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Food and nutrition for athletics: redefining the role and application

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Food and nutrition for athletics: redefining the role and application

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Editorial: Food and nutrition for athletics: redefining the role and application

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Editorial on the Research Topic
Food and nutrition for athletics: redefining the role and application

Food and nutrition play a vital role in athletes' health and performance, making it a timeless topic. Of course, this also applies to the general population. This Research Topic focuses on two main aspects: the impact of macronutrients on athletic performance and the effects of bioactive plant compounds on exercise performance.

Bagheri et al. reported that variations in muscle strength, power adaptation, and endurance after 16 weeks of either concurrent or resistance training with varying high-protein intakes were not linked to changes in lean mass among resistance-trained young males. Through meta-analysis, Zhao et al. concluded that protein intake offers modest benefits to athletes, especially in improving endurance. Subgroup analysis indicates that protein intake boosts muscle glycogen levels and that combining protein with carbohydrates is more effective for endurance athletes than consuming high protein alone. Kuhlman et al. reported that male collegiate gymnasts may have a high prevalence of low energy availability, primarily due to insufficient relative energy and carbohydrate intake. Ritson et al. observed a drug-free bodybuilder following evidence-based nutrition strategies over 18 weeks of low energy availability, resistance training, and a high-protein diet to attain extreme leanness, providing insights into the fluctuations of free triiodothyronine and total testosterone. Kripp et al. reported that in active and healthy individuals, a low-carbohydrate, high-fat diet negatively impacts individual blood lipid profiles compared to carbohydrate-rich diets. Noakes and Prins analyzed and highlighted the potential limitations of the exogenous carbohydrate ingestion prediction model for achieving a sub-2 h marathon, as proposed by Lukasiewicz et al. (1). Zhang et al. discovered that in free-living conditions, athletes' body composition is influenced by habitual water intake rather than hydration status.

Ikeda et al. found that kaempferol, a flavonoid found in edible plants, enhances sleep quality and may contribute to long-term improvements in quality of life, including physical activity, as demonstrated in a double-blind, placebo-controlled, crossover trial. Guo and Rezaei reviewed the effects of ashwagandha (*Withania somnifera*), an herbal plant from the Solanaceae family, highlighting its ability to enhance antioxidant response, alleviate stress-related conditions such as depression, anxiety, and insomnia, and improve

physical performance in sports such as maximum oxygen consumption, treadmill time to exhaustion, metabolic equivalents, and more. Wang et al. explored strategies to enhance the bioavailability of *Rhodiola rosea*, finding that its nano-dosage form significantly improves anti-exercise fatigue effects in rats, particularly when combined with aerobic exercise, compared to the normal form.

The Research Topic covered in this issue are limited. In the future, researchers can conduct in-depth studies on various areas such as the specific nutritional requirements of different types of sports (2), the effects and mechanisms of precise formulations on specific athletic performance (3), the potential toxicity and underlying mechanisms of long-term supplementation of certain nutrients (4), and the impact and mechanisms of specific nutrients on injury prevention or rehabilitation in athletes.

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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 benefits of ashwagandha (*Withania somnifera*) supplements on brain function and sports performance

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Ashwagandha or Withania somnifera is an herbal plant belonging to the Solanaceae family. Because of its wide range of phytochemicals, ashwagandha root extract has been used in numerous research studies, either alone or in conjunction with other natural plants, for various biomedical applications, which include its anti-microbial, anti-inflammatory, anti-stress, anti-tumor, cardioprotective, and neuroprotective properties. Additionally, it improves endothelial function, lowers reactive oxygen species, controls apoptosis, and improves mitochondrial function. These properties make it a useful treatment for a variety of conditions, including age-related symptoms, anxiety, neurodegenerative diseases, diabetes, stress, arthritis, fatigue, and cognitive/memory impairment. Despite the numerous benefits of ashwagandha supplementation, there have been just four meta-analyses on the herb's effectiveness in treating anxiety, neurobehavioral disorders, impotence, and infertility. Moreover, no reviews exist that examine how ashwagandha affects antioxidant response and physical sports performance. Consequently, the goal of this study was to analyze the scientific literature regarding the effects of ashwagandha consumption on antioxidant response and athletic performance.

KEYWORDS

ashwagandha, sports performance, brain function, antioxidant response, exercise, inflammation

Introduction

A resistance training program consists of exercises that force skeletal muscles to contract against external resistance. Such regimens frequently cause the body to increase muscle mass and produce greater strength (1, 2). However, studies have shown that prolonged and/or high-intensity exercise can damage muscle tissue. In addition, oxidative stress and inflammatory cytokines damage biomolecules, which may lead to additional muscle injury caused by the rise in free radical levels following muscle damage (3). Physical fitness affects the formation of reactive oxygen species (ROS) and causes changes in antioxidant enzymes, which can lead to different degrees of oxidative damage and lipid peroxidation (4, 5). Fatigue and compromised cellular and muscular function have been linked to oxidative stress (6, 7). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are antioxidant enzymes acting as the first line of defense to prevent the generation of free radicals and ROS (8).

Numerous investigations have evaluated the efficacy of dietary alterations and micronutrient supplements in mitigating the oxidative stress generated by exercise (9, 10).

Certain medicinal herbs can improve exercise capacity and prevent illness because they contain antioxidants and antifatigue compounds (11). It was reported that supplementation with ashwagandha may enhance the adaptations and gains produced by exercise, making it a helpful addition to a resistance training regimen. There are some justifications for this theory. Ashwagandha has been shown in studies to enhance muscular strength and coordination as well as cardiorespiratory endurance in healthy, normal individual (12, 13). Ashwagandha is classified as a "rasayana" or Ayurvedic rejuvenation therapy which has been used for ages to promote health, increase lifespan, slow down aging, revitalize the body, and produce overall wellbeing (14, 15). Ashwagandha has been reported to possess an extensive spectrum of pharmacological activity, including analgesic, anti-inflammatory, sedative, hypotensive, anxiolytic, immunomodulatory, central nervous system, cardiac, anabolic, and antioxidant properties (14, 16–18). Moreover, it increases thyroid activity and respiratory function, and relaxes smooth muscle (19). Human studies showed that ashwagandha was well tolerated and linked to an increase in testosterone (20, 21) and a decrease in cortisol (22). Ashwagandha may lessen