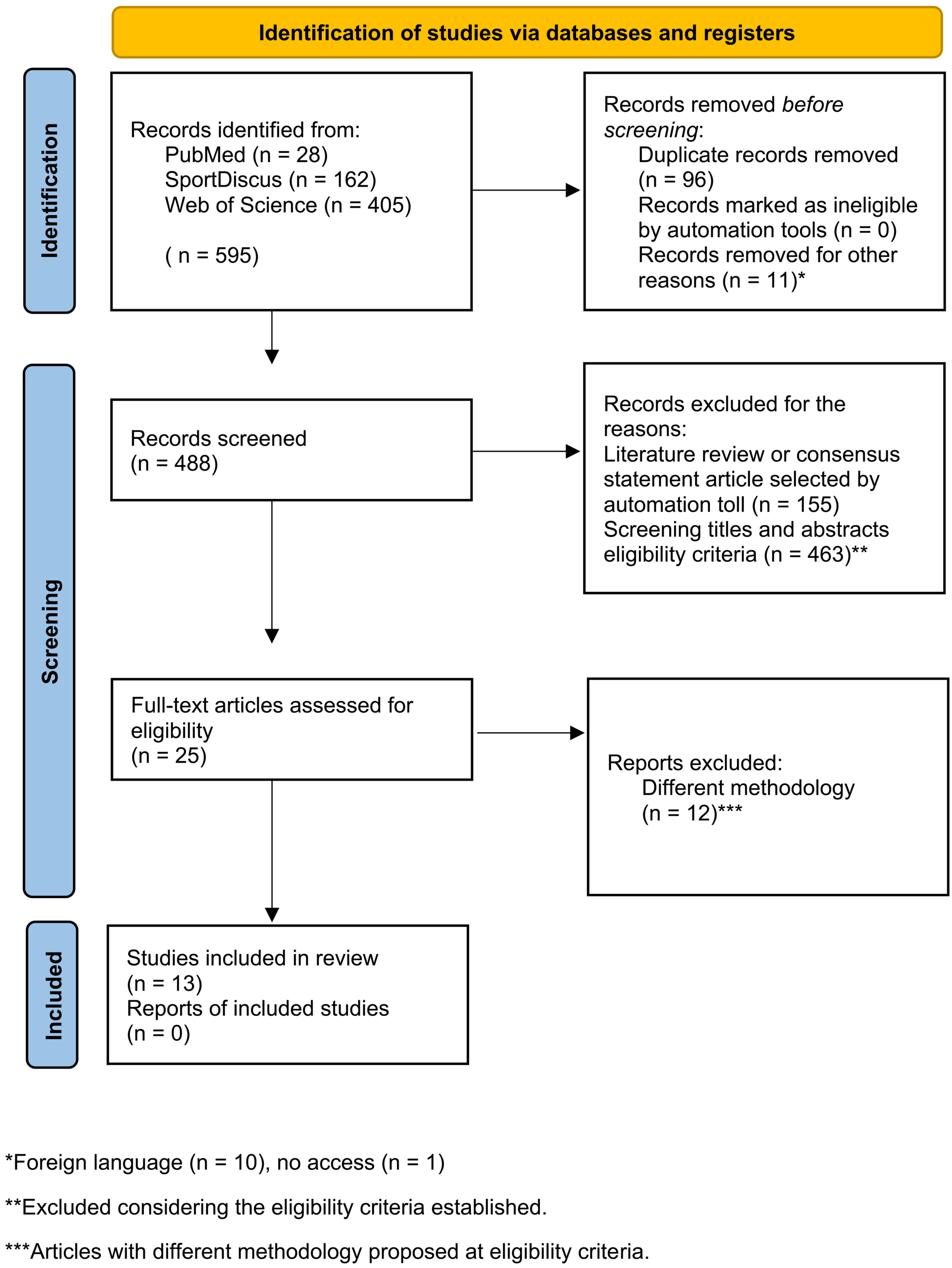

FIGURE 1
Flowchart diagram of the study selection process (PRISMA 2020) (15).

BM, FM, and blood T3 concentration, included in 10 out of 13 studies (76.9%).

While the 2023 IOC Consensus statement marked a significant milestone in selecting appropriate diagnostic markers for REDs, the authors emphasized the necessity for ongoing updates and revisions. This included refining the range of recognized *sequelae* associated with REDs and reassessing the markers themselves. Thus, a critical examination of the strengths and limitations of

TABLE 2 Overview of biomarkers used in REDs diagnosis and main outcomes ($n = 13$).

	REDs markers					
Study	BMD	RMR	Blood biomarkers	Anthropometric parameters	Nutritional intake	Performance
Hooper et al. (16)	—	↔ (pre-XC vs. post-XC) ↔ RMR ratio (any time point) ↑ (post-XC vs. pre-track) ↑ (pre-XC vs. pre-track)	T3 ↔ (any time point) Ferritin ↓ (pre-XC vs. post-XC) Ferritin ↑ (post-XC vs. pre-track) Vitamin D ↓ (pre-XC vs. post-XC) Vitamin D ↑ (post-XC vs. pre-track)	BMI ↔ (pre-XC vs. post-XC) BM ↔ (pre-XC vs. post-XC) BM ↑ (post-XC vs. pre-track) FFM ↔ (pre-XC vs. post-XC) FM ↔ (pre-XC vs. post-XC)	EA ↓ (vs. ACSM recommendations)	Performance relative to the PR ↔
Õnnik et al. (17)	LS-BMD ↔ (males, females) RF-BMD ↔ (males, females) TB-BMD ↔ (males, females)	—	LH ↔ FSH ↔prolactin ↔ testosterone ↔free T4 ↔ TSH ↔ T3 ↔GH ↔ IGF-1 ↔insulin ↓ (females) cortisol ↑ (males) WBC ↔ RBC ↓ (males) hemoglobin ↔ hematocrit ↓ (males) hematocrit ↔ (females) neutrophils ↔ (males) neutrophils ↑ (females) lymphocytes ↔ (males) lymphocytes ↓ (females) estradiol ↓ (males) estradiol ↔ (females) eosinophils ↔ basophils ↔	BMI males ↓ (vs. control) BMI females↓ (vs. control) BM females ↓ (vs. control) BM males ↓ (vs. control)	EI ↔ (males, females) relative EI (per kg body weight) ↑ (females) protein intake + relative value ↔ fat intake + relative value ↔ (males, females) carbohydrate intake + relative value ↔ (males) carbohydrate intake + relative value ↑ (females) dietary fiber intake ↔ dietary fiber intake relative value ↔ (males) dietary fiber intake relative value (per kg body weight) ↑ (females) sodium intake + relative value ↔ calcium intake ↑ calcium intake relative value ↔ (males) calcium intake relative value ↑ potassium intake ↔ potassium intake relative value ↑	VO_2 max ↔ (measured only in athletes) IAFF score ↔ (points) (measured only in athletes)
Torstveit et al. (18)	—	RMR ↔ RMR ratio ↔ low RMR ratio ↔	Cortisol ↑ (group high EXDS score vs. group low) Cortisol highest quartile of range ↑ (group high EXDS score vs. group low) high cortisol (number of subjects) ↔ testosterone ↔ T3 ↔ IGF-1 ↔ insulin ↔ glucose ↔ testosterone: cortisol ratio ↔ cortisol: insulin ratio ↔	BMI ↔ BM ↔ FFM ↔ FM ↔ sleeping heart rate ↔	EEE (kcal/day) ↑ (group high EXDS score vs. group low) EI ↔ carbohydrate intake ↔ protein intake ↔ fat intake ↔ fiber intake ↔ energy balance (kcal/day) ↓ (group high EXDS score vs. group low) EA ↔ low EA (number of subjects with low EA) ↔	VO_2 peak ↔ Active in sport ↔ exercise (hours/week) ↑ (group high EXDS score vs. group low)
Keay et al. (19)	↓ (Negative changes in both areas vs. before intervention) ↓ (negative changes in one areas vs. before intervention) ↑ (positive changes in both areas vs. before intervention) ↑ (positive changes in one areas vs. before intervention)	↓ (respondents with low EA) ↓ (respondents without skeletal loading exercise)	Testosterone ↔ testosterone/Z-score ↔ vitamin D ↑ (educated group vs. control group) vitamin D/Z-score ↔ T3 ↑ (educated group vs. control group) T3/Z-score ↔ albumin ↑ (educated group vs. control group) albumin/Z-score ↔ calcium ↔ alkaline phosphatase ↔ alkaline phosphatase/Z-score ↔ corrected calcium ↔ corrected calcium/Z-score ↔	—	EA ↑ (educated vs. control group)	Points gained over the racing season ↓ (group with negative changes in EA vs. before intervention) FTP ↓ (group with negative changes in EA vs. before intervention) points gained over the racing season ↑ (group with positive changes in EA vs. before intervention) FTP ↑ (group with positive changes in EA vs. before intervention)
Stenqvist et al. (20)	↔	Absolute RMR ↓ relative RMR ↓ RMR ratio ↓	Total testosterone ↑ free testosterone ↔ SHBG ↔ T3 ↓ cortisol ↑ insulin ↔ IGF-1 ↔ free testosterone: cortisol ratio ↔ total testosterone:cortisol ratio ↔	BMI ↔ BM ↔ FFM ↔ FM ↔	EI ↔ carbohydrate intake ↔ relative carbohydrate intake ↔ protein intake ↔ relative protein intake ↔ fat intake ↔ relative fat intake ↔	VO_2 peak ↔ FTP (W) ↑ FTP (W/kg) ↑ aerobic peak power output (W) ↑ training volume per week ↔

(*Continued*)

TABLE 2 Continued

Study	REDs markers					
	BMD	RMR	Blood biomarkers	Anthropometric parameters	Nutritional intake	Performance
Keay et al. (21)	↓[a]	—	Mean total testosterone ↓ (lower end of the reference range) mean vitamin D ↓ T3 ↔ (lower half of the reference range) albumin ↔ calcium ↔ alkaline phosphatase ↔	BMI[a] ↓ FM[a] ↓ VAT mass[a] ↓	—	FTP (W/kg) ↔ training load ↔
Stenqvist et al. (22)	L1–L4 Z-score ↔ Femur Z-score ↔	RMR ratio ↓ (low vs. normal RMR) relative RMR ↓ (low vs. normal RMR)	Testosterone ↔ free testosterone ↔ T3 ↔ cortisol ↔ total cholesterol ↔ LDL cholesterol ↔	BMI ↔ BM ↔ FFM ↔ FFM index ↔ FM ↔ FM index ↔	—	Training volume per month ↔
Mathisen et al. (23)	↔	RMR FA ↓ (baseline vs. 2 weeks before competition)	—	FM ↓ (FA vs. FR) BMI ↔ BM ↔ LBM ↔ adult BM difference ↔ history of ED (self-reported) ↔ current ED (self-reported) ↔	EI (kcal) ↑ (FA vs. FR) EI (kcal/kg LBM) ↑ (FA vs. FR) carbohydrate intake (g) ↑ (FA vs. FR) carbohydrate intake (g/kg BW) ↑ (FA vs. FR) protein intake (g/) ↑ (FA vs. FR) protein intake (g/kg BW) ↑ (FA vs. FR) fat (energy %) ↑ (FA vs. FR) dietary fiber ↔	Experience with regular exercise ≥5 years ↔ exercising ≥5 times per week current year ↔
Civil et al. (24)	Total BMD ↔ Z-score ↔	↔	Vitamin D ↔	BM ↓ (after week of observation) BMI ↔ WHR ↔ FM ↔ FFM ↔	EI ↔ DIT ↔ EA (calculated) ↔ TEE (total energy expenditure) ↑ energy balance ↓ NEAT ↑ EEE ↑ fiber intake ↑ fluid intake ↑ fat intake ↔ carbohydrate intake ↔ protein intake ↔	Training volume per week (self-reported) ↔
Lee et al. (25)	BMD ↔ Z-score ↔	REE ratio ↓ REEm/FFM ↓	T3 ↔ cortisol ↔ insulin ↔ GH ↔ IGF-1 ↑ (vs. REE ratio) testosterone ↔ leptin ↔	BM↔ BMI ↔ FM ↔ FFM ↔ sleeping energy expenditure ↔	EI ↓ DIT ↓ EEE ↔ EPOC ↔ NEAT ↔ hourly resting energy expenditure ↔ TEE ↔ 24 h energy balance ↔ 24 h EA ↔ within-day energy balance <0 kcal (h/day) ↔ within-day energy balance <−400 kcal (h/day) ↔ largest hourly deficit (kcal) ↔	VO_2 max ↔
Pritchett et al. (26)	Z-score ↓ (females) Z-score ↓ (males)	—	Testosterone ↓ (males) IGF-1[a] ↑ (females) progesterone ↓ T3 ↔ estradiol ↔	—	—	—
Gibson-Smith et al. (27)	—	—	Serum ferritin ↓ (females) transferrin saturation ↔ sum of 8 SF (serum ferritin) ↑	BM ↓ BMI ↓ FM ↑ arm girth ↓ waist girth ↓ calf girth ↓ gluteal girth ↔	EI (kcal·kgFFM-1·day-1) ↑ (females) carbohydrate intake ↔ protein intake ↔ fat intake ↔ iron intake ↔ iron intake density (mg/1,000 kcal) ↔	—
Kalpana et al. (28)	Z-score ↓ bone mineral content ↔ BMD ↔ T-score ↔	BMR ↓	Serum calcium ↔ serum vitamin D3 ↔ serum free T3 ↔ hemoglobin ↔ serum albumin ↔ serum creatine ↔ SGOT ↔ SGPT ↔	BM ↔ FM ↔ overall sleep quality ↓ LBM ↑	EA ↓ carbohydrate intake ↓ protein intake ↓ fat intake ↓ vitamin A intake ↓ vitamin B2, B6, B9 intake ↓ iron intake ↓ zinc intake ↓ fluid intake ↓ AEE ↑ daily energy expenditure ↑ EI ↓ daily energy expenditure/BMR ↔	Agility ↓ speed ↔

Pre-CX, athletes before cross-country season; Post-CX, athletes after cross-country season; Pre-track, athletes before track season; low RMR ratio, number of subjects with low RMR; BMD, bone mineral density; T3, triiodothyronine; T4, thyroxine; BMI, body mass index; BM, body mass; FM, fat mass; EXDS score, exercise dependence scale score; RMR, resting metabolic rate; EA, energy availability; EI, energy intake; FFM, fat-free mass; IGF-1, insulin-like growth factor 1; EEE, exercise energy expenditure; FTP, functional threshold power; GH, growth hormone, ALP, alkaline phosphatase; LBM, lean body mass; TEE, total energy expenditure; NEAT, non-exercise activity thermogenesis; DIT, dietary induced thermogenesis; TSH, thyroid-stimulating hormone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; WBC, white blood cells; RBC, red blood cells; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; LDL, low density lipoprotein; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; AEE, activity energy expenditure; PR, personal record; IAFF score, international association of athletics federations score.

Signs ↑ (increase) and ↓ (decrease) indicate a statistically significant result, and sign ↔ indicates a statistically insignificant result.

[a]The markers were evaluated via comparing groups with low vs. adequate energy availability.

TABLE 3 Frequency of markers measured among the studies.

Number of studies	Relative frequency	Markers
10	76.9	BMD, T3, BMI, BM, FM
9	69.2	RMR
8	61.5	Total testosterone, EI
7	53.9	Carbohydrate intake, protein intake, fat intake
6	46.2	EA, FFM, training volume
5	38.5	Vitamin D, cortisol, IGF-1
4	30.8	Insulin, dietary fiber intake, EEE
3	23.1	Albumin, calcium, energy balance, FTP, TEE
2	15.4	Ferritin, free testosterone, estradiol, GH, hemoglobin, ALP, LBM, iron intake, fluid intake, NEAT, DIT, VO_2max, VO2 peak
1	7.7	Free thyroxine, transferrin saturation, SHBG, prolactin, LH, FSH, progesterone, TSH, leptin, glucose, WBCs, RBCs, hematocrit, neutrophils, lymphocytes, basophils, creatine, SGOT, SGPT, total cholesterol, LDL, WHR, girth measurement, VAT, vitamin A intake, vitamin B2 intake, vitamin B6 intake, vitamin B9 intake, calcium intake, sodium intake, potassium intake, zinc intake, AEE, sleeping heart rate, overall sleep quality, sleeping energy expenditure, points gained over the racing season, performance relative to the PR, agility, IAFF score, speed, aerobic peak power

BMD, bone mineral density; T3, triiodothyronine; BMI, body mass index; BM, body mass; FM, fat mass; RMR, resting metabolic rate; EA, energy availability; EI, energy intake; FFM, fat-free mass; IGF-1, insulin-like growth factor 1; EEE, exercise energy expenditure; FTP, functional threshold power; GH, growth hormone, ALP, alkaline phosphatase; LBM, lean body mass; TEE, total energy expenditure; NEAT, non-exercise activity thermogenesis; DIT, dietary induced thermogenesis; TSH, thyroid-stimulating hormone; SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; WBCs, white blood cells; RBCs, red blood cells; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; LDL, low density lipoprotein; WHR, waist-to-hip ratio; VAT, visceral adipose tissue; AEE, activity energy expenditure; PR, personal record; IAFF score, international association of athletics federations score.

these markers, alongside evaluating their ability to reflect individuals' health status accurately, remains imperative.

Anthropometric parameters

Anthropometric parameters, such as BMI and body composition, are widely used in medical practice. According to the Centers for Disease Control and Prevention's recommendations for general practitioners (32), BMI is a simple, inexpensive, and non-invasive method of estimating body fat and health risk, requiring no special equipment. However, several studies have pointed to the inaccuracy of BMI, particularly among patients with different ethnic backgrounds or an inability to distinguish body weight between body fat and muscle mass (33, 34). Thus, although this calculation can provide valuable information in the REDs diagnostic process, as with other markers, it cannot be evaluated in isolation (35). According to REDs CAT2, BMI is considered a potential indicator in assessing REDs risk, underscoring that the need for further research to quantify the parameters and cutoffs more accurately (14).

To accurately determine body composition, it is necessary to use valid methods that contribute to an objective assessment of the athlete's overall condition. Body composition and adipose tissue thicknesses can be accessed via skinfold measurement. However, B-mode ultrasound is a more reliable and preferred method, which can provide good results even in lean individuals (36). Despite its costliness, the dual-energy x-ray absorptiometry (DXA) measurement is also the recommended method of choice as the gold standard for assessing body composition (37).

The authors of the IOC Consensus statement also pointed out that too much focus on anthropometric parameters and body composition can intensify the pressure placed on athletes, especially on adolescents under the age of 18 years (13, 38). It is, therefore, essential to identify valid and reliable methods and develop guidelines for interpreting, managing, and communicating with athletes (39).

Bone health

Biomarkers assessing bone health are among the most used, as shown by the results of this review. Impaired bone health has been associated with low energy availability from its onset. It was also included in the original definition of the female athlete triad (34), from which the concept of REDs was developed (1). Low energy availability affects bone health through reduced levels of hormones such as estrogen, leptin, and T3 associated with insulin-like growth factor 1 (IGF-1) secretion (40–43). In addition, inadequate intake of essential nutrients, including protein, calcium, or vitamin D, has been linked to the low energy intake associated with REDs (44, 45).

DXA is the most used method for measuring bone mineral density (46) and is also noted as a "preferred method" in the 2023 IOC Consensus statement (13). According to REDs CAT2, the authors recommend the following as a positive finding:

- *Premenopausal women and men aged <50 years: BMD Z-score <−1 at the lumbar spine, total hip, or femoral neck or decreased BMD Z-score from previous testing.*
- *Children/adolescents: BMD Z-score <−1 at the lumbar spine or total body less head or decreased BMD Z-score from the last testing (may be due to bone loss or insufficient bone gain)* (14).

Some previously published studies on energy availability have also used markers of bone turnover derived from blood samples. The research findings by Ihle and Loucks (47) suggest that changes may be apparent after 3 days of LEA. The findings of the study by Papageorgiou et al. (48) showed that 5 days of LEA below 15 kcal/day leads to changes in bone turnover markers in women, but no significant changes were found in men. A year later, Papageorgiou et al. (49) conducted another study involving a group of eumenorrheic women in whom 3-day LEA through dietary energy restriction resulted in changes in bone formation but not bone resorption. However, these bone turnover markers are not established due to the number of factors that may influence them. The time taken for the manifestation of changes might also be significantly influenced by variables such as the severity of LEA.

Moreover, markers reporting bone mineral density status should continually be assessed in the context of supplementary information, considering the specificity of each sport discipline. For example, the bone density of weightlifters generally reaches higher values than the reference range (50), and average values may indicate reduced BMD in these athletes.

Resting metabolic rate

RMR was assessed in nine of the 13 included studies, accounting for 69.2% (see Table 3). RMR represents the energy necessary to maintain homeostasis while at rest. Unlike basal metabolic rate, which necessitates strict conditions such as a 12-h fasting period and a thermoneutral environment, RMR can be measured throughout the day (51). The suppressed RMR associated with LEA may be explained by adaptive responses aimed at conserving energy (52).

Various methodologies are employed in studies to determine RMR. Indirect calorimetry is often called the gold standard but requires specialized equipment (53). Consequently, researchers usually resort to estimating RMR using predictive equations, such as those proposed by Cunningham (54), Harris and Benedict (55), or Owen et al. (56). Another approach is the RMR ratio, defined as the ratio between measured RMR and predicted RMR. Some studies suggest that the RMR ratio serves as a valid indicator of LEA (57, 58). However, it is advisable to evaluate RMR in conjunction with other markers due to variations in the degree of metabolic suppression among athletes. These variations are influenced by factors such as the severity of LEA (58).

The 2023 IOC Consensus statement recognizes RMR testing as a "used and recommended" method for identifying impaired energy metabolism. Specifically, the endorsed procedures include indirect or room calorimetry measurements (13). In addition, REDs CAT2 identifies RMR as a potential indicator, with a reduced or low RMR [<30 kcal/kg fat-free mass (FFM)/day] or an RMR ratio (<0.90) considered indicative of the condition (14). However, Sterringer and Larson-Meyer (59) pointed out that a threshold of 0.9 may not be appropriate for all cases. In particular, for studies using the Cunningham equation from 1991 (60) or DEXA measurement, a threshold of 0.9 may lead to an underestimation of the prevalence of LEA.

Blood biomarkers—hormone concentration

One of the most used markers in the included studies (76.9%) was T3. It is one of the hormonal agents released by the thyroid gland and is indispensable in energy metabolism and growth (61). T3 is also involved in the reproductive process (62) and bone tissue metabolism through the local production of IGF-1 (63). Although its concentration is strongly associated with metabolic functions, this marker still needs to be evaluated in the context of other methods. This is because its concentration may be affected by many conditions, such as circadian rhythms, thyroid disease, alterations in serum binding proteins, or other associated medical conditions (64, 65). Clinically or subclinically low total or free T3 is also considered one of the primary REDs indicators listed in REDs CAT2 (14), and clinically or subclinically low IGF-1 is included in the list of potential indicators.

Testosterone concentration is also a frequently used marker in the studies (61, 5%). Subclinically low total or free testosterone is listed in REDs CAT2 primary indicators; clinically low total or free testosterone is considered a severe primary indicator (counted as two primary indicators) (14). While disturbances in the menstrual cycle may affect the hypothalamic–pituitary–gonadal axis in women, this condition may not be detected as early in male athletes. Thus, for male athletes, in addition to testosterone levels, it is often necessary to consider self-reported data, such as the presence of low libido or decreased frequency of morning erections, in the diagnosis of REDs. Thus, as already mentioned, a combination of diagnostic methods is required. In women, low energy availability disrupts luteinizing hormone (LH) pulsatility, which further affects the hypothalamic–pituitary–gonadal axis, including levels of follicle-stimulating hormone (FSH), estrogens, and progesterone (66, 67). Two studies tested estradiol levels (17, 26), while one study tested levels of LH, FSH (17), and progesterone (26). However, the REDs CAT2 tool does not directly use these hormones as female reproductive cycle function indicators. Instead, it uses self-reported data on the presence of primary amenorrhea, secondary amenorrhea, or oligomenorrhea (14). LEA also affects other endocrine pathways such as cortisol, leptin, growth hormone, IGF-1 axis, sympathetic and parasympathetic tone, or thyroid hormones (66).

Calculation of energy availability

The calculation of energy availability has been used in 46.2% of the included studies (6 out of 13). Given that low energy availability is a direct etiological factor in developing REDs (1), its inclusion in diagnostic methods appears logical. The variables required for the calculation of energy availability can also be obtained in a non-invasive and non-burdensome way. The prevalence of low energy availability was detected in four of the thirteen included studies: 67% (16), 23% (18), 22% (24), and 46% (28). In another study, a prevalence of 28% was assessed through the SEAQ-I questionnaire (21). In conclusion, the mean observed prevalence of LEA across the studies is 39.5%.

However, previous studies have indicated that calculating energy availability carries a high risk of error (9). Sources of this inaccuracy can include energy intake, while data obtained through nutritional recall may underestimate actual intake by 10%–20% (68, 69), and even cases of an underestimation of 50% are not uncommon (70). The measurement of energy expenditure also needs to be evaluated cautiously. Various methods of assessing energy expenditure are used across studies, such as doubly labeled water technique, direct calorimetry, indirect calorimetry, accelerometry, heart rate monitoring, or pedometry (71). Nevertheless, using more accurate methods is often complicated by the high cost of these devices in research settings. Therefore, epidemiological studies frequently rely on self-reported methods, which can lead to significant inaccuracies in the observed outcomes (72). Thus, calculating energy availability may serve as a valuable complementary method for diagnosing REDs and could also be beneficial in determining the optimal therapeutic approach (73). However, like other markers, it should not be evaluated in isolation.

Reference markers according to IOC REDs CAT2

Although there is still no uniform and standardized methodological approach for the diagnosis of REDs, the new IOC Consensus statement of 2023 provides a comprehensive overview of:

(I) Preferred methods;
(II) Used and recommended methods; and
(III) Potential methods applicable for these purposes (13).

The IOC REDs CAT2 is closely aligned with this document and summarizes the LEA indicators, including symptoms and signs, that have emerged as current best practices for clinical assessment and research. Based on the evaluation of these indicators, an athlete may be included in one of the four-color traffic-light severity/risk categories. Each category is also associated with recommendations for athletic participation, athlete monitoring, medical intervention, or even full medical support, which may require the athlete's temporary exclusion from training and competition (14).

The authors of the REDs CAT2 emphasize that this advanced tool should not be used in isolation but in combination with clinical consideration and other tools, such as screening questionnaires. In addition, they warn that the tool's reliability decreases if all the included indicators cannot be assessed and that REDs CAT2 is not a substitute for professional clinical diagnosis, advice, and/or treatment (13). Nevertheless, REDs CAT2 represents a scientifically supported system for evaluating LEA indicators and was selected as a reference tool to assess the quality of the markers used in the included studies.

As this review primarily focuses on objective methods of practical measurement, some subjective indicators obtained through interviews or questionnaires were excluded from this comparison. However, as previously mentioned, objective and subjective methods cannot be entirely separated, and combining them is desirable. After excluding methods that are not objectively measurable, 15 markers were identified in the reference tool. Five of these markers are scored as primary or secondary indicators; 10 potential markers are not scored but are considered emerging. An overview of the included and excluded indicators and the results of the agreement can be found in Table 4. The highest agreement with the CAT2 REDs was achieved in the study of Stenqvist et al. (22) using 80% of the scored indicators. Six studies used 60% (19–21, 25, 26, 74), two used 40% (18, 28), three used 20% (16, 23, 24) of the scored indicators, while one study did not include any of these scored markers but only the potential ones (27).

Limitations

The main limitation, not only of this review but to the entire field of REDs, is that no single marker or group of markers can reliably indicate the presence of REDs in athletes at this time. Therefore, we can only determine athletes' risk levels as "low/moderate/high" rather than diagnosing the presence or absence of REDs. REDs cannot be diagnosed based on a single variable. Instead, several factors must be considered. Thus, this review can only provide an overview of the markers used in REDs diagnosis in current studies and highlight their frequency of use. The most commonly used markers were also analyzed with respect to the REDs CAT2 tool. Another potential source of error is the assessment of study quality and the risk of bias. Although three researchers performed these tasks independently, evaluating individual questions and the overall evaluation of the included categories might be influenced by subjective perceptions or interpretations of the questions related to the REDs topic.

Future directions

The process of diagnosing REDs is currently fragmented, with studies employing various methods and a broad range of markers in their methodologies, as evidenced by the findings of this review. In addition, determining the presence or absence of REDs is challenging. In response, it is crucial to identify reliable markers suitable for diagnosing REDs, establish diagnostic cutoffs, and develop guidelines for their evaluation (13). It is essential to approach this condition holistically, considering factors that may influence the final diagnosis, such as the age of the athletes, their overall nutritional status, or the type and intensity of their training schedule. Furthermore, the importance of interdisciplinary and multidisciplinary collaboration in diagnosing, treating, and preventing this syndrome cannot be overstated, as it is necessary to improve the future approach to REDs. The fragmentation of complex conditions like REDs can lead to erroneous conclusions and flawed therapeutic strategies (75). The prevention of REDs should not rely solely on the sports physician. Coaches, physiotherapists, nutritional therapists, psychiatrists, the athletes themselves and, when appropriate, their parents should all be involved in every part of this process–primary, secondary, and tertiary REDs prevention (73, 76).

Conclusion

This review is among the first articles to summarize the type and frequencies of markers used in REDs diagnosis in current

TABLE 4 Agreement of used markers according to IOC REDs CAT2 ($n = 13$ studies).

REDs indicator (14)	Hooper et al. (16)	Önnik et al. (74)	Torstveit et al. (18)	Keay et al. (19)	Stenqvist et al. (20)	Keay et al. (21)	Stenqvist et al. (22)	Mathisen et al. (23)	Civil et al. (24)	Lee et al. (25)	Pritchett et al. (26)	Gibson-Smith et al. (27)	Kalpana et al. (28)
Severe primary indicators (count as two primary indicators)													
Primary amenorrhea (females: primary amenorrhea is indicated when there has been a failure to menstruate by age 15 in the presence of normal secondary sexual development (two SDs above the mean of 13 years) or within 5 years after breast development if that occurs before age 10) or prolonged secondary amenorrhea (absence of 12 or more consecutive menstrual cycles) due to FHA[a]													
Clinically low free or total testosterone (males: below the reference range)[b]	—	Yes	Yes	Yes	Yes	Yes	Yes	—	—	Yes	Yes	—	—
Primary indicators													
Secondary amenorrhea (females: absence of 3–11 consecutive menstrual cycles) caused by FHA[a]													
Subclinically low total or free testosterone (males: within the lowest 25% (quartile) of the reference range)[b]	—	Yes	Yes	Yes	Yes	Yes	Yes	—	—	Yes	Yes	—	—
Subclinically or clinically low total or free T3 (within or below the lowest 25% (quartile) of the reference range)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	—	—	Yes	Yes	—	Yes
History of ≥1 high-risk (femoral neck, sacrum, pelvis) or ≥2 low-risk BSI (all other BSI locations) within the previous 2 years or absence of ≥6 months from training due to BSI in the previous 2 years[a]													
Premenopausal females and males <50 years old: BMD Z-score[a] <−1 at the lumbar spine, total hip or femoral neck or decrease in BMD Z-score from prior testing Children/adolescents: BMD Z-score[a] <−1 at the lumbar spine or TBLH or decrease in BMD Z-score from prior testing (can occur from bone loss or inadequate bone accrual)	—	Yes	—	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	—	Yes
A negative deviation of a pediatric or adolescent athlete's previous growth trajectory (height and/or weight)	—	—	—	—	—	—	—	—	—	—	—	—	—
An elevated score for the EDE-Q global (>2.30 in females; >1.68 in males) and/or clinically diagnosed DSM-5-TR-defined eating disorder[a] (only one primary indicator for either or both outcomes)[a]													
Secondary indicators													
Oligomenorrhea caused by FHA (>35 days between periods for a maximum of 8 periods/year)[a]													
History of 1 low-risk BSI (see high vs. low-risk definition above) within the previous 2 years and absence of <6 months from training due to BSI in the previous 2 years[a]													
Elevated total or LDL cholesterol (above reference range)	—	—	—	—	—	—	Yes	—	—	—	—	—	—
Clinically diagnosed depression and/or anxiety (only one secondary indicator for either or both outcomes)[a]													
Potential indicators (not scored, emerging)													
Subclinically or clinically low IGF-1 (within or below the lowest 25% (quartile) of the reference range)	—	Yes	Yes	—	Yes	—	—	—	—	Yes	Yes	—	—
Clinically low blood glucose (below the reference range)	—		Yes	—	—	—	—	—	—	—	—	—	—
Clinically low blood insulin (below the reference range)	—	Yes	Yes	—	Yes	—	—	—	—	Yes	—	—	—

(*Continued*)

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TABLE 4 Continued

REDs indicator (14)	Hooper et al. (16)	Õnnik et al. (74)	Torstveit et al. (18)	Keay et al. (19)	Stenqvist et al. (20)	Keay et al. (21)	Stenqvist et al. (22)	Mathisen et al. (23)	Civil et al. (24)	Lee et al. (25)	Pritchett et al. (26)	Gibson-Smith et al. (27)	Kalpana et al. (28)
Chronically poor or sudden decline in iron studies (e.g., ferritin, iron, transferrin) and/or hemoglobin	Yes	Yes	—	—	—	—	—	—	—	—	—	Yes	Yes
Lack of ovulation (via urinary ovulation detection)[a]													
Elevated resting AM or 24 h urine cortisol (above the reference range or significant change for an individual)	—	Yes	Yes	—	Yes	—	Yes	—	—	Yes	—	—	—
Urinary incontinence (females)[a]													
GI or liver dysfunction/adverse GI symptoms at rest and during exercise[a]													
Reduced or low RMR <30 kcal/kg FFM/day or RMR ratio <0.90	Yes	—	Yes	Yes	Yes	—	Yes	Yes	Yes	Yes	—	—	—
Reduced or low libido/sex drive (especially in males) and decreased morning erections[a]													
Symptomatic orthostatic hypotension	—	—	—	—	—	—	—	—	—	—	—	—	—
Bradycardia (HR <40 in adult athletes; HR <50 in adolescent athletes)	—	—	—	—	—	—	—	—	—	—	—	—	—
Low systolic or diastolic BP (<90/60 mm Hg)	—	—	—	—	—	—	—	—	—	—	—	—	—
Sleep disturbances	—	—	—	—	—	—	—	—	—	—	—	—	Yes
Psychological symptoms (e.g., increased stress, anxiety, mood changes, body dissatisfaction and/or body dysmorphia)[a]													
Psychology symptoms[a]													
Exercise dependence/addiction[a]													
Low BMI	Yes	Yes	Yes	—	Yes	Yes	Yes	Yes	Yes	Yes	—	Yes	—
Agreement													
Pointed indicators ($n = 5$)	20%	60%	40%	60%	60%	60%	80%	20%	20%	60%	60%	0%	40%
Potential indicators ($n = 11$)	27.3%	45.5%	54.5%	9.1%	45.5%	9.1%	27.3%	18.2%	18.2%	45.5%	9.1%	18.2%	18.2%
Overall	25%	50%	50%	25%	50%	25%	43.8%	18.8%	18.8%	50%	25%	12.5%	25%

BMD, bone mineral density; BMI, body mass index; BP, blood pressure; BSI, bone stress injuries; DSM-5-TR, diagnostic and statistical manual of mental disorders, fifth edition, text revision; DXA, dual-energy x-ray absorptiometry; EDE-Q, eating disorder examination questionnaire; FFM, fat-free mass; FHA, functional hypothalamic amenorrhea; GI, gastrointestinal; HR, heart rate; traffic-light severity/risk categories, insulin-like growth factor 1; ISCD, International Society for Clinical Densitometry; LDL, low-density lipoprotein; LSC, least significant change; RMR, resting metabolic rate; T3, triiodothyronine; T, testosterone; TBLH, total body less head.

[a]Gray rows show indicators that cannot be objectively measured and that, therefore, were excluded for the purpose of marker agreement in this review.

[b]Testosterone level, which is included in the "severe primary indicators" and "primary indicators" categories, was considered as one indicator (not counted twice) to calculate agreement in marker use.

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studies. A focus on unifying the methodology for diagnosing REDs is essential for future research, as the variety of markers and inconsistent methodologies may complicate the interpretation of results. This review identified that the most commonly used markers were BMD, anthropometrical parameters (e.g., BMI, BM, and FM), and T3 hormone concentration (76.9% of the included studies). RMR (69.2% of the included studies), testosterone concentration, and energy intake calculation (61.5% of the included studies) also had a high frequency of use. According to the REDs CAT2 (14), the highest agreement was achieved in the study by Stenqvist et al. (22) using 80% of the scored indicators. Six studies used 60% (19–21, 25, 26, 74), two used 40% (18, 28), three used 20% (16, 23, 24) of the scored indicators, while one study did not include any of these scored markers, only the potential ones (27).

The calculation of energy availability, a direct etiological factor for developing REDs, was used in 46.2% of the included studies. Despite its simplicity and broad applicability, this marker has the disadvantage of a potentially significant risk of error in calculating energy intake and expenditure during physical activity. Thus, it should be evaluated in combination with other methods.

This summary of the markers used in REDs diagnosis may help future researchers focus on the most widely used markers when planning research and facilitate interpreting research results. Incorporating new tools into research and medical care will likely take some time. Therefore, it remains relevant to highlight the inconsistency of methods used in current studies.

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

KD: Conceptualization, Data curation, Writing – original draft, Formal Analysis, Investigation, Methodology, Project administration, Visualization, Writing – review & editing. AP: Conceptualization, Supervision, Writing – review & editing, Visualization. AW: Data curation, Writing – review & editing, Formal Analysis. DP: Data curation, Writing – review & editing. MG: Supervision, Writing – review & editing, Conceptualization. MK: Supervision, Writing – review & editing, Conceptualization, Visualization.

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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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References

1. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the female athlete triad–relative energy deficiency in sport (RED-S). *Br J Sports Med*. (2014 Apr) 48(7):491–7. doi: 10.1136/bjsports-2014-093502

2. Malina RM, Spirduso WW, Tate C, Baylor AM. Age at menarche and selected menstrual characteristics in athletes at different competitive levels and in different sports. *Med Sci Sports*. (1978) 10(3):218–22.

3. Yeager KK, Agostini R, Nattiv A, Drinkwater B. The female athlete triad: disordered eating, amenorrhea, osteoporosis. *Med Sci Sports Exerc*. (1993) 25 (7):775–7. doi: 10.1249/00005768-199307000-00003

4. Souza MJD, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, et al. 2014 female athlete triad coalition consensus statement on treatment and return to play of the female athlete triad: 1st international conference held in San Francisco, California, May 2012 and 2nd international conference held in Indianapolis, Indiana, May 2013. *Br J Sports Med*. (2014) 48(4):289. doi: 10.1136/bjsports-2013-093218

5. Sundgot-Borgen J, Meyer NL, Lohman TG, Ackland TR, Maughan RJ, Stewart AD, et al. How to minimise the health risks to athletes who compete in weight-sensitive sports review and position statement on behalf of the *ad hoc* research working group on body composition, health and performance, under the auspices of the IOC Medical Commission. *Br J Sports Med*. (2013) 47(16):1012–22. doi: 10.1136/bjsports-2013-092966

6. Hamer J, Desbrow B, Irwin C. Are coaches of female athletes informed of relative energy deficiency in sport? A Scoping Review. *Women Sport Phys Act J*. (2021) 29 (1):38–46. doi: 10.1123/wspaj.2020-0062

7. Lodge MT, Ackerman KE, Garay J. Knowledge of the female athlete triad and relative energy deficiency in sport among female cross-country athletes and support staff. *J Athl Train*. (2022) 57(4):385–92. doi: 10.4085/1062-6050-0175.21

8. Loucks A. Energy availability, not body fatness, regulates reproductive function in women. *Exerc Sport Sci Rev*. (2003) 31:144–8. doi: 10.1097/00003677-200307000-00008

9. Heikura IA, Uusitalo ALT, Stellingwerff T, Bergland D, Mero AA, Burke LM. Low energy availability is difficult to assess but outcomes have large impact on bone injury rates in elite distance athletes. *Int J Sport Nutr Exerc Metab*. (2018) 28(4):403–11. doi: 10.1123/ijsnem.2017-0313

10. Sim A, Burns SF. Review: questionnaires as measures for low energy availability (LEA) and relative energy deficiency in sport (RED-S) in athletes. *J Eat Disord*. (2021) 9(1):41. doi: 10.1186/s40337-021-00396-7

11. Gould RJ, Ridout AJ, Newton JL. Relative energy deficiency in sport (RED-S) in adolescents—a practical review. *Int J Sports Med*. (2023) 44(4):236–46. doi: 10.1055/a-1947-3174

12. Areta JL, Taylor HL, Koehler K. Low energy availability: history, definition and evidence of its endocrine, metabolic and physiological effects in prospective studies in females and males. *Eur J Appl Physiol*. (2021) 121(1):1–21. doi: 10.1007/s00421-020-04516-0

13. Mountjoy M, Ackerman KE, Bailey DM, Burke LM, Constantini N, Hackney AC, et al. 2023 International Olympic Committee's (IOC) consensus statement on

relative energy deficiency in sport (REDs). *Br J Sports Med*. (2023) 57(17):1073–98. doi: 10.1136/bjsports-2023-106994

14. Stellingwerff T, Mountjoy M, McCluskey WT, Ackerman KE, Verhagen E, Heikura IA. Review of the scientific rationale, development and validation of the International Olympic Committee relative energy deficiency in sport clinical assessment tool: v.2 (IOC REDs CAT2)—by a subgroup of the IOC consensus on REDs. *Br J Sports Med*. (2023) 57(17):1109–21. doi: 10.1136/bjsports-2023-106914

15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Br Med J*. (2021) 372:n71. doi: 10.1136/bmj.n71

16. Hooper DR, Mallard J, Wight JT, Conway KL, Pujalte GGA, Pontius KM, et al. Performance and health decrements associated with relative energy deficiency in sport for division I women athletes during a collegiate cross-country season: a case series. *Front Endocrinol*. (2021) 12:524762. doi: 10.3389/fendo.2021.524762

17. Õnnik L, Mooses M, Suvi S, Haile DW, Ojiambo R, Lane AR, et al. Prevalence of triad–RED–S symptoms in high-level Kenyan male and female distance runners and corresponding control groups. *Eur J Appl Physiol*. (2022) 122(1):199–208. doi: 10.1007/s00421-021-04827-w

18. Torstveit MK, Fahrenholtz IL, Lichtenstein MB, Stenqvist TB, Melin AK. Exercise dependence, eating disorder symptoms and biomarkers of relative energy deficiency in sports (RED-S) among male endurance athletes. *BMJ Open Sport Exerc Med*. (2019) 5(1):e000439. doi: 10.1136/bmjsem-2018-000439

19. Keay N, Francis G, Entwistle I, Hind K. Clinical evaluation of education relating to nutrition and skeletal loading in competitive male road cyclists at risk of relative energy deficiency in sports (RED-S): 6-month randomised controlled trial. *BMJ Open Sport Exerc Med*. (2019) 5(1):e000523. doi: 10.1136/bmjsem-2019-000523

20. Stenqvist TB, Torstveit MK, Faber J, Melin AK. Impact of a 4-week intensified endurance training intervention on markers of relative energy deficiency in sport (RED-S) and performance among well-trained male cyclists. *Front Endocrinol*. (2020) 11:512365. doi: 10.3389/fendo.2020.512365

21. Keay N, Francis G, Hind K. Low energy availability assessed by a sport-specific questionnaire and clinical interview indicative of bone health, endocrine profile and cycling performance in competitive male cyclists. *BMJ Open Sport Exerc Med*. (2018) 4(1):e000424. doi: 10.1136/bmjsem-2018-000424

22. Stenqvist TB, Melin AK, Garthe I, Slater G, Paulsen G, Iraki J, et al. Prevalence of surrogate markers of relative energy deficiency in male Norwegian Olympic-level athletes. *Int J Sport Nutr Exerc Metab*. (2021) 31(6):497–506. doi: 10.1123/ijsnem.2020-0368

23. Mathisen TF, Heia J, Raustøl M, Sandeggen M, Fjellestad I, Sundgot-Borgen J. Physical health and symptoms of relative energy deficiency in female fitness athletes. *Scand J Med Sci Sports*. (2020) 30(1):135–47. doi: 10.1111/sms.13568

24. Civil R, Lamb A, Loosmore D, Ross L, Livingstone K, Strachan F, et al. Assessment of dietary intake, energy status, and factors associated with RED-S in vocational female ballet students. *Front Nutr*. (2018) 5:136. doi: 10.3389/fnut.2018.00136

25. Lee S, Moto K, Han S, Oh T, Taguchi M. Within-day energy balance and metabolic suppression in male collegiate soccer players. *Nutrients*. (2021) 13(8):2644. doi: 10.3390/nu13082644

26. Pritchett K, DiFolco A, Glasgow S, Pritchett R, Williams K, Stellingwerff T, et al. Risk of low energy availability in national and international level paralympic athletes: an exploratory investigation. *Nutrients*. (2021) 13(3):979. doi: 10.3390/nu13030979

27. Gibson-Smith E, Storey R, Ranchordas M. Dietary intake, body composition and iron status in experienced and elite climbers. *Front Nutr*. (2020) 7:122. doi: 10.3389/fnut.2020.00122

28. Kalpana K, Cherian KS, Khanna GL. Energy availability and RED-S risk assessment among Kho-Kho players in India. *Sport Sci Health*. (2022) 19:1–8. doi: 10.1007/s11332-022-00996-z

29. Limaye D, Shankar RP, Limaye V, Sydymanov A, Otzipka C, Ziesenis P, et al. Development of a quantitative scoring method for STROBE checklist. *Acta Pol Pharm Drug Res*. (2018) 75(5):1095–106. doi: 10.32383/appdr/84804

30. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *Br Med J*. (2016) 355. doi: 10.1136/bmj.i4919

31. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC relative energy deficiency in sport clinical assessment tool (RED-S CAT). *Br J Sports Med*. (2015) 49(21):1354–1354. doi: 10.1136/bjsports-2015-094873

32. Centers for Disease Control and Prevention. *Body Mass Index: Considerations for Practitioners* (2011). Available online at: https://www.cdc.gov/obesity/downloads/bmiforpactitioners.pdf.

33. Humphreys S. The unethical use of BMI in contemporary general practice. *Br J Gen Pract*. (2010) 60(578):696–7. doi: 10.3399/bjgp10X515548

34. Jeong SM, Lee DH, Rezende LFM, Giovannucci EL. Different correlation of body mass index with body fatness and obesity-related biomarker according to age, sex and race-ethnicity. *Sci Rep*. (2023) 13:3472. doi: 10.1038/s41598-023-30527-w

35. Mountjoy M, Sundgot-Borgen JK, Burke LM, Ackerman KE, Blauwet C, Constantini N, et al. IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *Br J Sports Med*. (2018) 52(11):687–97. doi: 10.1136/bjsports-2018-099193

36. Müller W, Fürhapter-Rieger A, Ahammer H, Lohman TG, Meyer NL, Sardinha LB, et al. Relative body weight and standardised brightness-mode ultrasound measurement of subcutaneous fat in athletes: an international multicentre reliability study, under the auspices of the IOC medical commission. *Sports Med Auckl NZ*. (2020) 50(3):597–614. doi: 10.1007/s40279-019-01192-9

37. Ackland TR, Lohman TG, Sundgot-Borgen J, Maughan RJ, Meyer NL, Stewart AD, et al. Current status of body composition assessment in sport: review and position statement on behalf of the *ad hoc* research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. *Sports Med Auckl NZ*. (2012) 42(3):227–49. doi: 10.2165/11597140-000000000-00000

38. Mathisen TF, Ackland T, Burke LM, Constantini N, Haudum J, Macnaughton LS, et al. Best practice recommendations for body composition considerations in sport to reduce health and performance risks: a critical review, original survey and expert opinion by a subgroup of the IOC consensus on relative energy deficiency in sport (REDs). *Br J Sports Med*. (2023) 57(17):1148–58. doi: 10.1136/bjsports-2023-106812

39. Ackerman KE, Stellingwerff T, Elliott-Sale KJ, Baltzell A, Cain M, Goucher K, et al. #REDS (relative energy deficiency in sport): time for a revolution in sports culture and systems to improve athlete health and performance. *Br J Sports Med*. (2020) 54(7):369–70. doi: 10.1136/bjsports-2019-101926

40. Ackerman KE, Misra M. Neuroendocrine abnormalities in female athletes. In: Gordon CM, LeBoff MS, editors. *The Female Athlete Triad: A Clinical Guide*. Boston, MA: Springer (2015). p. 85–109. doi: 10.1007/978-1-4899-7525-6_6

41. De Souza MJ, Williams NI. Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Hum Reprod Update*. (2004) 10(5):433–48. doi: 10.1093/humupd/dmh033

42. Misra M, Miller KK, Almazan C, Ramaswamy K, Lapcharoensap W, Worley M, et al. Alterations in cortisol secretory dynamics in adolescent girls with anorexia nervosa and effects on bone metabolism. *J Clin Endocrinol Metab*. (2004) 89(10):4972–80. doi: 10.1210/jc.2004-0723

43. Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, et al. Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. *Endocrinology*. (1998) 139(10):4264–8. doi: 10.1210/endo.139.10.6254

44. Grinspoon SK, Baum HB, Kim V, Coggins C, Klibanski A. Decreased bone formation and increased mineral dissolution during acute fasting in young women. *J Clin Endocrinol Metab*. (1995) 80(12):3628–33. doi: 10.1210/jcem.80.12.8530611

45. Shapses SA, Heymsfield SB, Ricci TA. "Voluntary weight reduction increases bone turnover and loss." In: Burckhardt P, Dawson-Hughes B, Heaney RP, editors. *Nutritional Aspects of Osteoporosis: A Serono Symposia SA Publication*. New York, NY: Springer (1998). p. 180–4. Proceedings in the Serono Symposia USA Series. doi: 10.1007/978-1-4612-2228-6_19

46. Morgan SL, Prater GL. Quality in dual-energy x-ray absorptiometry scans. *Bone*. (2017) 104:13–28. doi: 10.1016/j.bone.2017.01.033

47. Ihle R, Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res*. (2004) 19(8):1231–40. doi: 10.1359/JBMR.040410

48. Papageorgiou M, Elliott-Sale KJ, Parsons A, Tang JCY, Greeves JP, Fraser WD, et al. Effects of reduced energy availability on bone metabolism in women and men. *Bone*. (2017) 105:191–9. doi: 10.1016/j.bone.2017.08.019

49. Papageorgiou M, Dolan E, Elliott-Sale KJ, Sale C. Reduced energy availability: implications for bone health in physically active populations. *Eur J Nutr*. (2018) 57(3):847–59. doi: 10.1007/s00394-017-1498-8

50. Karlsson MK, Johnell O, Obrant KJ. Bone mineral density in weight lifters. *Calcif Tissue Int*. (1993) 52(3):212–5. doi: 10.1007/BF00298721

51. Fullmer S, Benson-Davies S, Earthman CP, Frankenfield DC, Gradwell E, Lee PSP, et al. Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *J Acad Nutr Diet*. (2015) 115(9):1417–446.e2. doi: 10.1016/j.jand.2015.04.003

52. De Souza MJ, Koltun KJ, Williams NI. The role of energy availability in reproductive function in the female athlete triad and extension of its effects to men: an initial working model of a similar syndrome in male athletes. *Sports Med*. (2019) 49(2):125–37. doi: 10.1007/s40279-019-01217-3

53. O'Neill JER, Corish CA, Horner K. Accuracy of resting metabolic rate prediction equations in athletes: a systematic review with meta-analysis. *Sports Med*. (2023) 53(12):2373–98. doi: 10.1007/s40279-023-01896-z

54. Cunningham JJ. A reanalysis of the factors influencing basal metabolic rate in normal adults. *Am J Clin Nutr*. (1980) 33(11):2372–4. doi: 10.1093/ajcn/33.11.2372

55. Harris JA, Benedict FG. A biometric study of human basal metabolism. *Proc Natl Acad Sci U S A*. (1918) 4(12):370–3. doi: 10.1073/pnas.4.12.370

56. Owen O, Kavle E, Owen R, Polansky M, Caprio S, Mozzoli M, et al. A reappraisal of caloric requirements in healthy women. *Am J Clin Nutr*. (1986) 44(1):1–19. doi: 10.1093/ajcn/44.1.1

57. De Souza MJ, Hontscharuk R, Olmsted M, Kerr G, Williams NI. Drive for thinness score is a proxy indicator of energy deficiency in exercising women. *Appetite*. (2007) 48(3):359–67. doi: 10.1016/j.appet.2006.10.009

58. Staal S, Sjödin A, Fahrenholtz I, Bonnesen K, Melin AK. Low RMR ratio as a surrogate marker for energy deficiency, the choice of predictive equation vital for correctly identifying male and female ballet dancers at risk. *Int J Sport Nutr Exerc Metab*. (2018) 28(4):412–8. doi: 10.1123/ijsnem.2017-0327

59. Sterringer T, Larson-Meyer DE. RMR ratio as a surrogate marker for low energy availability. *Curr Nutr Rep*. (2022) 11(2):263–72. doi: 10.1007/s13668-021-00385-x

60. Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. *Am J Clin Nutr*. (1991) 54(6):963–9. doi: 10.1093/ajcn/54.6.963

61. Martin B, Golden E, Carlson OD, Egan JM, Mattson MP, Maudsley S. Caloric restriction: impact upon pituitary function and reproduction. *Ageing Res Rev*. (2008) 7(3):209–24. doi: 10.1016/j.arr.2008.01.002

62. Elliott-Sale KJ, Tenforde AS, Parziale AL, Holtzman B, Ackerman KE. Endocrine effects of relative energy deficiency in sport. *Int J Sport Nutr Exerc Metab*. (2018) 28 (4):335–49. doi: 10.1123/ijsnem.2018-0127

63. Harvey CB, O'Shea PJ, Scott AJ, Robson H, Siebler T, Shalet SM, et al. Molecular mechanisms of thyroid hormone effects on bone growth and function. *Mol Genet Metab*. (2002) 75(1):17–30. doi: 10.1006/mgme.2001.3268

64. Welsh KJ, Soldin SJ. How reliable are free thyroid and total T3 hormone assays? *Eur J Endocrinol*. (2016) 175(6):R255–63. doi: 10.1530/EJE-16-0193

65. Russell W, Harrison RF, Smith N, Darzy K, Shalet S, Weetman AP, et al. Free triiodothyronine has a distinct circadian rhythm that is delayed but parallels thyrotropin levels. *J Clin Endocrinol Metab*. (2008) 93(6):2300–6. doi: 10.1210/jc.2007-2674

66. Dipla K, Kraemer RR, Constantini NW, Hackney AC. Relative energy deficiency in sports (RED-S): elucidation of endocrine changes affecting the health of males and females. *Horm Athens Greece*. (2021) 20(1):35–47. doi: 10.1007/s42000-020-00214-w

67. Sale C, Elliott-Sale KJ. Nutrition and athlete bone health. *Sports Med Auckl NZ*. (2019) 49(Suppl 2):139–51. doi: 10.1007/s40279-019-01161-2

68. Freedman LS, Commins JM, Moler JE, Willett W, Tinker LF, Subar AF, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for potassium and sodium intake. *Am J Epidemiol*. (2015) 181 (7):473–87. doi: 10.1093/aje/kwu325

69. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*. (1986) 124(1):17–27. doi: 10.1093/oxfordjournals.aje.a114366

70. Schoeller DA. Limitations in the assessment of dietary energy intake by self-report. *Metab Clin Exp*. (1995) 44:18–22. doi: 10.1016/0026-0495(95)90204-X

71. Ndahimana D, Kim EK. Measurement methods for physical activity and energy expenditure: a review. *Clin Nutr Res*. (2017) 6(2):68–80. doi: 10.7762/cnr.2017.6.2.68

72. Welk G. *Physical Activity Assessments for Health-Related Research*. Champaign, IL: Human Kinetics (2002). p. 269. Available online at: http://catalog.hathitrust.org/api/volumes/oclc/49376240.html (accessed August 30, 2023).

73. Torstveit MK, Ackerman KE, Constantini N, Holtzman B, Koehler K, Mountjoy ML, et al. Primary, secondary and tertiary prevention of relative energy deficiency in sport (REDs): a narrative review by a subgroup of the IOC consensus on REDs. *Br J Sports Med*. (2023) 57(17):1119–26. doi: 10.1136/bjsports-2023-106932

74. Õnnik L, Mooses M, Suvi S, Haile DW, Ojiambo R, Lane AR, et al. Correction to: prevalence of triad–RED-S symptoms in high-level Kenyan male and female distance runners and corresponding control groups. *Eur J Appl Physiol*. (2022) 122 (1):209. doi: 10.1007/s00421-021-04836-9

75. Schofield K, Thorpe H, Sims S. Compartmentalised disciplines: why low energy availability research calls for transdisciplinary approaches. *Perform Enhanc Health*. (2020) 8:100172. doi: 10.1016/j.peh.2020.100172

76. Todd E, Elliott N, Keay N. Relative energy deficiency in sport (RED-S). *Br J Gen Pract*. (2022) 72(719):295–7. doi: 10.3399/bjgp22X719777

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The association between diet and sleep with internalising symptoms in young athletes: a serial multiple mediation models

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Introduction: Athletes frequently experience anxiety and depression at rates similar to or higher than non-athletes. A balanced diet can alleviate athletes internalizing symptoms. Moreover, diet and sleep are all associated with internalising symptoms. Our study investigates how sleep quality mediates the impact of dietary habits on internalizing symptoms in athletes.

Methods: The current cross-sectional study involved 758 Chinese young athletes. The adapted Australian Athletes Diet Index was used to assess dietary patterns, and sleep was measured using the Athletes Sleep Screening Questionnaire. The Generalised Anxiety Disorder 7 scale and the Patient Health Questionnaire 9-item scale were used to assess symptoms of anxiety and depression. Structural Equation Modelling (SEM) analysis was used to examine the mediating role of sleep quality in young athletes. Raw scores of chronotypes, sleep quality, anxiety and depression were calculated for the statistical analysis.

Results: No significant indirect effects were found in adolescents. In adult athletes, diet mediated the relationship between chronotype and sleep quality ($\beta = -0.028$, $p < 0.001$). Sleep quality mediated the association between diet and anxiety ($\beta = -0.060$, $p = 0.001$), and depression ($\beta = -0.076$, $p = 0.001$). Additionally, diet and sleep quality mediated the association between chronotypes and anxiety ($\beta = -0.028$, $p = 0.001$), and depression ($\beta = -0.028$, $p = 0.001$).

Conclusion: Sleep quality mediated the relationship between dietary patterns and internalising symptoms in young adult athletes. Additionally, diet and sleep quality mediated the association between chronotypes and internalising symptoms in young adult athletes.

KEYWORDS
diet, sleep, internalising symptoms, athletes, mediation

1 Background

The main feature of internalising symptoms is the disturbance of mood or emotional state (1). These symptoms typically manifest as anxiety, depression, mood disorders, and social withdrawal (2, 3). These symptoms are characterised by negative emotional states and a lack of visible, externalised behaviours (4). In 2019, there were 280 million individuals worldwide experiencing depression and 301 million suffering from anxiety disorders (5). The prevalence of internalising symptoms is also notable in athletes. Recent studies suggest that elite athletes, due to the unique pressures of competitive sports, are particularly vulnerable to mental disorders (6, 7). A systematic review revealed that the prevalence of anxiety and depression in athletes varies but is often higher than or comparable to their non-athlete counterparts (6). Internalising symptoms can significantly impact an athlete's physical performance and mental health. Depression and anxiety, for example, can lead to decreased motivation, impaired concentration, and suboptimal performance in sports (8). The high demands and stress associated with competitive sports can exacerbate these internalising symptoms, further affecting athletes' overall well-being and career trajectory (9, 10).

To date, there is a limited number of studies focused on the relationship between diet and internalising symptoms in athletes. However, several studies indicated a positive association between better dietary habits and greater mental health. Dietary habits can influence mental health, where a balanced diet is associated with a reduced risk of depression and anxiety (11, 12). Previous studies suggested that a healthy diet rich in fruits, vegetables, and protein intake has benefits for reducing internalising symptoms in both the general population and athletes (12, 13). Conversely, poor dietary habits were associated with mental disorders in athletes (14). For instance, diets high in processed foods and sugars have been linked to increased risk of depression and anxiety (11, 12). Diet is one of the foundations for reaching athletic performance, with athletes' dietary strategies, particularly those before and after competitions, being essential for recovery and adaptation (15). Effective dietary can enhance the adaptive response to fatigue, promote muscle function and boost exercise tolerance (16). Therefore, the monitoring of diets is important for athletes' competition performance (17). However, scientific evidence indicates that there are some issues in the dietary behaviours of athletes. Specifically, there is an imbalanced intake of vitamins, including insufficient Vitamin D and excessive levels of phosphorus, iron and zinc (18). Elite athletes also demonstrate excessive consumption of vitamins, meat and sweets compared to non-elite athletes (19). Alcohol consumption among athletes poses a significant dietary problem (20). Research suggests that an athlete's diet can impact their sleep, stress and overall well-being (21). Moreover, the understanding of nutrition among national-level athletes is profoundly limited and poorer than that of the general population of the same age (22, 23). Furthermore, a comparison between elite and non-elite athletes did not show substantial benefits in nutritional knowledge (24).

Sleep and internalising symptoms are closely related in athletes (25–28). Furthermore, sleep quality is even more closely linked to athletes' mental health and is a significant predictor of internalising symptoms in athletes (29). Sleep deprivation is a contributing factor to impaired mental health in elite athletes (30). Chronic sleep deprivation and accumulated sleep debt can lead to severe mental wellness issues in athletes (31). The connection between sleep and mental health can be achieved by enhancing the recovery of the immune and nervous systems (32). Conversely, poor sleep quality and quantity have been consistently linked to higher levels of anxiety and depression among athletes (33). A longitudinal study on athletes indicated that those with poor sleep patterns were more likely to report symptoms of depression and anxiety, compared to those with adequate sleep (34). More importantly, diet plays a crucial role in the quality of sleep for athletes (35). It has been known that nutritional choices can either promote restorative sleep or contribute to sleep disturbances (35). Scientific evidence has also shown that a diet high in carbohydrates can facilitate the onset of sleep, while high-fat diets may adversely affect sleep quality (32, 36). Moreover, one qualitative review synthesised the effect of nutrition interventions on athletes' sleep and indicated that sleep deprivation may lead to alterations in dietary habits (28).

The health behaviours of elite athletes are an ongoing concern, encompassing aspects such as diet and sleep. Athletes' health behaviours can impact their training status and even directly affect their performance in competitions, ultimately influencing the trajectory of their careers (19). Therefore, the health behaviours and mental well-being of athletes during the Olympic preparation cycle are crucially important for optimising their performance (37). Although diet and sleep are associated with internalising symptoms, there is few studies have explored their interactive relationship in athletes. This gap is particularly notable given the high prevalence of mental health issues among athletes and the potential impact of these factors on their performance and well-being. The current study aims to address this gap by exploring how dietary habits and sleep patterns collectively influence internalising symptoms in athletes. Understanding the role of sleep as a mediator in this relationship is crucial, as it may offer insights into effective strategies for improving athletes' mental health and, consequently, their sports performance. Therefore, the primary aim of this study is to investigate the association between diet and sleep with internalising symptoms in young athletes. Moreover, this study also explores the interactive relationship between unhealthy behaviours and internalising symptoms in young athletes.

2 Methods

2.1 Study design

This study was conducted using an online survey developed through the web-based questionnaire tool "Wen Juan Xing" (WJX.cn). The survey consisted of four sections: demographic data, health behaviours, and mental health. Participants were obtained by contacting coaches of university-level sports teams. Before participating in the study, athletes were provided with information about the research, and their informed consent was obtained. For athletes under the age of 18, parental or coach consent was required for participation in the survey. This study received approval from the Ethics Review Committee of the Shanghai University of Sport (Approval No. 102772022RT113).

Data were collected anonymously, and aggregate statistical analysis results were shared with the athletes or their guardians. The data collection was conducted from October to December 2022. Most of this period coincided with China's COVID-19 epidemic prevention phase. On December 6, China announced new epidemic prevention regulations, initiating the comprehensive relaxation of epidemic control measures across society. However, the implementation of these measures continued in various locations until the end of December. Throughout the duration of this study, the training regimes of the athletes surveyed remained normal.

2.2 Participants

The sample size for this study was calculated using the G-power sample calculator, ensuring a probability level (α err prob) of $p = 0.05$, a small-to-medium effect size $d = 0.3$, and a statistical power (1-β) of 0.90, which necessitated a minimum sample size of 109. To ensure a sufficient number of athlete participants, there were no restrictions on the level or age of the athletes participating in the study, other than being between the ages of 13 and 29. The athletic levels considered were National First-Class, National Elite, and International Elite. Data from athletes below National First-Class or those not meeting the criteria (non-athletes) and duplicate entries were excluded.

According to the standards of the General Administration of Sport of China, National First-Class Athletes are those who meet the standards in national school leagues or national youth U-series competitions. National-Level Athletes are those qualified to participate in national competitions, while International-Level Athletes generally refer to those qualified to participate in international or intercontinental competitions. The standards for athlete levels vary by sport, as detailed in the standards of the General Administration of Sport of China (38).

2.3 Measures

This study targeted high-level competitive athletes in China, specifically those who have been engaged in long-term sports training and have participated in provincial-level or higher competitions. The survey incorporated internationally recognised scales suitable for high-level athletes, facilitating the identification of health behaviour issues in this group. The comprehensive questionnaire included the following sections.

2.3.1 Diet measurement

The dietary behaviour questionnaire was adapted from the core nutritional section of the Australian Athlete Diet Index (ADI). The ADI is a scale specifically designed for assessing the nutritional behaviours of athletes (39). It demonstrates good reliability (ICC = 0.80, 95% CI 0.69, 0.87; $p < 0.001$), and Bland–Altman plots (mean 1.9; limits of agreement −17.8, 21.7) did not show systematic bias ($y = 4.57 - 0.03 \times x$) (95% CI −0.2, 0.1; $p = 0.70$) (40). Due to regional and cultural differences, the structure of the ADI core nutrition section was used, with item content referencing the "Chinese Elite Athlete Nutrition Guide" for the dietary requirements of athletes. The athletes were required to recall their dietary patterns over the past week. The questionnaire contains 13 items, covering the total variety of fruit, vegetables, grains, whole grains, dairy products, low-fat dairy products, total meat, poultry, fish, sweets, processed meats, takeout and dining out, and alcohol intake. The adapted questionnaire was retested for reliability in the athlete population, showing a test–retest reliability (ICC = 0.711, 95% CI 0.69, 0.87; $p < 0.001$). Higher raw scores represent healthier diet behaviours.

2.3.2 Sleep measurement

The Athlete Sleep Screening Questionnaire (ASSQ) was used to assess sleep, including sleep quantity, quality, timing, insomnia, sleep-disordered breathing, and travel-related sleep issues, categorising athletes into four groups based on a "Sleep Difficulty Score": none, mild, moderate, and severe clinical problem. The ASSQ shows good agreement with personal assessments by physicians specialising in elite athlete sleep (Cohen's kappa = 0.84), with a diagnostic sensitivity of 81%, specificity of 93%, positive predictive value of 87%, and negative predictive value of 90%. It is the only universally used sleep screening questionnaire for athletes and has been clinically validated (41, 42). The ASSQ is a 16-item multiple-choice questionnaire that calculates the "Sleep Difficulty Score (SDS)" using five questions based on total sleep time, sleep satisfaction, and symptoms of insomnia. The SDS, along with scores from other questions assessing circadian rhythm and sleep-disordered breathing, are translated into specific intervention recommendations. The ASSQ has been proven effective and reliable in the athletic population (41). The first five items of the ASSQ assess sleep disorders (e.g., In the past month, how many hours do you sleep at night?), with a scoring system of 4 and 5 points. A total score of 1–17 is calculated, with ≥8 indicating the presence of a sleep disorder. Chronotype can be defined as an individual's inherent propensity towards a preference for morningness or eveningness (43). In terms of ASSQ Chronotype, the raw score ≤ 4 indicates the athlete is an evening type.

2.3.3 Anxiety and depression measurement

The Generalised Anxiety Disorder 7 (GAD-7) scale and the Patient Health Questionnaire 9-item (PHQ-9) scale were used to assess symptoms of anxiety and depression in athletes. The GAD-7 assesses anxiety through seven items, such as "Over the last 2 weeks, how often have you been bothered by the following problem? (1) Feeling nervous, anxious, or on edge. (2) Not being able to stop or control worrying" Each item is scored from 0 ("Not at all") to 3 ("Nearly every day"), with a maximum score of 21. A total score between 0 and 21, with ≥10 indicating anxiety symptoms. The PHQ-9 measures depression through nine items, such as: "Over the past 2 weeks, how often have you been bothered by any of the following problems?" (1) Little interest or pleasure in doing things. (2) Feeling down, depressed, or hopeless. Each item is scored from 0 ("Not at all") to 3 ("Nearly every day"), with a maximum score of 27. A total score of 0–27 is calculated, with ≥10 indicating depressive symptoms.

2.4 Data analysis

Descriptive statistics and correlations were calculated using IBM SPSS v26. The correlation analysis was controlled for variables such as age, sex, BMI, athlete grade, training hours/day, training days/week, and perceived training intensity. Structural Equation Modelling (SEM) analysis was conducted in IBM AMOS v24. The SEM analysis aimed to examine the relationships between diet, sleep quality scores, chronotype scores, and the variables of depression and anxiety. Dietary patterns were the independent variable. Sleep quality was treated as a mediating variable, with anxiety and depression as dependent variables. Since the given model met these criteria, maximum likelihood was used to calculate estimates. Following Kline's recommendations, the chi-square test, Comparative Fit Index (CFI > 0.9), Goodness of fit (GFI > 0.9), Index Root Mean Square Error of Approximation (RMSEA < 0.05), and Standardised Root Mean Square Residual (SRMR < 0.05) were calculated to assess the goodness of fit for each model (44). Indirect effects were tested using a bias-corrected bootstrap with 5,000 bootstrap samples. Confidence intervals were calculated at the 95% level. The significance level was set at 0.05.

3 Results

The descriptive statistics of demographic, health behaviour, and mental health variables from the questionnaire, as well as the mean comparisons among subgroups, are presented in Table 1. In total, 815 online questionnaire responses were confirmed. After applying

TABLE 1 Descriptive statistics for demographic variables and the health questionnaires.

Variables	Total (*N* = 758)	Male (*N* = 388)	Female (*N* = 370)	*T* value	*p*	International elite (*N* = 50)	National elite (*N* = 274)	National first-class athletes (*N* = 434)	*F* value	*p*
Age	19.36 ± 3.17	19.76 ± 3.25	18.95 ± 3.04	3.540	<0.001	23.06 ± 3.62	20.01 ± 3.25	18.52 ± 2.62	64.335	<0.001
BMI	22.48 ± 8.15	23.54 ± 9.48	21.38 ± 6.31	3.667	<0.001	23.15 ± 4.88	22.14 ± 4.19	22.63 ± 10.11	0.482	0.618
Training hours/day	5.21 ± 10.87	4.82 ± 2.78	5.61 ± 15.29	−1.001	0.317	5.66 ± 1.79	5.18 ± 2.99	5.17 ± 14.16	0.046	0.955
Training days/week	5.82 ± 2.62	5.89 ± 2.31	5.76 ± 3.0	0.682	0.496	6.14 ± 1.29	5.99 ± 3.09	5.68 ± 2.40	1.635	0.196
Diet	55.25 ± 14.47	57.69 ± 13.61	52.69 ± 14.92	4.825	<0.001	56.83 ± 13.14	53.11 ± 14.99	56.42 ± 14.15	4.747	0.009
Chronotype	6.55 ± 1.88	6.71 ± 1.86	6.37 ± 1.90	2.481	0.013	6.08 ± 2.23	6.58 ± 1.76	6.58 ± 1.92	1.643	0.194
Sleep quality	5.83 ± 2.89	5.59 ± 2.85	6.08 ± 2.92	−2.341	0.019	5.84 ± 3.19	6.01 ± 2.94	5.72 ± 2.83	0.847	0.429
Anxiety	4.85 ± 4.44	4.22 ± 4.22	5.51 ± 4.56	−4.041	<0.001	5.28 ± 5.51	5.39 ± 4.50	4.47 ± 4.23	3.883	0.021
Depression	5.69 ± 4.91	4.98 ± 4.73	6.45 ± 5.00	−4.160	<0.001	5.60 ± 5.86	6.22 ± 4.98	5.37 ± 4.73	2.509	0.082

**$p < 0.01$, *$p < 0.05$. ASSQ, The Athlete Sleep Screening Questionnaire; SDS, Sleep Difficulty Score; Chronotype, Eveningness–tendency to sleep late; SMHRT-1, Sport Mental Health Assessment Tool 1.

eligibility criteria, 758 of these were selected as the sample data for the study. Athletes from 22 different sports participated in the survey. The top five most represented sports were: Track and Field (22.0%), Diving (15.2%), Swimming (12.0%), Gymnastics (9.8%), and Tennis (8.6%). Finally, this study ultimately included questionnaire data from 758 athletes (19.36 ± 3.17 years). Of these, 388 were male (51.19%) and 370 were female (48.81%); there were 50 International Elite athletes, 274 National Elite athletes, and 434 National First-Class athletes. In the comparison between male and female samples, female athletes scored lower than male athletes in overall diet scores (52.69 ± 14.92 vs. 57.69 ± 13.61, $p < 0.01$), had higher scores in sleep difficulties (6.08 ± 2.92 vs. 5.59 ± 2.85, $p < 0.05$), and scored lower in late sleeping (reverse scoring: 6.37 ± 1.90 vs. 6.71 ± 1.86, $p < 0.05$). Female athletes also had higher scores in anxiety and depression than male athletes (5.51 ± 4.56 vs. 4.22 ± 4.22, $p < 0.01$; 6.45 ± 5.00 vs. 4.98 ± 4.73, $p < 0.01$), with the only non-significant statistical differences being in Training hours/day ($p = 0.317$) and Training days/week ($p = 0.496$). In the comparison among different athletic levels, significant statistical differences were observed in the age (23.06 ± 3.62 vs. 20.01 ± 3.25 vs. 18.52 ± 2.62, $p < 0.001$), diet (56.83 ± 13.14 vs. 53.11 ± 14.99 vs. 56.42 ± 14.15, $p = 0.009$), and anxiety (5.28 ± 5.51 vs. 5.39 ± 4.50 vs. 4.47 ± 4.23, $p = 0.021$) scores of International Elite athletes, National Elite athletes, and National First-Class athletes, while other indicators showed no significant statistical differences ($p > 0.05$).

The correlation coefficients among indicators are shown in Table 2. After controlling for variables such as age, sex, BMI, athlete grade, training hours/day, training days/week, and perceived training intensity, the athletes' diet raw scores were significantly negatively correlated with sleep quality raw scores ($r = -0.128$, $p < 0.01$), and significantly positively correlated with chronotypes raw scores ($r = 0.138$, $p < 0.01$), anxiety raw scores ($r = 0.091$, $p < 0.05$), and depression raw scores ($r = 0.099$, $p < 0.01$). Sleep quality raw scores were significantly positively correlated with anxiety ($r = 0.384$, $p < 0.01$) and depression raw scores ($r = 0.450$, $p < 0.01$). There was also a significant positive correlation between anxiety and depression raw scores ($r = 0.821$, $p < 0.01$).

TABLE 2 Zero-order correlation of variables (*N* = 750).

Measure	Diet	SDS	Chronotype	Anxiety
SDS	−0.136**			
Chronotype	0.134**	−0.058		
Anxiety	−0.091*	0.386**	0.004	
Depression	−0.101**	0.451**	−0.034	0.821**

*Significant differences between groups, $p < 0.05$; **significant differences between groups, $p < 0.01$.

Tables 3, 4 display the direct effects of chronotypes, diet and sleep on anxiety and depression raw scores in young athletes. In adolescents, sleep quality was associated with anxiety ($\beta = 0.431$, 95%CI = 0.299–0.552, $p < 0.01$) and depression ($\beta = 0.382$, 95%CI = 0.289–0.563). In adults, chronotype was positively associated with diet ($\beta = 0.167$, 95%CI = 0.074–0.253, $p < 0.01$). Diet was negatively related to sleep quality ($\beta = -0.168$, 95%CI = −0.242 to −0.091, $p < 0.01$). Additionally, sleep quality was positively associated with anxiety ($\beta = 0.359$, 95%CI = 0.275–0.432, $p < 0.01$) and depression ($\beta = 0.451$, 95%CI = 0.377–0.522, $p < 0.01$).

Tables 5, 6 show the indirect effects of chronotype, diet, and sleep quality on anxiety and depression raw scores. In adolescents, we did not find any significant indirect effects. In adults, diet mediated the relationship between chronotype and sleep quality ($\beta = -0.028$, $p < 0.001$). Sleep quality mediated the association between diet and anxiety ($\beta = -0.060$, $p = 0.001$), and depression ($\beta = -0.076$, $p = 0.001$). Additionally, diet and sleep quality mediated the association between chronotypes and anxiety ($\beta = -0.028$, $p = 0.001$), and depression ($\beta = -0.028$, $p = 0.001$).

4 Discussion

To the best of the author's knowledge, this is the first study to explore the mediating role of sleep in the relationship between diet

TABLE 3 Direct effects of chronotype, diet and sleep duration on anxiety.

Path	Overall sample			Adolescents			Adults		
	Standardised estimates	95%CI		Standardised estimates	95%CI		Standardised estimates	95%CI	
		Lower	Upper		Lower	Upper		Lower	Upper
Chronotype → diet	0.152***	0.075	0.227	0.078	−0.077	0.235	0.167**	0.074	0.253
Chronotype → sleep quality	−0.058	−0.132	0.020	−0.066	−0.211	0.079	−0.047	−0.129	0.046
Diet → sleep quality	−0.135***	−0.199	−0.066	−0.053	−0.177	0.073	−0.168**	−0.242	−0.091
Diet → anxiety	−0.064	−0.132	0.001	−0.058	−0.166	0.055	−0.072	−0.144	0.011
Sleep quality → anxiety	0.382***	0.313	0.449	0.431**	0.299	0.552	0.359**	0.275	0.432

***$p < 0.001$, **$p < 0.010$, *$p < 0.050$.

TABLE 4 Direct effects of chronotype, diet and sleep duration on depression.

Path	Overall sample			Adolescents			Adults		
	Standardised estimates	95%CI		Standardised estimates	95%CI		Standardised estimates	95%CI	
		Lower	Upper		Lower	Upper		Lower	Upper
Chronotype → diet	0.152***	0.075	0.227	0.078	−0.077	0.235	0.167**	0.074	0.253
Chronotype → sleep quality	−0.058	−0.132	0.020	−0.066	−0.210	0.082	−0.047	−0.129	0.046
Diet → sleep quality	−0.135***	−0.199	−0.066	−0.053	−0.171	0.078	−0.168**	−0.242	−0.091
Diet → anxiety	−0.061	−0.122	0.001	−0.083	−0.197	0.032	−0.056	−0.129	0.021
Sleep quality → anxiety	0.445***	0.376	0.510	0.382***	0.289	0.563**	0.451**	0.377	0.522

***$p < 0.001$, **$p < 0.010$, *$p < 0.050$.

TABLE 5 Indirect effects on anxiety.

Paths	Overall		Adolescents		Adults	
	Standardised estimates	*p*	Standardised estimates	*p*	Standardised estimates	*p*
Chronotype → diet → sleep quality	−0.020***	0.001	−0.004	0.296	−0.028***	0.001
Chronotype → diet → anxiety	−0.010*	0.039	−0.005	0.240	−0.012	0.067
Chronotype → sleep quality → anxiety	−0.022	0.134	−0.028	0.369	−0.017	0.314
Diet → sleep quality → anxiety	−0.051***	0.001	−0.023	0.395	−0.060***	0.001
Chronotype → diet → sleep quality → anxiety	−0.020***	0.001	−0.004	0.288	−0.028***	0.001

***$p < 0.001$, **$p < 0.010$, *$p < 0.050$. Model fit index (adults): chi-square (χ^2/DF = 1.066, $p = 0.302$), SRMR = 0.013, RMSEA = 0.011, CFI = 0.999, GFI = 0.999.

and internalising symptoms in young athletes. The results indicate that there are significant associations between chronotype and diet, diet and sleep quality, sleep quality and anxiety/depression. Furthermore, our findings revealed two significant mediating pathways: (1) diet → sleep quality → anxiety/depression and (2) chronotype → diet → sleep quality → anxiety/depression in young adult athletes. Additionally, we did not find any indirect effects in adolescent athletes.

Our findings suggest a positive association between chronotype and diet raw scores in young adult athletes, which is consistent with the previous studies (45–47). The previous study investigated adults aged 18–65 years and found that those with a morning chronotype were intended to consume higher energy, protein and fat intake, and reduced carbohydrate intake (45). Conversely, evening chronotypes consumed lower protein intake (45). In university athletes, a cross-sectional study indicated that evening chronotype athletes consumed higher amounts of carbohydrates, confectionary, and sweet beverages compared to their morning chronotype counterparts (46). Furthermore, Moss and colleagues found higher chronotype raw scores were linked to increased gain consumption (47). Morning

TABLE 6 Indirect effects on depression.

Paths	Overall		Adolescents		Adults	
	Standardised estimates	p	Standardised estimates	p	Standardised estimates	p
Chronotype → diet → sleep quality	−0.020***	0.001	−0.004	0.296	−0.028***	0.001
Chronotype → diet → depression	−0.009*	0.036	−0.006	0.233	−0.009	0.124
Chronotype → sleep quality → depression	−0.026	0.142	−0.029	0.363	−0.021	0.317
Diet → sleep quality → depression	−0.060***	0.001	−0.023	0.373	−0.076***	0.001
Chronotype → diet → sleep quality → depression	−0.020***	0.000	−0.004	0.280	−0.028***	0.001

***$p < 0.001$, **$p < 0.010$, *$p < 0.050$. Model fit index (adults): chi-square (χ^2/DF = 0.002, $p = 0.964$), SRMR = 0.001, RMSEA = 0.000, CFI = 1.000, GFI = 1.000.

chronotypes were identified as individuals consuming more breakfasts, while evening chronotypes displayed preferences for more snacks, caffeine, alcohol, and later eating patterns (45, 48, 49). A scoping review also suggested that evening chronotypes were associated with higher consumption of sweets, caffeine, and alcohol, as well as unhealthier behaviours such as delayed meal times, irregular breakfast eating, breakfast skipping and excessive calorie intake during the night (50).

The current study found that dietary patterns were associated with sleep quality in young athletes. This finding aligns with previous studies (47, 51). For instance, Moss and colleagues found endurance athletes who consumed fewer grains had poorer sleep quality (47). Cando and colleagues found nutrition intake was associated with both sleep duration and quality in elite female athletes (51). Additionally, our findings suggest a positive association between sleep quality and anxiety/depression raw scores in young athletes. This finding is consistent with several previous studies (52–54). Grandner and colleagues found that poor sleep quality was associated with a higher risk of anxiety (Unstandardised $B = 0.459$, 95%CI = 0.294–0.624, $p < 0.0001$) and depression (Unstandardised $B = 0.52$, 95%CI = 0.239–0.801, $p = 0.0003$) (53). Moreover, Potter and colleagues investigated 162 high school athletes and found poor sleep quality was associated with higher anxiety ($\beta = 0.391$, 95%CI = 0.263–0.520, $p < 0.001$) and depressive symptoms ($\beta = 0.456$, 95%CI = 0.346–0.565, $p < 0.001$) (54). We also found that sleep quality was associated with anxiety/depression in adolescent athletes. Adolescents may experience poor sleep quality due to maturational development (e.g., a circadian phase delay) and societal and psychosocial factors (e.g., increased bedtime autonomy, and screen time) (55, 56). It is well-documented that poor sleep quality is associated with higher levels of anxiety and depressive symptoms (54, 57). This can partially explain the association between sleep quality and anxiety/depression in adolescent athletes. Our findings also support the strongest association between sleep quality and internalising symptoms in young athletes. However, mediating analysis suggests that no direct effects are observed between diet and anxiety/depression, as well as chronotypes and sleep quality in young athletes.

In terms of indirect effects, the current study found that sleep quality mediated the relationship between diet and internalising symptoms in young adult athletes. The previous study has indicated that unhealthy dietary patterns in conjunction with inadequate sleep may constitute significant risk factors for the onset of mental health problems (58). Our study extends this finding by demonstrating that sleep quality mediates the association between dietary patterns and anxiety/depression in young adult athletes. Accordingly, diet can impact sleep through various mechanisms such as circulating gut hormones, stimulating the production of serotonin and melatonin, acting on GABAergic or serotonergic neurons, or other unidentified mechanisms such as the bioactive peptides and the nonprotein nitrogen fraction of diet (59, 60). For these reasons, unhealthier dietary patterns may lead to poor sleep quality in young athletes (61). Moreover, the relationship between sleep and anxiety may be explained by shared neurocircuity and there is a complex network of receptor systems, including dopamine, serotonin and adenosine (62). Additionally, sleep may impact depression via increasing inflammatory cytokines, biochemical pathways such as changes in cholinergic and monoaminergic neurons, genetic factors and circadian rhythm (63). Taken together, the relationship between diet and internalising symptoms may be explained by the effects of diet on sleep quality, which may, in turn, impact internalising symptoms in young athletes. Moreover, diet and sleep quality mediated the association between chronotype and internalising symptoms. According to existing evidence, morning chronotype people were more likely to have a healthier and regular lifestyle when compared to their evening counterparts (48, 49, 59). In comparison with the mediating effects of sleep quality, the indirect effect of chronotype on internalising symptoms is smaller than the mediating effect of sleep quality. This finding indicates that chronotype may be associated with insufficient sleep duration through poor dietary behaviours, which could subsequently be related to anxiety and depression. It implies that coaches, athletes, and other stakeholders should be aware that both a healthier diet and better sleep quality potentially have benefits for reducing the risk of internalising symptoms in athletes.

This study represents one of the initial attempts to examine the mediating effects of sleep quality in the relationship between diet and internalising symptoms in young athletes. However, several limitations should be considered when interpreting the findings. Firstly, a cross-sectional study design was employed to examine the relationship between diet, sleep and internalising symptoms in young athletes. More experimental studies are needed to establish the causality of this association. Secondly, self-reported measures were used to assess diet, sleep and internalising symptoms. To enhance our understanding of this association, further research would benefit from incorporating more objective measures. Thirdly, as the sample comprises Chinese young athletes, improving the generalisability of the results is recommended by including a larger sample size and participants from diverse cultural backgrounds. Lastly, given that the data collection was conducted between October and December 2022 during the period of COVID-19. The findings of the current study should be interpreted with caution.

5 Conclusion

The results suggest significant direct effects of chronotype on diet, diet on sleep quality, and sleep quality on anxiety/depression in young adult athletes. Moreover, the mediating models indicate that sleep quality mediated the relationship between dietary patterns and internalising symptoms in young adult athletes. Additionally, diet and sleep quality mediated the association between chronotype internalising symptoms in young adult athletes.

Data availability statement

The datasets presented in this article are not readily available because of the restrictions of ethics. Requests to access the datasets should be directed to Lei Wang: wl2119@163.com.

Ethics statement

The studies involving humans were approved by the Ethics Review Committee of Shanghai University of Sport (Approval No. 102772022RT113). 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

YG: Formal analysis, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. LW: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

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References

1. Hughes EK, Gullone E. Internalizing symptoms and disorders in families of adolescents: a review of family systems literature. *Clin Psychol Rev*. (2008) 28:92–117. doi: 10.1016/j.cpr.2007.04.002

2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5, vol. *5*. Washington, DC: American Psychiatric Association (2013).

3. Zahn-Waxler C, Klimes-Dougan B, Slattery MJ. Internalizing problems of childhood and adolescence: prospects, pitfalls, and progress in understanding the development of anxiety and depression. *Dev Psychopathol*. (2000) 12:443–66. doi: 10.1017/S0954579400003102

4. Eisenberg N, Spinrad TL, Eggum ND. Emotion-related self-regulation and its relation to children's maladjustment. *Annu Rev Clin Psychol*. (2010) 6:495–525. doi: 10.1146/annurev.clinpsy.121208.131208

5. World Health Organization. Depressive disorder (depression). Geneva, Switzerland: World Health Organization (2023).

6. Gorczynski PF, Coyle M, Gibson K. Depressive symptoms in high-performance athletes and non-athletes: a comparative meta-analysis. *Br J Sports Med*. (2017) 51:1348–54. doi: 10.1136/bjsports-2016-096455

7. Rice SM, Purcell R, De Silva S, Mawren D, McGorry PD, Parker AG. The mental health of elite athletes: a narrative systematic review. *Sports Med*. (2016) 46:1333–53. doi: 10.1007/s40279-016-0492-2

8. Palazzolo J. Anxiety and performance. *L'encephale*. (2020) 46:158–61. doi: 10.1016/j.encep.2019.07.008

9. Chang CJ, Putukian M, Aerni G, Diamond AB, Hong ES, Ingram YM, et al. American Medical Society for Sports Medicine position statement: mental health issues and psychological factors in athletes: detection, management, effect on performance, and prevention—executive summary. *Clin J Sport Med*. (2020) 30:91–5. doi: 10.1097/JSM.0000000000000799

10. Reyes-Bossio M, Corcuera-Bustamante S, Veliz-Salinas G, Villas Boas Junior M, Delgado-Campusano M, Brocca-Alvarado P, et al. Effects of psychological interventions on high sports performance: a systematic review. *Front Psychol*. (2022) 13:1068376. doi: 10.3389/fpsyg.2022.1068376

11. Christensen N, van Woerden I, Aubuchon-Endsley NL, Fleckenstein P, Olsen J, Blanton C. Diet quality and mental health status among division 1 female collegiate athletes during the COVID-19 pandemic. *Int J Environ Res Public Health*. (2021) 18:13377. doi: 10.3390/ijerph182413377

12. McCabe EM, Ketcham CJ, Hall EE. Good food, good mood: perspectives on the relationship between nutrition and mental health with division I collegiate athletic programs. *Front Sports Act Living*. (2021) 3:692601. doi: 10.3389/fspor.2021.692601

13. Gerber M, Jakowski S, Kellmann M, Cody R, Gygax B, Ludyga S, et al. Macronutrient intake as a prospective predictor of depressive symptom severity: an exploratory study with adolescent elite athletes. *Psychol Sport Exerc*. (2023) 67:102442. doi: 10.1016/j.psychsport.2023.102442

14. Suel E. The relationship between emotional eating and general health among professional basketball players. *Prog Nutr*. (2020) 22:1–7. doi: 10.23751/pn.v22i2-S.10575

15. Burke L. Fueling strategies to optimize performance: training high or training low? *Scand J Med Sci Sports*. (2010) 20:48–58. doi: 10.1111/j.1600-0838.2010.01185.x

16. Malsagova KA, Kopylov AT, Sinitsyna AA, Stepanov AA, Izotov AA, Butkova TV, et al. Sports nutrition: diets, selection factors, recommendations. *Nutrients*. (2021) 13:3771. doi: 10.3390/nu13113771

17. Kerksick CM, Arent S, Schoenfeld BJ, Stout JR, Campbell B, Wilborn CD, et al. International Society of Sports Nutrition position stand: nutrient timing. *J Int Soc Sports Nutr*. (2017) 14:33. doi: 10.1186/s12970-017-0189-4

18. Ersoy N, Kalkan I, Ersoy G. Assessment of nutrition status of Turkish elite young male soccer players in the pre-competition period. *Prog Nutr*. (2019) 21:12–8. doi: 10.23751/pn.v21i1.7127

19. Diehl K, Yarmoliuk T, Mayer J, Zipfel S, Schnell A, Thiel A, et al. Eating patterns of elite adolescent athletes: results of a cross-sectional study of 51 Olympic sports. *German J Sports Med*. (2013) 2013:126–31. doi: 10.5960/dzsm.2012.067

20. Reardon CL, Bindra A, Blauwet C, Budgett R, Campriani N, Currie A, et al. Mental health management of elite athletes during COVID-19: a narrative review and recommendations. *Br J Sports Med*. (2021) 55:608–15. doi: 10.1136/bjsports-2020-102884

21. Von Rosen P, Olofsson O, Väsbom S, Heijne A. Correlates of health in adolescent elite athletes and adolescents: a cross-sectional study of 1016 adolescents. *Eur J Sport Sci*. (2019) 19:707–16. doi: 10.1080/17461391.2018.1552721

22. Spendlove JK, Heaney SE, Gifford JA, Prvan T, Denyer GS, O'Connor HT. Evaluation of general nutrition knowledge in elite Australian athletes. *Br J Nutr*. (2012) 107:1871–80. doi: 10.1017/S0007114511005125

23. Rathnayake H, Wickramatilake CM, Senanayake DW. Knowledge, attitudes and practices on diet and nutrition in a group of national-level athletes in the developing world. *Med Sport*. (2021) 74:56–65. doi: 10.23736/S0025-7826.21.03811-4

24. Mitchell L, Lehane P, McCarthy M, O'Shea K, Tracey M, Whyte T, et al. Nutrition knowledge of elite and non-elite Gaelic footballers. *Sci Med Footb*. (2022) 6:159–63. doi: 10.1080/24733938.2021.1906440

25. Gupta L, Morgan K, Gilchrist S. Does elite sport degrade sleep quality? A systematic review. *Sports Med*. (2017) 47:1317–33. doi: 10.1007/s40279-016-0650-6

26. Samuels C. Sleep, recovery, and performance: the new frontier in high-performance athletics. *Neurol Clin*. (2008) 26:169–80. doi: 10.1016/j.ncl.2007.11.012

27. Huskey A, Kim K, Emert S, Auerbach A, Webb R, Skog M, et al. 315 athlete sleep and mental health: differences by gender, race, and ethnicity. *Sleep*. (2021) 44:A125–6. doi: 10.1093/sleep/zsab072.314

28. Halson SL. Sleep in elite athletes and nutritional interventions to enhance sleep. *Sports Med*. (2014) 44:13–23. doi: 10.1007/s40279-014-0147-0

29. Murphy J, Gladney C, Sullivan P. Exploring the relationship between sleep quality, sleep hygiene, and psychological distress in a sample of Canadian varsity athletes. *J Clin Sport Psychol*. (2021) 16:220–33. doi: 10.1123/jcsp.2020-0037

30. Facer-Childs ER, Mascaro L, Hoffman D, Mansfield D, Drummond SP, Rajaratnam SM. Sleep as a major determinant for mental health outcomes in elite Australian football league (AFL) athletes. *Med Sci Sports Exerc*. (2022) 54:665–72. doi: 10.1249/MSS.0000000000002825

31. Knufinke M, Nieuwenhuys A, Maase K, Moen MH, Geurts SA, Coenen AM, et al. Effects of natural between-days variation in sleep on elite athletes' psychomotor vigilance and sport-specific measures of performance. *J Sports Sci Med*. (2018) 17:515–24.

32. Doherty R, Madigan S, Warrington G, Ellis J. Sleep and nutrition interactions: implications for athletes. *Nutrients*. (2019) 11:822. doi: 10.3390/nu11040822

33. Juliff LE, Halson SL, Peiffer JJ. Understanding sleep disturbance in athletes prior to important competitions. *J Sci Med Sport*. (2015) 18:13–8. doi: 10.1016/j.jsams.2014.02.007

34. Hrozanova M, Moen F, Pallesen S. Unique predictors of sleep quality in junior athletes: the protective function of mental resilience, and the detrimental impact of sex, worry and perceived stress. *Front Psychol*. (2019) 10:1256. doi: 10.3389/fpsyg.2019.01256

35. Barnard J, Roberts S, Lastella M, Aisbett B, Condo D. The impact of dietary factors on the sleep of athletically trained populations: a systematic review. *Nutrients*. (2022) 14:3271. doi: 10.3390/nu14163271

36. Gratwicke M, Miles KH, Pyne DB, Pumpa KL, Clark B. Nutritional interventions to improve sleep in team-sport athletes: a narrative review. *Nutrients*. (2021) 13:1586. doi: 10.3390/nu13051586

37. Schubring A, Halltén M, Barker-Ruchti N, Post A. Balancing risk-taking and self-care: the ecology of athlete health behaviour during the Olympic qualification phase. *Int Rev Sociol Sport*. (2023) 58:1326–48. doi: 10.1177/10126902231168098

38. General Administration of Sport of China. (2021). Technical grade standards for athletes in each event. Available at: https://www.sport.gov.cn/n4/n207/n209/c23399926/content.html (Accessed July 21, 2021).

39. Capling L, Gifford JA, Beck KL, Flood VM, Slater GJ, Denyer GS, et al. Development of an athlete diet index for rapid dietary assessment of athletes. *Int J Sport Nutr Exerc Metab*. (2019) 29:643–50. doi: 10.1123/ijsnem.2019-0098

40. Capling L, Gifford JA, Beck KL, Flood VM, Halar F, Slater GJ, et al. Relative validity and reliability of a novel diet quality assessment tool for athletes: the athlete diet index. *Br J Nutr*. (2021) 126:307–19. doi: 10.1017/S000711452000416X

41. Bender AM, Van Dongen HP, Samuels CH. Sleep quality and chronotype differences between elite athletes and non-athlete controls. *Clocks Sleep*. (2018) 1:3–12. doi: 10.3390/clockssleep1010002

42. Samuels C, James L, Lawson D, Meeuwisse W. The athlete sleep screening questionnaire: a new tool for assessing and managing sleep in elite athletes. *Br J Sports Med*. (2015) 50:418–22. doi: 10.1136/bjsports-2014-094332

43. Vitale JA, Weydahl A. Chronotype, physical activity, and sport performance: a systematic review. *Sports Med*. (2017) 47:1859–68. doi: 10.1007/s40279-017-0741-z

44. Kline RB. *Principles and practice of structural equation modeling*. *4th Edn*. New York: The Guilford Press (2016).

45. Günal AM. Sleep, activity, and diet in harmony: unveiling the relationships of chronotype, sleep quality, physical activity, and dietary intake. *Front Nutr*. (2023) 10:10. doi: 10.3389/fnut.2023.1301818

46. Nakade M, Takeuchi H, Uezato T, Krejci M, Tsuji F, Kawada T, et al. Questionnaire study on the comparison among circadian typology, sleep habit, physical and mental health and meal habit in Japanese university athletes. *Psychology*. (2015) 6:1562–9. doi: 10.4236/psych.2015.612153

47. Moss K, Zhang Y, Kreutzer A, Graybeal AJ, Porter RR, Braun-Trocchio R, et al. The relationship between dietary intake and sleep quality in endurance athletes. *Front Sports Act Living*. (2022) 4:810402. doi: 10.3389/fspor.2022.810402

48. van der Merwe C, Münch M, Kruger R. Chronotype differences in body composition, dietary intake and eating behavior outcomes: a scoping systematic review. *Adv Nutr*. (2022) 13:2357–405. doi: 10.1093/advances/nmac093

49. Teixeira GP, Guimarães KC, Soares AGN, Marqueze EC, Moreno CR, Mota MC, et al. Role of chronotype in dietary intake, meal timing, and obesity: a systematic review. *Nutr Rev*. (2023) 81:75–90. doi: 10.1093/nutrit/nuac044

50. Mazri FH, Manaf ZA, Shahar S, Mat Ludin AF. The association between chronotype and dietary pattern among adults: a scoping review. *Int J Environ Res Public Health*. (2020) 17:68. doi: 10.3390/ijerph17010068

51. Condo D, Lastella M, Aisbett B, Stevens A, Roberts S. Sleep duration and quality are associated with nutrient intake in elite female athletes. *J Sci Med Sport*. (2022) 25:345–50. doi: 10.1016/j.jsams.2021.11.045

52. Alvaro PK, Roberts RM, Harris JK. A systematic review assessing bidirectionality between sleep disturbances, anxiety, and depression. *Sleep*. (2013) 36:1059–68. doi: 10.5665/sleep.2810

53. Grandner MA, Hall C, Jaszewski A, Alfonso-Miller P, Gehrels J-A, Killgore WD, et al. Mental health in student athletes: associations with sleep duration, sleep quality, insomnia, fatigue, and sleep apnea symptoms. *Athl Train Sports Health Care*. (2021) 13:e159–67. doi: 10.3928/19425864-20200521-01

54. Potter MN, Howell DR, Dahab KS, Sweeney EA, Albright JC, Provance AJ. Sleep quality and quality of life among healthy high school athletes. *Clin Pediatr*. (2020) 59:170–7. doi: 10.1177/0009922819892050

55. Mason L, Connolly J, Devenney LE, Lacey K, O'Donovan J, Doherty R. Sleep, nutrition, and injury risk in adolescent athletes: a narrative review. *Nutrients*. (2023) 15:5101. doi: 10.3390/nu15245101

56. Saidi O, Souabni M, Del Sordo GC, Maviel C, Peyrel P, Maso F, et al. Association between low energy availability (LEA) and impaired sleep quality in young Rugby players. *Nutrients*. (2024) 16:609. doi: 10.3390/nu16050609

57. Stracciolini A, McCracken CM, Meehan WP, Milewski MD. Lack of sleep among adolescent athletes is associated with a higher prevalence of self-reported history of anxiety and depression. *J Clin Sport Psychol*. (2021) 17:176–88. doi: 10.1123/jcsp.2021-0004

58. De Souza NL, Esopenko C, Conway FN, Todaro SM, Buckman JF. Patterns of health behaviors affecting mental health in collegiate athletes. *J Am Coll Heal*. (2021) 69:495–502. doi: 10.1080/07448481.2019.1682591

59. Peuhkuri K, Sihvola N, Korpela R. Diet promotes sleep duration and quality. *Nutr Res*. (2012) 32:309–19. doi: 10.1016/j.nutres.2012.03.009

60. Godos J, Grosso G, Castellano S, Galvano F, Caraci F, Ferri R. Association between diet and sleep quality: a systematic review. *Sleep Med Rev*. (2021) 57:101430. doi: 10.1016/j.smrv.2021.101430

61. Fiedler R, Heidari J, Birnkraut T, Kellmann M. Digital media and mental health in adolescent athletes. *Psychol Sport Exerc*. (2023) 67:102421. doi: 10.1016/j.psychsport.2023.102421

62. Chellappa SL, Aeschbach D. Sleep and anxiety: from mechanisms to interventions. *Sleep Med Rev*. (2022) 61:101583. doi: 10.1016/j.smrv.2021.101583

63. Fang H, Tu S, Sheng J, Shao A. Depression in sleep disturbance: a review on a bidirectional relationship, mechanisms and treatment. *J Cell Mol Med*. (2019) 23:2324–32. doi: 10.1111/jcmm.14170

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Performance variables and nutritional status analysis from Moroccan professional and adolescent football players during the competition period: a descriptive study

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Introduction: Nutrition plays an integral role in optimizing football players' performance during training sessions and matches and maintaining their overall health throughout the season. This study aimed to evaluate how well the dietary practices of professional and adolescent football players in Morocco during the competitive period met international macronutrient recommendations, and to explore the relationship between their nutritional status and aerobic performance, as measured by the Yo-Yo IRL1 test.
Methods: A total of 277 footballers from Morocco's professional league, "Botola-Pro", were monitored over a seven-day (training microcycle) during the competitive period. The dietary intake was assessed through self-reported methods and 24-hour recalls. Relevant body composition was measured with bioelectrical impedance (BI), and aerobic performance was evaluated using the Yo-Yo IR test.
Results: The results indicated significant variations in performance and nutritional status across different categories and age groups. The nutritional status of the players didn't match the UEFA recommendations ($p < 0.001$). We found that higher intake levels of carbohydrates and proteins were positively correlated with the total distance covered by the players ($p < 0.001$, $r = 0.63$, $R^2 = 0.4$ for carbohydrates; $p < 0.001$, $r = 0.59$, $R^2 = 0.35$ for proteins). Conversely, a higher proportion of energy derived from fats in the diet was negatively correlated with the distance covered ($p < 0.001$, $r = -0.64$, $R^2 = 0.41$).
Conclusion: These findings suggest that optimizing carbohydrates and protein intake while managing fat consumption is crucial for enhancing sporting performance. This information is essential for tailoring training programs and nutritional regimens based on the competition level.

KEYWORDS

nutritional status, carbohydrates proteins and fat intake, professional and adolescent football players, competitive period, aerobic performance, the Yo-Yo IR test

1 Introduction

Soccer, widely regarded as the world's most popular sport, is marked by repeated bursts of high-intensity activity (1). It requires a complex set of physical qualities, skillfully combining intense phases of running, sprints, changes in direction, and jumps with moments of relative recovery (2, 3). These diverse performances, ranging from explosive accelerations to powerful movements, demand a delicate balance between anaerobic efforts and aerobic endurance (4).

Making good nutritional choices can enhance the health and performance of football players, the type, quantity, and timing of food, fluids, and supplements consumed can significantly impact players' performance and recovery during and between matches (5). However, the rapidly evolving nature of the game, both technically and tactically, underscores the importance for medical staff and players to make informed nutritional decisions (6). These decisions are vital to supporting training loads and optimizing physical performance. A recent meta-analysis revealed that over the past two decades, from 2000 to 2019, players whether professionals, amateurs, adults, or adolescents have faced challenges in effectively meeting their nutritional needs, particularly concerning energy and carbohydrates intake (7).

Several recommendations have been implemented over time, with the most recent being the UEFA consensus (5)suggesting that one day before the match (D-1), the match day (D), and the day after the match (D + 1), carbohydrate (CHO) intake should be increased to replenish muscle glycogen reserves. It's recommended to consume 6–8 g/day/kg of body weight (BW) (5).

The proteins, composed of amino acids, play a crucial role in the repair and growth of muscle tissues solicited during aerobic efforts (8).They also contribute to the synthesis of new enzymes and molecules essential for performance. Muscle recovery after an intensive football match involves protein synthesis, where amino acids from dietary proteins are used to repair damaged muscle fibers (9). This muscular regeneration helps minimize fatigue, prevent injuries, and support long-term athletic performance (10). Moreover, in the context of football, where endurance, speed, and strength are crucial, proteins play a key role in the synthesis of enzymes and molecules necessary for energy production (11). Enzymes facilitate metabolic processes that provide the necessary energy to sustain the intensity of the game (12–14). Adequate protein intake in the diet thus contributes to optimizing the performance of football players on the field. The UEFA recommendations suggest a protein intake ranging from 1.2 to 2.2 g/day/kg of body weight (BM) for players (5), and according to recent studies conducted primarily in Europe, the USA, Australia, and Asia, most adults often meet this ratio (7). The scientific community suggests that adolescents should also have sufficient protein intake during this crucial phase to promote normal growth and development in young footballers (15).

The dietary fat plays a crucial role in training nutrition by serving as an energy source, facilitating the absorption of fat-soluble vitamins, and providing essential fatty acids. Athletes are recommended to tailor their fat intake to meet protein and carbohydrate requirements within overall energy goals (16). Additionally, adherence to community guidelines regarding minimal trans fatty acid intake and cautious consumption of saturated fats is advised (17) The UEFA consensus recommends that the percentage ratio for fat intake should fall within the range of 20%–35% of total energy intake (TEI) (5).

In sport performance, the aerobic capacity (AC), represents an essential element of physical fitness (PF) that defines the ability of players to maintain their optimal performance levels throughout a match or an intense training session (12). Many research studies have been carried out in the last few years to investigate the relationship between running distance covered, and physical performances during football games, both at the semi-elite and youth levels (18–23), and at the professional level (1, 12, 24–28). The results of these studies confirm a strong correlation between physical fitness and the running demands during matches, highlighting that total distance covered and peak speed in endurance tests are associated with both total distance and high-intensity distance covered during play. In this context, field tests are considered a more appropriate alternative to laboratory tests (19, 29), mainly due to their relevance to the sport, low cost, and their ease of implementation. Considering these aspects, it will appropriate use field tests during the competitive season frequently.

The physical fitness (PF) of football players engaged in significant competitive levels has been the subject of thorough analysis over the past decades, utilizing various testing protocols, including the Yo-Yo IR1 test developed by Bangsbo et al. (30) which involves covering the greatest possible distance while following a running pattern that includes periods of intense effort and recovery. The assessment of seasonal variability in players' physical capacity within different European football leagues has been conducted using this protocol and similar ones (27, 31–34).

On the other hand, several scientific studies have shown that footballers, whether adults or adolescents, struggle to meet their nutritional needs, whether in terms of energy or macronutrients (7). Furthermore, adolescents face a paradox: they must not only meet the demands of sports performance but also satisfy the specific needs related to their growth phase… (significant changes, physically psychologically …). The combination of intense training, competitive demands, and physiological changes can increase adolescents' vulnerability to phenomena such as fatigue, overtraining, and injuries. Prolonged fatigue not only can affects sports performance but also impacts negatively the overall wellbeing and growth of adolescents (26, 35). On the flip side, making optimal nutrition choices is crucial for promoting health and performance. The quality, quantity, and timing of food, fluids, and supplements consumption play a pivotal role in influencing the players' performance and recovery time both during and between matches (5, 36).

Following this introduction, it is essential to highlight the crucial importance of the relationship between nutritional status and aerobic performance, whether during matches or training. This relationship is influenced by various factors, including the level of competition, age category, workload intensity, exertion, and other parameters. However, to date, and to our knowledge, a few studies have explored these relationships, particularly in Morocco (37), North Africa, and across the African continent. Therefore, this study aims to (I) evaluate the dietary habits and nutritional status in terms of macronutrients among Moroccan adolescent and adult football players, to determine if their diet aligns with the international recommendations of the UEFA scientific community for adults and professionals, as well as with Moroccan nutritional recommendations for pre-adolescents; (II) examine the impact of this nutritional status on aerobic performance, measured by the distance covered during the Yo-Yo IR1 test.

In this context, it is crucial to examine several questions to deepen our understanding of the interactions between nutrition and performance in Moroccan football. This study aims to address the following questions:

1. Do Moroccan football players adhere to macronutrient recommendations?
2. Do players with higher levels of aerobic performance better comply with macronutrient nutritional recommendations?
3. Is there a correlation between the level of competition and the nutritional status of players?
4. How do players' dietary habits evolve from adolescence to adulthood, and are these changes aligned with nutritional recommendations for different levels of competition?
5. What is the impact of nutritional status on aerobic performance, as measured by the distance covered during the Yo-Yo IR1 test?

These questions seek to clarify the complex relationships between nutritional intake and sports performance, considering the specificities of Moroccan players across various levels of competition.

2 Material and methods

2.1 Study design

An observational study protocol was established to assess potential relationships between macronutrient intake, energy intake, and physical performance measures expressed in distance covered following a validated field test Yo-Yo TR1 (30). The aim was to investigate the impact of nutritional status on performance in a total of 253 male soccer players in Morocco of varying ages (from 12 to 25), from academic to professional level competition. The study was conducted during the second half of the return phase (after transition phase) of the Moroccan "Botola-Pro" National league Championship competitive season 2022–2023. Each age category or group was monitored over a period of 7 consecutive days, including one microcycle training from Monday to Saturday, as illustrated in Figure 1.

Dietary survey and anthropometric measurements were conducted daily from monday to sunday at 10 a.m. before training sessions. It's noteworthy that the physical test was conducted during the Sunday session, and no other activity was carried out before or during this testing period to ensure the validity of the protocol and to avoid any other factors that could influence the results. A warm-up of 5–10 min was performed based on the qualities of each player, as well as their category and competition level.

This study has been approved by the regional ethics committee of the Ibn RORCHD University Hospital Center of Casablanca governed by Morocco's Ministry of Health (Approval No. 22/2022) and has been conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The participants have given their consent to be involved in the study and were given the option to opt out at any moment.

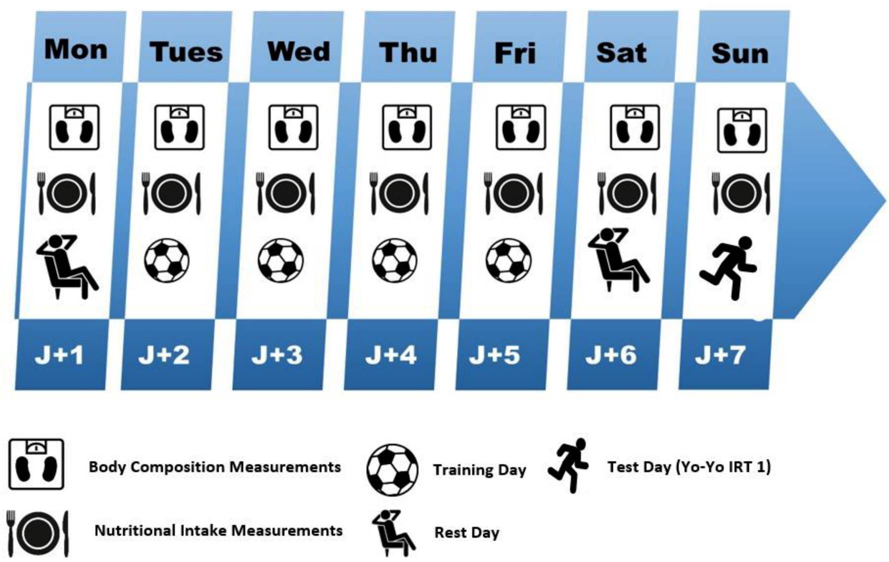

FIGURE 1
The study design.

2.2 Participants

A total of 277 male Moroccan football players from 14 clubs competing in the "Botola-Pro" Moroccan championship were included in the study, with careful distribution made across age groups and competitive levels. The eligibility criteria applied to this study excluded goalkeepers (a number of 14) because the physical effort exerted by goalkeepers differs from that of other players, and our objective is to evaluate the aerobic performance of outfield players. Also excluded were the players who did not complete performance tests (a number of 4) or the dietary questionnaires (totaling 5), as well as those with injuries or illnesses during the research period (a number of 2).

In total 253 participants representing 14 football teams with players (ranging from pre-adolescence to adulthood, from academic to professional levels), were followed, analyzed, and processed till the end of the study and distributed as follows:

The *Senior Professional group level "S-PROF"* was made up of 49 players from two professional clubs that were involved in the "Botola-Pro" competition. Fifty players from two teams competing in the national elite tournament made up the senior elite group, or "S-ELIT". Fifty players from two teams competing in the young elite division of the national elite youth championship made up the "Y-ELIT" group of young players. Finally, 55 players from three teams in the U15 and U14 categories (referred to as "U15/U14-ACDM") who were competing in the academic championship were also included in our study. Furthermore, 49 competitors in the U13 and U12 divisions were also included.

2.3 Anthropometry

InBody Co., Ltd.'s InBody 120 bioelectrical impedance analyzer was used to collect anthropometric data. This analyzer produces a current of 150 µA (± 50 µA) while operating at frequencies of 20 and 100 kHz. It offers a range of complete findings, including weight, body mass index (BMI), body fat mass (BFM), body muscle mass (BMM), and Resting Metabolic Rate (RMR). The precision errors less than 2% for lean body mass (FFM), fat mass (FM), and percentage of body fat (%BF) (38, 39).

A stadiometer (Portable Stadiometer-Seca 225, Hamburg, Germany) with an accuracy of ±0.1 mm was used to measure the subjects' height; to assure accuracy, athletes took off their shoes.

The World Health Organization (WHO) curves were used in conjunction with a particular approach to calculate BMI for participants in the Under 18 group. These globally accepted curves ensure an accurate evaluation of weight status while accounting for changes linked to growth and physiological development (40).

2.4 Physical performance test

To assess the participants' physical performances, we opted for the Yo-Yo intermittent recovery test level 1 (Yo-Yo IRT 1) developed by Bangsbo et al. (30), a recognized measure in the field of aerobic endurance evaluation, this test involves a series of repeated runs with progressively reduced recovery intervals. Participants are pushed to their limits in terms of speed and endurance, providing a reliable assessment of their aerobic capacities. The Yo-Yo IRT 1 test is often favored for its specificity to the demands of football, challenging players' ability to sustain high-intensity efforts throughout the test. the distance traveled is expressed in meters (m) and the results obtained through the Yo-Yo test will contribute to our in-depth understanding of the participants' physical performance profiles within the context of our study.

2.5 Nutritional status measurement

A rigorous protocol was implemented for the collection of nutritional data, aiming to comprehensively assess the intake of macronutrients, including carbohydrates, proteins, and fats, as well as dietary fiber and cholesterol among the examined players. For each category, the 24-hour dietary recall method was systematically applied over a period of seven consecutive days (Figure 1). This approach involved the use of standard household units, such as spoons, glasses, and containers, to enable a precise estimation of the quantities consumed (41, 42). Additionally, the Remote Food Photographic Method (RFPM) was integrated into the protocol as a complementary method to meticulously estimate caloric intake. This methodological decision aligns with recommendations from scientific studies, particularly the work of Martin et al. (43). The utilization of RFPM added an extra dimension to our assessment of energy intake, providing a detailed visual perspective on the dietary habits of players throughout the study duration.

2.6 Dietary analysis

A careful analysis of dietary intake was conducted using the Nutrilog 3.30 software, a tool based on the 2020 Ciqual nutritional database (44, 45). This software provides an advanced platform that accurately determines the levels of macronutrients such as carbohydrates, proteins, and fats, as well as micronutrients, fibers, and various other food components. Leveraging the updated Ciqual database, Nutrilog software facilitated a thorough analysis of participants' nutritional intake, thereby offering a comprehensive insight into the composition of their dietary patterns during the study period.

2.7 Statistical analysis

The statistical analysis of the data collected was conducted using SPSS version 27 (IBM, SPSS Statistics, Version 27, Chicago IL), as the primary analytical tool. The mean (mean) was employed to represent the central tendency of the data and the

Standard deviation (SD) was used to assess the dispersion or variability around the mean.

To assess the normality of variables, the Kolmogorov-Smirnov test was applied. The *T*-test was used form comparisons of the means between macronutrient intake and UEFA recommended values for professional and elite and young groups, as well as between means of Moroccan recommendations for academic levels (U15/U14 and U13/U12). And a one-way analysis of variance (ANOVA) with post-hoc testing and the Tukey test to identify the level of macronutrient consumption that could enhance the athletic performance of the studied football players. For carbohydrates, players were categorized into four groups based on the quantity consumed: less than 4 g/kg body weight, between 4 and 5 g/kg body weight, between 5 and 6 g/kg body weight, and over 6 g/kg body weight. For protein intake, participants were classified into three groups: less than 1.6 g/kg body weight, between 1.6 and 2.2 g/kg body weight, and over 2.2 g/kg body weight. To explore the impact of fat intake on sports performance, participants were grouped into four categories based on the quantity of fats consumed (between 20% and 25%, between 25% and 30%, between 30% and 35%, and over 35%).

The Pearson correlation test was applied to examine the relationships between various performance variables and dietary intake. Statistical significance was assessed with a threshold of $p < 0.05$, indicating a level of significance. Additionally, a 95% confidence interval (CI) was set to enhance result precision. These analytical procedures, along with the defined significance criteria, were rigorously adhered to ensure the validity and reliability of the conclusions drawn from this study.

3 Results

3.1 Anthropometric characteristic's

Table 1 presents a descriptive analysis of the anthropometric characteristics of the 253 players studied, distributed across different age categories. Taking into account the level of competition, body mass index, basal metabolism, and body fat percentage, this table shows a trend of increasing values according to age, except for the body fat percentage, which decreases with age and increases with the level of competition with averages of 14.3% (±1.2) to 10.2% (±2.3).

3.2 Performance

The performance results for Yo-Yo IRT measurements are presented in Table 2, indicating means (m) with standard deviations (±SD), detailed for each category. The normality of all variables was tested using the Kolmogorov-Smirnov test, which displayed a *p*-value greater than 0.05. To determine significant differences in performance, especially between professional and elite levels, as well as between academic levels, the ANOVA test was utilized with post-hoc testing and Tukey's test. The results revealed statistically significant variations (F = 175.86) among the means of the S-PROF, S-ELIT, Y-ELIT, U15/14-ACDM, and U13/12-ACDM categories ($p < 0.001$). Professional players (S-PROF) exhibited superior performances, covering a distance of 2,451.5 m ± 162.9, while S-ELIT, U15/14-ACDM, and U13/12-ACDM achieved distances of 2,200.8 m ± 380.8; 2,101.6 m ± 331.2; 1,677.8 m ± 209.0; and 1,175.1 m ± 174.5, respectively.

Significant differences were observed between the S-ELIT, U15/14-ACDM, and U13/12-ACDM categories ($p < 0.001$), as well as between the Y-ELIT, U15/14-ACDM, and U13/12-ACDM categories ($p < 0.001$). However, no difference was found between the S-ELIT and Y-ELIT categories, with a *p*-value of 0.34.

3.3 Dietary status

Table 3 presents data on the athletes (*N* = 253 participants) macronutrient consumption as means with standard deviations (±SD). The total energy intake (TEI) ranges from 2,414 to 3,128 kcal (Figure 2A, Table 3). For the U15/14 and U13/12 categories (academic level), the energy intake aligns with Moroccan recommendations, with values of 2,521 ± 260 and 2,414 ± 300 Kcal, respectively. However, it significantly deviates for competitive-level footballers according to UEFA standards, with a $p < 0.001$. This trend is also evident in carbohydrate (CHO) consumption in g/kg of body mass, showing values of 4.5 g/kg BM for U15/14 and 4.2 g/kg BM for U13/12.

Moreover, the S-PROF (3,128 ± 295 Kcal), S-ELIT (2,966 ± 242 Kcal), and Y-ELIT (2,708 ± 233 Kcal) groups exhibit slightly lower levels compared to UEFA recommendations for professional and elite players, with significant differences of

TABLE 1 Descriptive analysis of the sample characteristics (participants players 253).

Variables	S-PROF		S-ELIT		Y-ELIT		U15/U14 ACDM		U13/U12 ACDM	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Age (years)	25.2	3.4	21.5	2.4	16.0	0.4	14.8	0.4	12.8	0.4
Height (cm)	179.8	6.4	177.9	4.4	172.5	4.1	165.1	5.8	155.1	6.2
Weight (kg)	72.6	6.0	70.6	5.1	63.1	4.9	55.3	4.6	47.2	7.1
BMI	22.4	1.2	22.3	1.2	21.2	1.2	20.3	1.2	19.5	2.3
BFM (%)	10.2	2.3	12.1	1.3	12.5	1.2	13.4	1.2	14.3	1.2
BM (Kcal)	1794	109	1782	84	1689	81	1552	84	1404	119

BMI, body mass index; BFM, body fat mass; BM, Basal metabolic; SD, standard deviation; *p*-value at 0,05. Senior professional "S-PROF" senior elite group level "S-ELIT", Young elite group level "Y-ELIT", U15 and U14 categories academic level "U15/14-ACDM". U13 and U12 categories academic level "U13/12-ACDM".

TABLE 2 The physical test (Yo-Yo IR1) and significance differences by ANOVA statistical test for All groups of football players.

(I) categories (*N* = 253)	Mean (m)	±SD	(J) Group of categories	Mean difference (I-J)	Sig.	95% Confidence interval	
						Lower bound	Upper bound
S-PROF	2451.5	162.9	S-ELIT	250.7306[a]	<0.001	103.688	397.773
			Y-ELIT	349.9306[a]	<0.001	202.888	496.973
			U15/14-ACDM	773.7124[a]	<0.001	630.016	917.409
			U13/12-ACDM	1276.4286[a]	<0.001	1128.645	1424.212
S-ELIT	2200.8	380.8	S-PROF	−250.7306[a]	<0.001	−397.773	−103.688
			Y-ELIT	99.2000	0.340	−47.098	245.498
			U15/14-ACDM	522.9818[a]	<0.001	380.047	665.916
			U13/12-ACDM	1025.6980[a]	<0.001	878.655	1172.741
Y-ELIT	2101.6	331.2	S-PROF	−349.9306[a]	<0.001	−496.973	−202.888
			S-ELIT	−99.2000	0.340	−245.498	47.098
			U15/14-ACDM	423.7818[a]	<0.001	280.847	566.716
			U13/12-ACDM	926.4980[a]	<0.001	779.455	1073.541
U15/14-ACDM	1677.8	209.0	S-PROF	−773.7124[a]	<0.001	−917.409	−630.016
			S-ELIT	−522.9818[a]	<0.001	−665.916	−380.047
			Y-ELIT	−423.7818[a]	<0.001	−566.716	−280.847
			U13/12-ACDM	502.7161[a]	<0.001	359.020	646.413
U13/12-ACDM	1175.1	174.5	S-PROF	−1276.4286[a]	<0.001	−1424.212	−1128.645
			S-ELIT	−1025.6980[a]	<0.001	−1172.741	−878.655
			Y-ELIT	−926.4980[a]	<0.001	−1073.541	−779.455
			U15/14-ACDM	−502.7161[a]	<0.001	−646.413	−359.020

Senior professional "S-PROF" senior elite group level "S-ELIT", Young elite group level "Y-ELIT", U15 and U14 categories academic level "U15/14-ACDM". U13 and U12 categories academic level "U13/12-ACDM".
[a]The mean difference is significant at the 0.01 level (ANOVA test).

$p < 0.001$ and $p < 0.01$ concerning the recommended values for energy intake. This trend is also reflected in carbohydrate intake (CHO) in g/kg of body weight, with quantities of 5.0 g/kg, 4.8 g/kg, and 4.7 g/kg of body mass (BM) respectively for the S-PROF, S-ELIT, and Y-ELIT groups, displaying statistical significance of $p < 0.01$ (Figure 2B, Table 3). Also, the percentage of carbohydrates (CHO%) varies from 53%, 52%, and 50%.

However, the protein intake (PRO) in the S-PROF, S-ELIT, and Y-ELIT groups aligns with UEFA recommendations, with values of 1.7 g/kg, 1.6 g/kg, and 1.6 g/kg of body mass (BM) with a value of 15% of total energy consumption. As for players in the U15/14-ACDM and U13/12-ACDM categories, they adhere to Moroccan recommendations but are slightly below the guidelines for competitive athletes, with a value of 1.4 g/kg for each category (Figure 2C, Table 3).

Moreover, an increased consumption of fats (FAT%) is clearly evident: Only the professionals (27%) do not exceed the limits stated as recommendations; the other categories often exceed the recommended levels, presenting values of 35.2%, 36.3%, and 39.2%, respectively, for the Y-ELIT, U15/14 ACDM, and U13/12ACDM categories (Figure 2D, Table 3). Simultaneously, an excess of cholesterol levels (Choles) has been observed, with values ranging from 324 to 415 mg. The young players exhibit higher values compared to professionals (324 mg ± 63) overall, in Y-ELIT, U15/14ACDM, and U13/12ACDM categories: 385 mg ± 63, 415 mg ± 63, and 371 mg ± 73, respectively.

Regarding fiber intake, players have quantities ranging from 19.2 to 27.1 g. Young players exhibit lower levels of fiber compared ($p < 0{,}01$) to those of professionals and elite-level players.

4 Discussion

The main objective of this study was twofold: firstly, to assess and describe the dietary habits as well as the nutritional status in terms of macronutrients among Moroccan football players, taking into account their level of competition. This analysis explored the evolution of these aspects from adolescence to adulthood, including the transition to the professional level, in order to assess whether these dietary patterns align with international recommendations (5, 46).

Secondly, to establish whether there are correlations between dietary choices and physical performance in Moroccan's football players at different stages of development.

4.1 The players show deficiencies in meeting the recommended macronutrient intake

Like all other findings on an international scale (6, 47, 48), Moroccan players exhibit energy and carbohydrate intake levels below the established recommendations, a recurring observation across all player categories. This deficit may be attributed to specific dietary habits, which seem not to fully meet the nutritional requirements of high-level sports. In contrast, protein intake generally adheres to the recommendations, particularly among adults, indicating a better awareness of the importance of protein in the athletic diet. However, fat intake consistently

TABLE 3 Macronutrient intake compared to recommendations of all football players groups.

Total (*N* = 253)	Mean of variables and recommendations (5)			*p-value*
S-PROF (*N* = 50)	Mean	±SD	DRIs	
TEI (Kcal)	3128	295	3400–4300	<0.01
CHO%	53	4	———-	——
CHO (g/kg BM)	5.0	0.5	6–8	<0.01
PRO (g/kg BM)	1.7	0.2	1.6–2.2	0.07
PRO%	15	2	———-	——
FAT (g/kg BM)	1.5	0.2	———-	——
FAT%	31.8	4.0	20–35	<0.01
Fiber (g)	27.1	1.7	20–40	<0.01
Choles (mg)	324	84	< 300 mg	<0.01
S-ELIT (*N* = 50)				
TEI (Kcal)	2966	242	3400–4300	<0.001
CHO%	52	4	———-	——
CHO (g/kg BM)	4.8	0.6	6–8	<0.01
PRO (g/kg BM)	1.6	0.2	1.6–2.2	0.63
PRO%	15	2	———-	——
FAT (g/kg BM)	1.6	0.2	———-	——
FAT%	33.2	4.5	20–35	<0.01
Fiber (g)	24.8	2.8	20–40	<0.01
Choles (mg)	404	66	< 300 mg	<0.01
Y-ELIT (*N* = 50)				
TEI (Kcal)	2708	233	3400–4300	<0.001
CHO%	50	6	———-	——
CHO (g/kg BM)	4.7	0.9	6–8	<0.01
PRO (g/kg BM)	1.6	0.2	1.6–2.2	0.17
PRO%	15	2	———-	——
FAT (g/kg BM)	1.7	0.3	———-	——
FAT%	35.2	5.8	20–35	<0.01
Fiber (g)	25.4	2.8	20–40	<0.01
Choles (mg)	385	63	< 300 mg	<0.01
U15/U14 ACDM (*N* = 50)				
TEI (Kcal)	2521	260	2500–3000	<0.05
CHO%	50	6	———-	——
CHO (g/kg BM)	4.5	0.7	5	<0.01
PRO (g/kg BM)	1.4	0.2	1.2	
PRO%	13	2	———-	——
FAT (g/kg BM)	1.8	0.3	———-	——
FAT%	36.3	6.4	20–35	<0.01
Fiber (g)	21.6	3.2	20–40	<0.01
Choles (mg)	415	63	< 300 mg	<0.01
U13/U12 ACDM (*N* = 50)				
TEI (Kcal)	2414	300	2500–3000	<0.05
CHO%	47	6	———-	<0.01
CHO (g/kg BM)	4.2	0.7	5	<0.001
PRO (g/kg BM)	1.4	0.2	1.2	
PRO%	13	1	———-	——
FAT (g/kg BM)	2.1	0.3	———-	——
FAT%	39.2	6.3	20–35	<0.01
Fiber (g)	19.2	3.6	20–40	<0.01
Choles (mg)	371	73	< 300 mg	<0.01

TEI, Total energy intake; CHO, Carbohydrates; PRO, Proteins. FAT% ration of fat energy; Choles: Cholesterols. Senior professional "S-PROF" senior elite group level "S-ELIT", Young elite group level "Y-ELIT", U15 and U14 categories academic level "U15/14-ACDM". U13 and U12 categories academic level "U13/12-ACDM" DRIs: Dietary Recommendation Intake value.

exceeds recommendations across all categories, likely due to the consumption of fast food that is high in fat.

This situation, also observed in previous studies (7), is very common among high-level athletes, where nutritional imbalances are often noted, especially in competition. This raises concerns about the nutritional balance of the players, suggesting that a reevaluation of dietary choices is necessary to align their diet with international guidelines and optimize their sports performance.

The analysis of data on energy intake and macronutrient consumption among the studied football players demonstrate notable variations. The total energy intake (TEI) generally ranges between 2,414 and 3,128 kcal with significant differences observed among categories based on the level of competition. (Table 3). The S-PROF (3,128 ± 295 Kcal), S-ELIT (2,966 ± 242 Kcal), and Y-ELIT (2,708 ± 233 Kcal) groups show slightly lower energy intake levels compared to UEFA (5) (Union of European Football Associations) consensus sports nutrition recommendations, a significant divergence was found with a *p*-value 0.01, for professional and elite players. While the academic-level categories U15/14 ACDM (2,521 ± 260 kcal) and U13/12 ACDM 2,414 ± 300 Kcal diverge considerably from UEFA standards for competitive-level players *p*-value 0.001. (Table 3). Although they align more closely with Moroccan recommendations, there are still disparities to consider.

The observed levels of energy intake are comparable to those reported in other countries among football players. Similar studies conducted with senior football players have reported estimated energy intakes ranging from 2,164 ± 498 kcal to 3,442 ± 158 kcal (7, 49, 50). A study that appears more closely aligned with our results is the one concerning the professional players conducted in Japan by Ebine et al. (51) (Japanese Professional Players aged 22 ± 2 years, 69.8 ± 4.7 kg) with an energy intake of 3,113 ± 581 for a period of 7 consecutive days. For elite players, the study conducted by Raizel et al. in 2017 (52) for a sample of 20.7 ± 2.0 years showed an TEI intake of 2,924 ± 460 kcal, and for players in the Dutch Eredivisie, the study by Bettonviel et al. (11); (Dutch Eredivisie Professional Players, for 20 players, 20 ± 4 years, 73 ± 8 kg, in 4 days in-season) reported a value at 2,988 ± 583 kcal. As for young players, the research by Galanti et al. (53), in 2015 for a population of 15–16 years old shows an energy intake of 2,844 kcal. Regarding the academic level of pre-adolescents (U13 and U12), the study by Hannon et al. (54); conducted in 2020 for players in the U12/13 category, 12 ± 0 years old, 43.0 ± 4.8 kg, indicates an intake of 2,659 kcal ± 187. All these investigations suggest that energy intakes are below the recommended levels, emphasizing the players' struggle to meet their energy needs adequately.

On the other hand, disparities in Total Energy Intake (TEI) among the groups likely result from a combination of differences in anthropometric profiles, particularly age, basal metabolic rate (RMR), and physical load among the teams. In terms of performance, it's relevant to note that professional players, as illustrated in the figure Figure 2A, have also covered the greatest

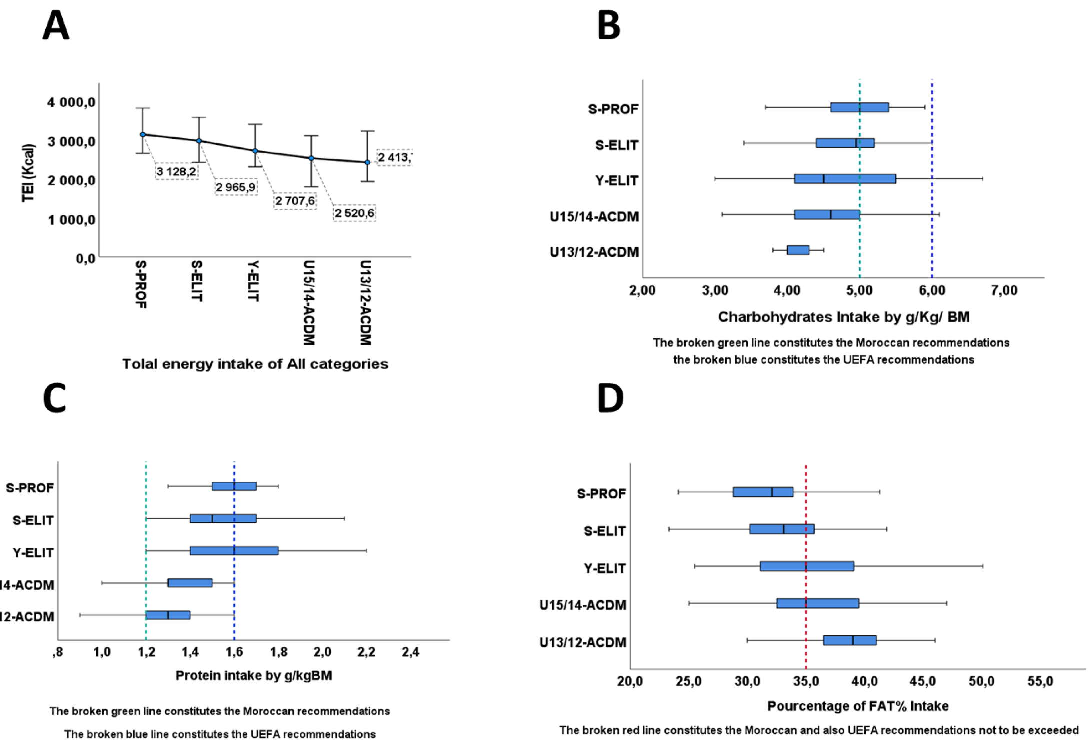

FIGURE 2
The distribution of macronutrients consumed by players from different categories in accordance with the recommendations. **(A)** The distribution of Total Energy intake of all categories. **(B)** The distribution of carbohydrates intake by g/kg/BM of all categories. **(C)** The distribution of protein intake by g/kg/MB of all categories. **(D)** The distribution of Fat intake of all categories.

distance compared to other categories, while U13/12 players recorded the smallest distance.

Furthermore, following to the Pearson test, a positive correlation links was detected between TEI and age ($p = 0.001$ and $r^2 = 0.48$) Figure 3D, Table 4. Also, between the TEI and RMR ($p = 0.001$ and $r^2 = 0.69$) Figure 3E, Table 4, as well as between TEI and the distance covered during the field test ($p = 0.001$ and $r^2 = 0.38$) Figure 3C, Table 4. Consequently, it would be advisable to consider age, resting metabolic rate (RMR), and the level of competition when developing age-specific training programs and diets to achieve adequate energy intake and meet the physical and physiological requirements of this activity.

4.2 Performance was significantly correlated with macronutrient intake levels, particularly carbohydrates, across the studied groups

4.2.1 CHO and performance

The distance covered during the performance test and the amount of carbohydrates consumed (CHO) showed a positive correlation, with a p-value of 0.001 (Table 4). This connection is visually depicted in Figure 3A, illustrating a linear correlation with a coefficient of determination of $R^2 = 0.40$. Further insights from the ANOVA test revealed that players who consumed more than 6 g of carbohydrates per day achieved the better performance (p-value 0.001, $F = 46.82$) compared to the other groups. These results confirm that an increased consumption of carbohydrates is associated with enhanced performance in terms of the distance covered.

These findings were confirmed by a linear analysis for each category, aiming to determine the extent to which carbohydrate intake affects performance. They illustrate a linear correlation with coefficient of determination values of $R2 = 0.77$, $R2 = 0.505$, $R2 = 0.44$, $R2 = 0.578$, and $R2 = 0.4639$ for players S-PROF, S-ELIT, Y-ELIT, U15/14-ACDM, and U13/12-ACDM respectively in Figures 3A–F. Macronutrients in particular carbohydrates (CHO), play a vital role by providing the necessary energy, promoting recovery, and contributing to the overall wellness protection of players (42), the carbohydrates, the main source of energy for the body, are broken down into glucose during digestion, and then converted into adenosine triphosphate (ATP) through glycolysis, providing the necessary energy for aerobic activities (11). In relation to the physical demands of football, And it's known that the anaerobic system provides rapid but limited energy (10), the aerobic endurance comes into play during longer periods of game and recovery between actions. The ability to maintain an effective balance between these two energy systems is crucial for ensuring optimal performance throughout the match (1).

Our results, as previously mentioned, demonstrate the players show deficiencies in meeting the recommended of carbohydrate values, a finding also observed in similar studies conducted with senior football players have reported varied estimated

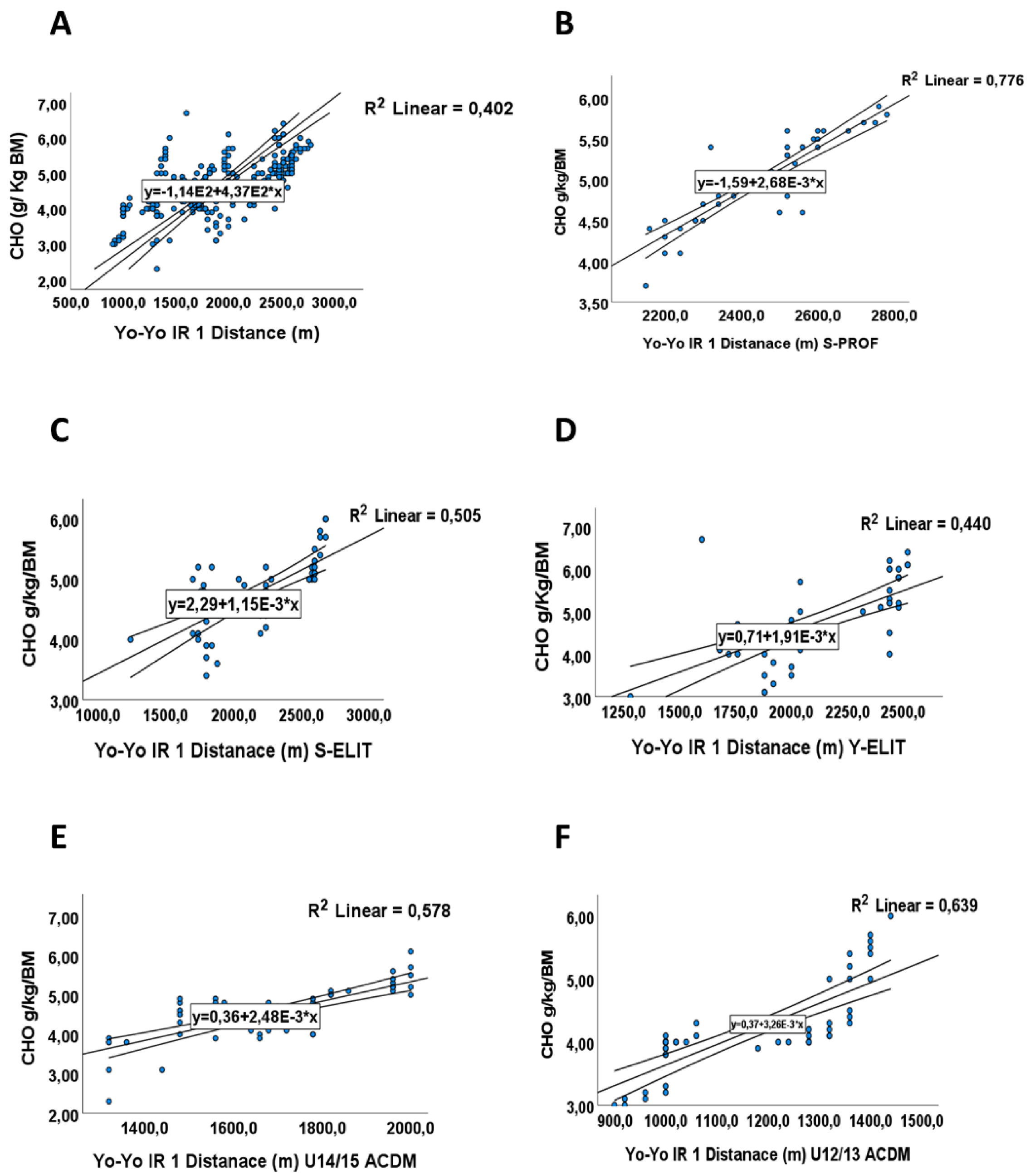

FIGURE 3
The relationship between mean distance (m) in Yo-Yo IR test and Carbohydrate's intake in g/kg/BM (CHO). **(A)** The relationship between mean distance (m) and Carbohydrate's intake in g/kg/BM (CHO) for all groups. **(B)** The relationship between mean distance covered (m) and Carbohydrate's intake in g/kg/BM (CHO) for S-PROF (Senior Professionel players). **(C)** The relationship between mean distance covered (m) and Carbohydrate's intake in g/kg/BM (CHO) for S-ELIT (Senior Elit players). **(D)** The relationship between mean distance covered (m) and Carbohydrate's intake in g/kg/BM (CHO) for Y-ELIT (Young Elit players). **(E)** The relationship between mean distance covered (m) and Carbohydrate's intake in g/kg/BM (CHO) for 14/15 ACDM (Academic players). **(F)** The relationship between mean distance covered (m) and Carbohydrate's intake in g/kg/BM (CHO) for 12/13 ACDM (Academic players).

carbohydrate (CHO) intakes. A study closely aligned with our results was conducted by Raizel et al. in 2017 (52) for Spanish senior players, reporting a CHO intake of 5.4 ± 1.9, and by Hassapidou (55) for the senior players with an age of 24.8 (5.5) and a CHO intake of 5.3 ± 1.9. Książek et al. in 2020 (56) also reported an average consumption of 5.1 g CHO/kg BM (body mass) in professional Polish football players. For adolescents, Naughton et al. in 2016 (57) studied adolescents players with an average age of 14.4 ± 0.5 and a CHO intake of 4.7 ± 1.4. All these investigations suggest that CHO intakes are below the recommended levels, emphasizing the players' struggle to meet their energy needs adequately. The low consumption of carbohydrates (CHO) generally corresponds to a low overall energy intake. As discussed earlier, the literature also confirms improvements in

TABLE 4 The correlation between distance covered and age, RMR, TEI and Macronutrients intake of all groups among football players.

Variables and Pearson correlation		Distance covered (m)	Age (years)	RMR	TEI	CHO (g/kg BM)	PRO (g/kg BM)	FAT %
Distance covered (m)	Pearson correlation							
	Sig. (2-tailed)							
Age (years)	Pearson correlation	0.717[a]						
	Sig. (2-tailed)	<0.001						
RMR	Pearson correlation	0.640[a]	0.692[a]					
	Sig. (2-tailed)	<0.001	<0.001					
TEI	Pearson correlation	0.620[a]	0.694[a]	0.831[a]				
	Sig. (2-tailed)	<0.001	<0.001	<0.001				
CHO (g/kg BM)	Pearson correlation	0.634[a]	0.299[a]	0.179[a]	0.442[a]			
	Sig. (2-tailed)	<0.001	<0.001	<0.004	<0.001			
PRO (g/kg BM)	Pearson correlation	0.595[a]	0.407[a]	0.255[a]	0.399[a]	0.617[a]		
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001		
FAT%	Pearson correlation	−0.646[a]	−0.391[a]	−0.215[a]	0.375[a]	−0.858[a]	−0.719[a]	
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

TEI, total energy intake; CHO, carbohydrates; PRO, proteins. FAT% ration of fat energy; Choles, cholesterols.
[a]Correlation is significant at the 0.01 level (2-tailed).

soccer performance, both at the technical level (58, 59), and in physical aspects, due to the consumption of a carbohydrate-rich diet in CHO. To ensure adequate energy intake, it is highly recommended to adopt improved nutritional periodization (60)

All these studies suggest that CHO intakes are below the recommended levels, underscoring the challenges players face in adequately fulfilling their energy requirements. A low total calorie intake is typically correlated with low carbohydrate consumption (CHO). A carbohydrate-rich diet (CHO), as previously mentioned, has been linked to increases in soccer performance, both technically (58), physically (59), and at the technical level. It is strongly advised to use enhanced nutritional periodization in order to guarantee appropriate energy intake (60).

However, CHO play a crucial role in optimizing football players' performance by providing a rapid and effective source of energy. During a match or intensive training, simple carbohydrates, such as those found in energy drinks and fruits, are quickly converted into glucose, offering immediate energy to the muscles and brain, this rapid availability of glucose helps maintain a high level of performance and prevents premature fatigue (61). Additionally, complex carbohydrates, such as those found in pasta and rice, are essential for glycogen storage in the muscles and liver (62). An adequate glycogen store allows players to sustain prolonged efforts and improve their endurance. After exertion, consuming sufficient sugars promotes quick recovery by replenishing depleted glycogen reserves, which is crucial for effectively preparing for the next training session or competition (5). Furthermore, adequate glucose intake supports concentration, coordination, and quick decision-making, all essential aspects of high-level play (6).

4.3 Protein's intake and performance

The Pearson correlation test revealed a significant positive association between protein intake levels and performance, with a correlation coefficient R^2 of 0.64 Figure 4A and (p-value 0.001 (Table 4). These results were validated through linear analysis within each category, aiming to assess the impact of protein intake on performance. They demonstrate a linear relationship, as evidenced by coefficient of determination values of $R2 = 0.05$, $R2 = 0.167$, $R2 = 0.384$, $R2 = 0.374$, and $R2 = 0.360$ for players S-PROF, S-ELIT, Y-ELIT, U15/14-ACDM, and U13/12-ACDM respectively in Figures 4B–F). This indicates that despite the importance of proteins for the energy process, this nutrient has a greater impact on the performance of young people and adolescents compared to adults, due to the growth and development phase of adolescents.

Nevertheless, when examining protein intake (PRO) in the S-PROF, S-ELIT, and Y-ELIT groups, it is noteworthy that their consumption aligns closely with the guidelines set by UEFA (Table 3). Specifically, protein intake values for these groups stand at 1.7 g/kg, 1.6 g/kg, and 1.6 g/kg of body mass (BM) respectively, constituting 15% of their total caloric intake. On the other hand, players falling under the U15/14-ACDM and U13/12-ACDM categories adhere to Moroccan dietary recommendations, albeit slightly below the suggested levels for competitive athletes. In these categories, protein intake has reported at 1.4 grams per kilogram of body mass, indicating a marginally lower adherence to the guidelines established for athletes engaged in competitive sports. A meta-analysis revealed that protein intake fluctuated within the range of 1.8–2.0 g/kg/day among junior athletes and 1.8–1.9 g/kg/day in their senior counterparts (7).

On the other hand, These results are consistent with the consensus within the scientific community, indicating that maintaining sufficient protein intake could play a pivotal role in enhancing athletes' physical performance (63). Essentially, this recommendation is underscored by the fact that adult athletes are often advised to increase their protein consumption compared to inactive individuals (47).

However, Proteins are essential for football players due to their crucial role in muscle repair and growth for young peoples. During

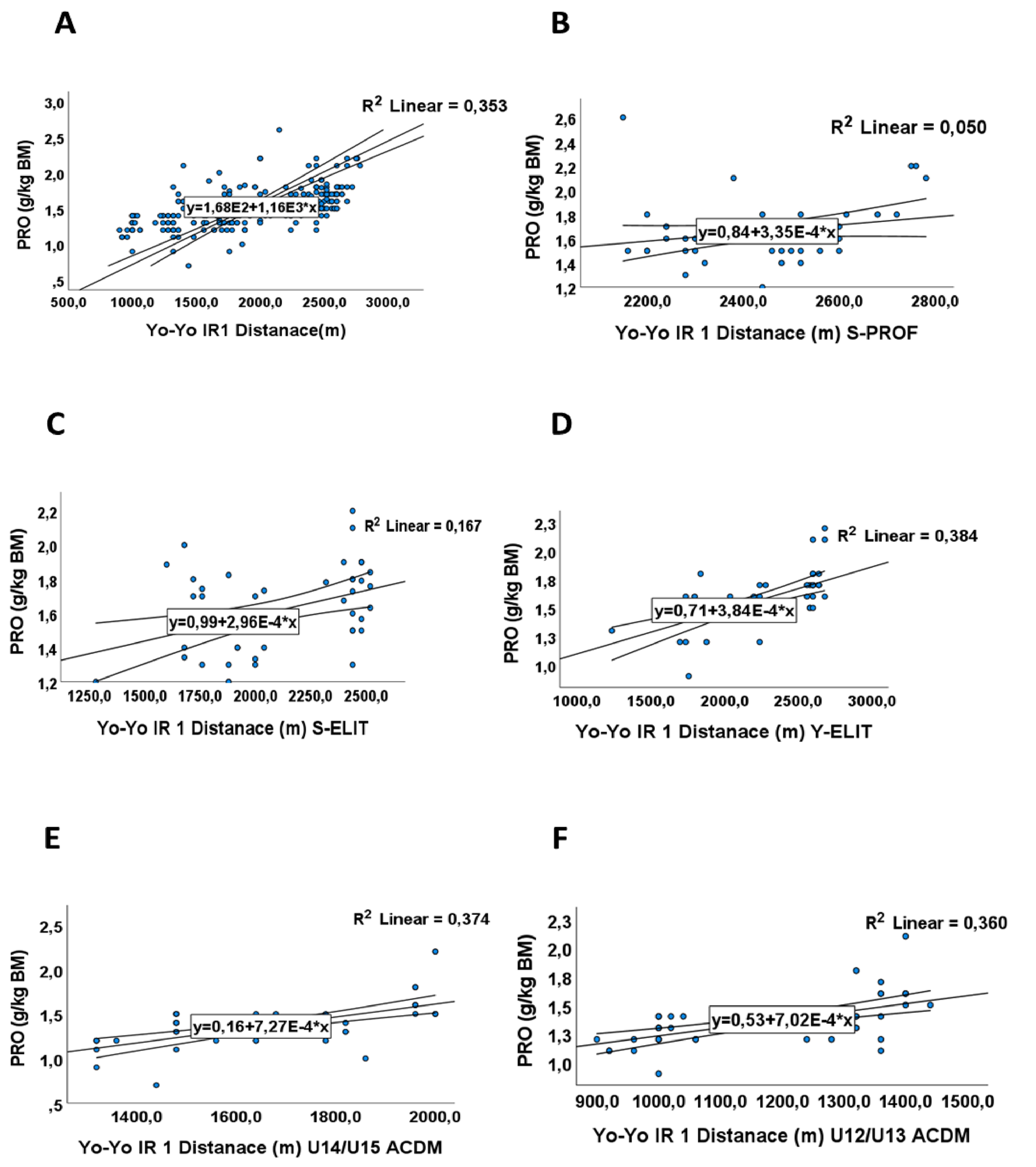

FIGURE 4
The relationship between mean distance (m) in Yo-Yo IR test and proteins intake in g/kg/BM (PRO). **(A)** The relationship between mean distance (m) and proteins intake in g/kg/BM for all groups. **(B)** The relationship between mean distance covered (m) and proteins intake in g/kg/BM for S-PROF (Senior Professionel players). **(C)** The relationship between mean distance covered (m) and proteins intake in g/kg/BM for S-ELIT (Senior Elit players). **(D)** The relationship between mean distance covered (m) and proteins intake in g/kg/BM for Y-ELIT (Young Elit players). **(E)** The relationship between mean distance covered (m) and proteins intake in g/kg/BM for U14/15 ACDM (Academic players). **(F)** The relationship between mean distance covered (m) and proteins intake in g/kg/BM for U12/13 ACDM (Academic players).

intense training and matches, muscles undergo micro-tears that require repair to strengthen and rebuild muscle tissue. Proteins provide the necessary amino acids for this recovery process, thereby promoting muscle adaptation and performance enhancement (15). Additionally, adequate protein intake helps maintain optimal muscle mass, which is vital for strength, speed, and agility on the field. After exertion, proteins aid in reducing muscle soreness and accelerating recovery, allowing players to return to peak performance more quickly. By incorporating sufficient protein into their diet, footballers can maximize their physical potential and improve their ability to meet the high demands of the sport (63).

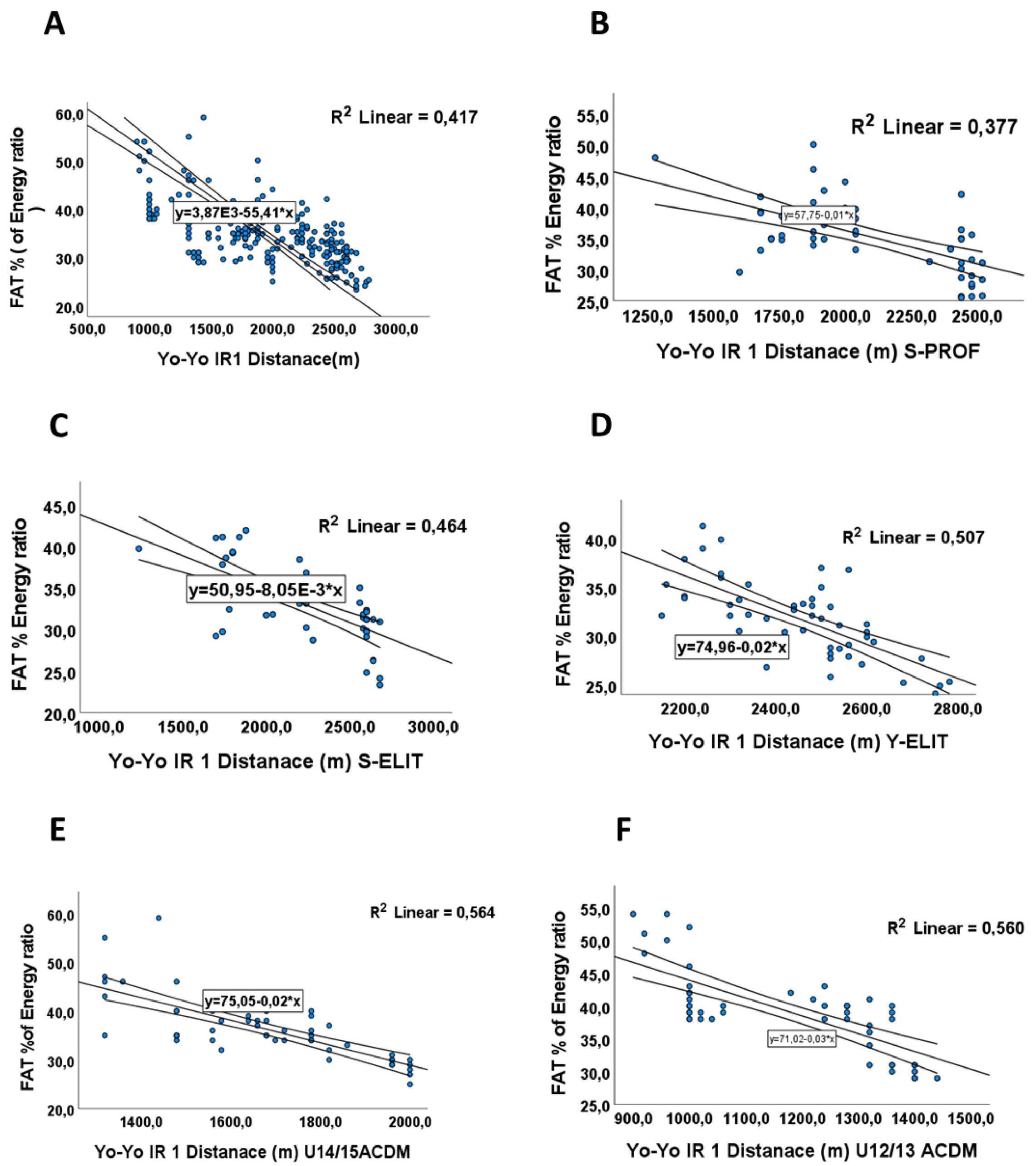

FIGURE 5
The relationship between mean distance (m) in Yo-Yo IR test and Fat intake in (FAT %). **(A)** The relationship between mean distance (m) and Fat intake in (FAT %) for all groups. **(B)** The relationship between mean distance covered (m) and Fat intake in (FAT %) for S-PROF (Senior Professionel players). **(C)** The relationship between mean distance covered (m) and Fat intake in (FAT %) for S-ELIT (Senior Elit players). **(D)** The relationship between mean distance covered (m) and Fat intake in (FAT %) for Y-ELIT (Young Elit players). **(E)** The relationship between mean distance covered (m) and Fat intake in (FAT %) for U14/15 ACDM (Academic players). **(F)** The relationship between mean distance covered (m) and Fat intake in (FAT %) for U12/13 ACDM (Academic players).

4.4 Fat intake and performance

The acceptable macronutrient distribution range for fats is 20 to 35%, the soccer players are recommended to consume less than 30% of their total energy needs from fats (5). Our findings indicate that only the professionals (27%) do not exceed the limits stated as recommendations (Table 3), the other categories often surpass the recommended levels, so that the U13/12 ACDM>U15/14 ACDM>Y-ELI>S-ELIT T>S-PROF. Concurrently, there has been an excess of cholesterol (Choles) measured, with values varying from 324 to 415 mg. The young players show a higher value overall [Y-ELIT (385 mg ± 63), U15/14 ACDM (415 mg ± 63), and U13/12 ACDM (371 mg ± 73)] than the S-PROF (324 mg ± 63).

A negative correlation was observed between the amount of fat consumed and distance covered with a *p*-value = 0,001, (r = −0.64) Table 4, and $R^2 = -0.41$ Figure 5A, the Players' performance was affected by higher fat consumption, especially if it was more than 35%, according to the ANOVA test (with post-hoc testing and the Tukey test) that showed a ($p < 0.001$, $F = 43.95$). Our results aligned with other research on elite or professional athletes (7).

These results were validated through linear analysis within each category, aiming to assess the impact of fat intake on performance. They demonstrate a linear relationship, as evidenced by coefficient of determination values of R2 = −0.377, R2 = −0.464, R2 = −0.507, R2 = −0.564, and R2 = −0.560 for players S-PROF, S-ELIT, Y-ELIT, U15/14-ACDM, and U13/12-ACDM respectively in Figures 5B–F.

The negative values of the coefficient of determination (R^2) observed for fat intake and performance across all player categories suggest an inverse relationship between fat intake and performance. These findings imply that higher fat intake might be associated with lower performance levels among football players in each category. It noted that fats play a crucial role in footballers' nutrition by providing a concentrated and sustained source of energy. While carbohydrates are the primary energy source for high-intensity efforts, lipids become an important energy source during periods of low intensity and prolonged activities. Essential fatty acids, found in vegetable oils, nuts, and fatty fish, are also crucial for cell health and hormone production. Furthermore, lipids help protect internal organs and regulate body temperature, which is important for maintaining optimal performance. Balanced fat consumption enhances endurance and supports effective recovery by providing a sustained energy reserve. However, it is important to avoid unhealthy fats, such as trans fats and excessive saturated fats found in processed foods and fried items, as they can negatively impact cardiovascular health and overall athletic performance.

As a conclusion the nutritional deficiencies in macronutrients observed among professional and adult players can be attributed to several complex factors. Firstly, limited access to a varied and balanced diet, particularly in terms of fiber from fruits and vegetables, as well as essential oils such as omega-3 and omega-6, remains a significant challenge. Additionally, the lack or absence of adequate meal planning around training sessions and matches exacerbates this issue. The absence of personalized nutritional monitoring, combined with insufficient management of dietary intake and hydration, also contributes to diets that fail to meet the demands of the sport. Furthermore, psychological pressures related to performance and social influences, such as the dietary choices of teammates or trends observed on social media, can lead to eating behaviors that are not aligned with the specific needs of professional football. For adolescent and young players, nutritional deficiencies are often the result of a combination of factors specific to this stage of development. Eating habits acquired during childhood, often shaped by family environment and cultural customs, may not always meet the high energy demands of high-level sports. Additionally, these young athletes are particularly susceptible to the influence of social media, which frequently disseminates unvalidated nutritional information. The lack of regular follow-up by nutritionists or dietitians exacerbates this situation, as nutritional needs evolve rapidly during adolescence. The prevalence of fast food and processed foods, compounded by social constraints and a busy schedule, also contributes to an imbalance in macronutrients. Finally, inadequate meal planning, combined with often demanding training and school schedules, further limits young players' ability to adhere to the nutritional recommendations necessary to support their growth and sports performance.

4.5 Limitation

The present study primarily focuses on analyzing players' performance and nutritional status without considering their on-field positions. Therefore, one limitation is the lack of consideration for the effort exerted and the nutritional intake based on playing positions and the tactical schemes of the coaches. Ultimately, the main objective was to gain a general overview of the teams and various studied categories, rather than focusing on specific criteria related to players' positions. Additionally, we did not account for cooking methods or the type of diet followed by the players. Our aim was to provide a general understanding of dietary intake without delving into these specific aspects. Future studies may consider these elements to better contextualize the Moroccan football landscape.

Despite these limitations, this study presents several strengths. It is one of the few studies that analyze the nutritional intake of Moroccan football players, providing valuable insights into their dietary habits in comparison with international recommendations (FIFA, UEFA). Additionally, the study takes into account a significant sample of players from different age categories, which offers a comprehensive overview of the nutritional practices across various levels of play. Moreover, the study's focus on traditional Moroccan meals, adapted for sports performance, brings a unique cultural perspective to the field of sports nutrition, contributing to a better understanding of how local dietary habits may impact performance in high-level athletes.

5 Conclusion

This study provides a comprehensive overview of dietary habits and their impact on the performance of Moroccan football players. It is evident that players exhibit shortcomings relative to nutritional recommendations, particularly regarding carbohydrate intake. Furthermore, a significant correlation has observed between protein intake and performance among young people and adolescents, highlighting the importance of this nutrient for athletic development. However, additional adjustments in diet may be necessary to fully meet recommendations. Regarding fat intake, an inverse relationship has observed with performance across all player categories. These findings underscore the crucial importance of a balanced diet tailored to players' specific needs to maximize their athletic performance, as well as the necessity of preventive measures such as age-appropriate training programs and appropriate nutritional guidance.

For these reasons, we recommend several measures to improve the nutrition of professional and adult football players, as well as adolescents and young footballers. It is essential to ensure regular access to a varied and balanced diet, including fiber, vitamins, and essential fatty acids such as omega-3 and omega-6. Nutritional programs should incorporate specific meal planning tailored to the demands of training sessions and competitions. The implementation of personalized nutritional monitoring, conducted by qualified dietitians or nutritionists, is crucial for adjusting diets according to the individual needs of athletes. For young players, early nutritional education will be recommended to promote healthy eating habits and mitigate the impact of scientifically unfounded diets. Additionally, appropriate management of hydration and dietary intake must be ensured. Finally, it is important to raise awareness among players about the effects of psychological pressures and social influences on their dietary choices, while developing stress management strategies that preserve the quality of their nutrition.

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.

Ethics statement

This study was approved by the regional ethics committee of the Ibn RORCHD University Hospital Center of Casablanca governed by Morocco's Ministry of Health. number (Approval No. 22/2022) and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent for participation in this study was provided by the participants and the participants' legal guardians/next of kin for the minors included.

Author contributions

MO: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HL: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. AD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. RS: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. HT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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.

Publisher's note

References

1. Bangsbo J, Mohr M, Krustrup P. Physical and metabolic demands of training and match-play in the elite football player. *J Sports Sci.* (2006) 24:665–74. doi: 10.1080/02640410500482529

2. Bush M, Barnes C, Archer DT, Hogg B, Bradley PS. Evolution of match performance parameters for various playing positions in the English premier league. *Hum Mov Sci.* (2015) 39:1–11. doi: 10.1016/j.humov.2014.10.003

3. Barnes C, Archer DT, Hogg B, Bush M, Bradley PS. The evolution of physical and technical performance parameters in the English premier league. *Int J Sports Med.* (2014) 35:1095–100. doi: 10.1055/s-0034-1375695

4. Bangsbo J. *Aerobic and Anaerobic Training in Soccer: Special Emphasis on Training of Youth Players.* Copenhagen: Stormtryk (2007). p. 212.

5. Collins J, Maughan RJ, Gleeson M, Bilsborough J, Jeukendrup A, Morton JP, et al. UEFA Expert group statement on nutrition in elite football. Current evidence to inform practical recommendations and guide future research. *Br J Sports Med.* (2021) 55:416. doi: 10.1136/bjsports-2019-101961

6. Abreu R, Figueiredo P, Beckert P, Marques JP, Amorim S, Caetano C, et al. Portuguese Football federation consensus statement 2020: nutrition and performance in football. *BMJ Open Sport Exerc Med.* (2021) 7:e001082. doi: 10.1136/bmjsem-2021-001082

7. Steffl M, Kinkorova I, Kokstejn J, Petr M. Macronutrient intake in soccer players—a meta-analysis. *Nutrients*. (2019) 11:1305. doi: 10.3390/nu11061305

8. Bagheri R, Robinson I, Moradi S, Purcell J, Schwab E, Silva T, et al. Muscle protein synthesis responses following aerobic-based exercise or high-intensity interval training with or without protein ingestion: a systematic review. *Sports Med*. (2022) 52:2713–32. doi: 10.1007/s40279-022-01707-x

9. Tipton KD, Wolfe RR. Protein and amino acids for athletes. *J Sports Sci*. (2004) 22:65–79. doi: 10.1080/0264041031000140554

10. Phillips SM, Van Loon LJC. Dietary protein for athletes: from requirements to optimum adaptation. *J Sports Sci*. (2011) 29:S29–38. doi: 10.1080/02640414.2011.619204

11. Bettonviel A EO, Brinkmans N YJ, Russcher K, Wardenaar FC, Witard OC. Nutritional Status and daytime pattern of protein intake on match, post-match, rest and training days in senior professional and youth elite soccer players. *Int J Sport Nutr Exerc Metab*. (2016) 26:285–93. doi: 10.1123/ijsnem.2015-0218

12. Stølen T, Chamari K, Castagna C, Wisløff U. Physiology of soccer: an update. *Sports Med Auckl NZ*. (2005) 35:501–36. doi: 10.2165/00007256-200535060-00004

13. Billat V, Billat V, Billat V, Billat V, Billat V, Billat V, et al. Physiologie du sport et de l'exercice).

14. Randell RK, Clifford T, Drust B, Moss SL, Unnithan VB, De Ste Croix MBA, et al. Physiological characteristics of female soccer players and health and performance considerations: a narrative review. *Sports Med*. (2021) 51:1377–99. doi: 10.1007/s40279-021-01458-1

15. Williams C. Macronutrients and performance. *J Sports Sci*. (1995) 13:S1–S10. doi: 10.1080/02640419508732271

16. American Dietetic Association, Dietitians of Canada, American College of Sports Medicine, Rodriguez NR, Di Marco NM, Langley S. American College of sports medicine position stand. Nutrition and athletic performance. *Med Sci Sports Exerc*. (2009) 41:709–31. doi: 10.1249/MSS.0b013e31890eb86

17. Kerksick CM, Wilborn CD, Roberts MD, Smith-Ryan A, Kleiner SM, Jäger R, et al. ISSN exercise & sports nutrition review update: research & recommendations. *J Int Soc Sports Nutr*. (2018) 15:38. doi: 10.1186/s12970-018-0242-y

18. Castagna C, Impellizzeri F, Cecchini E, Rampinini E, Alvarez JCB. Effects of intermittent-endurance fitness on match performance in young male soccer players. *J Strength Cond Res*. (2009) 23:1954–9. doi: 10.1519/JSC.0b013e3181b7f743

19. Castagna C, Manzi V, Impellizzeri F, Weston M, Barbero Alvarez JC. Relationship between endurance field tests and match performance in young soccer players. *J Strength Cond Res*. (2010) 24:3227–33. doi: 10.1519/JSC.0b013e3181e72709

20. Fernandes-da-Silva J, Castagna C, Teixeira AS, Carminatti LJ, Guglielmo LGA. The peak velocity derived from the carminatti test is related to physical match performance in young soccer players. *J Sports Sci*. (2016) 34:2238–45. doi: 10.1080/02640414.2016.1209307

21. Vieira LHP, Carling C, Barbieri FA, Aquino R, Santiago PRP. Match running performance in young soccer players: a systematic review. *Sports Med Auckl NZ*. (2019) 49:289–318. doi: 10.1007/s40279-018-01048-8

22. Paul DJ, Nassis GP. Testing strength and power in soccer players: the application of conventional and traditional methods of assessment. *J Strength Cond Res*. (2015) 29:1748–58. doi: 10.1519/JSC.0000000000000807

23. Rebelo A, Brito J, Seabra A, Oliveira J, Krustrup P. Physical match performance of youth football players in relation to physical capacity. *Eur J Sport Sci*. (2014) 14:S148–56. doi: 10.1080/17461391.2012.664171

24. Rampinini E, Coutts A, Castagna C, Sassi R, Impellizzeri F. Variation in top level soccer match performance. *Int J Sports Med*. (2007) 28:1018–24. doi: 10.1055/s-2007-965158

25. Reilly T, Drust B, Clarke N. Muscle fatigue during football match-play. *Sports Med Auckl NZ*. (2008) 38:357–67. doi: 10.2165/00007256-200838050-00001

26. Mohr M, Krustrup P, Bangsbo J. Fatigue in soccer: a brief review. *J Sports Sci*. (2005) 23:593–9. doi: 10.1080/02640410400021286

27. Carling C, Le Gall F, McCall A, Nédélec M, Dupont G. Squad management, injury and match performance in a professional soccer team over a championship-winning season. *Eur J Sport Sci*. (2015) 15:573–82. doi: 10.1080/17461391.2014.955885

28. Carling C. Interpreting physical performance in professional soccer match-play: should we be more pragmatic in our approach? *Sports Med Auckl NZ*. (2013) 43:655–63. doi: 10.1007/s40279-013-0055-8

29. Fanchini M, Schena F, Castagna C, Petruolo A, Combi F, McCall A, et al. External responsiveness of the yo-yo IR test level 1 in high-level male soccer players. *Int J Sports Med*. (2015) 36:735–41. doi: 10.1055/s-0035-1547223

30. Bangsbo J, Iaia FM, Krustrup P. The yo-yo intermittent recovery test: a useful tool for evaluation of physical performance in intermittent sports. *Sports Med*. (2008) 38:37–51. doi: 10.2165/00007256-200838010-00004

31. Impellizzeri FM, Rampinini E, Castagna C, Bishop D, Ferrari Bravo D, Tibaudi A, et al. Validity of a repeated-sprint test for football. *Int J Sports Med*. (2008) 29:899–905. doi: 10.1055/s-2008-1038491

32. Bradley PS, Carling C, Archer D, Roberts J, Dodds A, Di Mascio M, et al. The effect of playing formation on high-intensity running and technical profiles in English FA premier league soccer matches. *J Sports Sci*. (2011) 29:821–30. doi: 10.1080/02640414.2011.561868

33. Rago V, Krustrup P, Martín-Acero R, Rebelo A, Mohr M. Training load and submaximal heart rate testing throughout a competitive period in a top-level male football team. *J Sports Sci*. (2020) 38:1408–15. doi: 10.1080/02640414.2019.1618534

34. Mohr M, Krustrup P. Yo-Yo intermittent recovery test performances within an entire football league during a full season. *J Sports Sci*. (2014) 32:315–27. doi: 10.1080/02640414.2013.824598

35. Buchheit M, Simpson BM, Garvican-Lewis LA, Hammond K, Kley M, Schmidt WF, et al. Wellness, fatigue and physical performance acclimatisation to a 2-week soccer camp at 3600 m (ISA3600). *Br J Sports Med*. (2013) 47:i100–6. doi: 10.1136/bjsports-2013-092749

36. Collins J, McCall A, Bilsborough J, Maughan R. Football nutrition: time for a new consensus? *Br J Sports Med*. (2017) 51:1577–8. doi: 10.1136/bjsports-2016-097260

37. Oukheda M, Bouaouda K, Mohtadi K, Lebrazi H, Derouiche A, Kettani A, et al. The cardiorespiratory endurance (VO_2max), body composition and macronutrient's intake in the Pre-competitive period: a correlation study among Moroccan professional soccer players. *Int J Hum Mov Sports Sci*. (2024) 12:288–301. doi: 10.13189/saj.2024.120203

38. Ling CHY, De Craen AJM, Slagboom PE, Gunn DA, Stokkel MPM, Westendorp RGJ, et al. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin Nutr*. (2011) 30:610–5. doi: 10.1016/j.clnu.2011.04.001

39. Fürstenberg A, Davenport A. Assessment of body composition in peritoneal dialysis patients using bioelectrical impedance and dual-energy x-ray absorptiometry. *Am J Nephrol*. (2011) 33:150–6. doi: 10.1159/000324111

40. WHO Consultation on Obesity 1999: Geneva S, Organization WH. *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*. World Health Organization (2000). p. 252. Available online at: https://apps.who.int/iris/handle/10665/42330 (accessed September 19, 2023).

41. Briggs MA, Rumbold PLS, Cockburn E, Russell M, Stevenson EJ. Agreement between two methods of dietary data collection in male adolescent academy-level soccer players. *Nutrients*. (2015) 7:5948–60. doi: 10.3390/nu7075262

42. Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, et al. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr*. (1994) 72:619–43. doi: 10.1079/bjn19940064

43. Martin CK, Han H, Coulon SM, Allen HR, Champagne CM, Anton SD. A novel method to remotely measure food intake of free-living individuals in real time: the remote food photography method. *Br J Nutr*. (2008) 101:446–56. doi: 10.1017/S0007114508027438

44. Garcin M, Doussot L, Mille-Hamard L, Billat V. Athletes' dietary intake was closer to French RDA's than those of young sedentary counterparts. *Nutr Res N Y N*. (2009) 29:736–42. doi: 10.1016/j.nutres.2009.10.004

45. *Table of Nutritional Composition of Foods (CIQUAL): ANSES Supplements its Data and Publishes its Work Priorities for the Coming Years*. Anses - Agence Natl Sécurité Sanit L'alimentation L'environnement Trav (2020). Avaible online at: https://data.europa.eu/data/datasets/5369a15fa3a729239d2065b7?locale=en (accessed July 25, 2023).

46. Abhatoo: Guide Marocain de Nutrition: A l'usage des professionnels de santé. http://www.abhatoo.net.ma/maalama-textuelle/developpement-durable/societe-durable/sante-humaine/nutrition-et-soins-de-sante/malnutrition/guide-marocain-de-nutrition-a-l-usage-des-professionnels-de-sante (accessed July 25, 2023).

47. Briggs MA, Cockburn E, Rumbold PLS, Rae G, Stevenson EJ, Russell M. Assessment of energy intake and energy expenditure of male adolescent academy-level soccer players during a competitive week. *Nutrients*. (2015) 7:8392–401. doi: 10.3390/nu7105400

48. Iglesias-Gutiérrez E, García Á, García-Zapico P, Pérez-Landaluce J, Patterson ÁM, García-Rovés PM. Is there a relationship between the playing position of soccer players and their food and macronutrient intake? *Appl Physiol Nutr Metab*. (2012) 37:225–32. doi: 10.1139/h11-152

49. Oukheda M, Bouaouda K, Lebrazi H, Mohtadi K, Derouiche A, Saile R, et al. Evaluation of antioxidant vitamins intake of a professional soccer team in Morocco during a week of competition and training. *Curr Dev Nutr*. (2023) 7:101779. doi: 10.1016/j.cdnut.2023.101779

50. Oukheda M, Bouaouda K, Mohtadi K, Lebrazi H, Derouiche A, Kettani A, et al. Association between nutritional status, body composition, and fitness level of adolescents in physical education in Casablanca, Morocco. *Front Nutr*. (2023) 10:1268369. doi: 10.3389/fnut.2023.1268369

51. Ebine N, Rafamantanantsoa HH, Nayuki Y, Yamanaka K, Tashima K, Ono T, et al. Measurement of total energy expenditure by the doubly labelled water method in professional soccer players. *J Sports Sci*. (2002) 20:391–7. doi: 10.1080/026404102317366645

52. Raizel R, da Mata Godois A, Coqueiro AY, Voltarelli FA, Fett CA, Tirapegui J, et al. Pre-season dietary intake of professional soccer players. *Nutr Health*. (2017) 23:215–22. doi: 10.1177/0260106017737014

53. Galanti G, Stefani L, Scacciati I, Mascherini G, Buti G, Maffulli N. Eating and nutrition habits in young competitive athletes: a comparison between soccer players and cyclists. *Transl Med UniSa*. (2014) 11:44–7.

54. Hannon MP, Close GL, Morton JP. Energy and macronutrient considerations for young athletes. *Strength Cond J*. (2020) 42:109–19. doi: 10.1519/SSC.0000000000000570

55. Hassapidou MN, Grammatikopoulou MG, Liarigovinos T. Dietary intakes of Greek professional football players. *Nutr Food Sci*. (2000) 30:191–4. doi: 10.1108/00346650010330234

56. Książek A, Zagrodna A, Słowińska-Lisowska M. Assessment of the dietary intake of high-rank professional male football players during a preseason training week. *Int J Environ Res Public Health*. (2020) 17:8567. doi: 10.3390/ijerph17228567

57. Naughton RJ, Drust B, O'Boyle A, Morgans R, Abayomi J, Davies IG, et al. Daily distribution of carbohydrate, protein and fat intake in elite youth academy soccer players over a 7-day training period. *Int J Sport Nutr Exerc Metab*. (2016) 26:473–80. doi: 10.1123/ijsnem.2015-0340

58. Russell M, Kingsley M. The efficacy of acute nutritional interventions on soccer skill performance. *Sports Med Auckl NZ*. (2014) 44:957–70. doi: 10.1007/s40279-014-0184-8

59. Ali A, Williams C, Nicholas CW, Foskett A. The influence of carbohydrate-electrolyte ingestion on soccer skill performance. *Med Sci Sports Exerc*. (2007) 39:1969–76. doi: 10.1249/mss.0b013e31814fb3e3

60. Close GL, Hamilton DL, Philp A, Burke LM, Morton JP. New strategies in sport nutrition to increase exercise performance. *Free Radic Biol Med*. (2016) 98:144–58. doi: 10.1016/j.freeradbiomed.2016.01.016

61. Thomas D, Burke L, Erdman K. Nutrition and athletic performance. *Med Sci*. (2016) 48:543–68. doi: 10.1249/MSS.0000000000000852

62. Bergström J, Hermansen L, Hultman E, Saltin B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand*. (1967) 71:140–50. doi: 10.1111/j.1748-1716.1967.tb03720.x

63. Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am J Physiol Endocrinol Metab*. (2007) 292:E71–76. doi: 10.1152/ajpendo.00166.2006

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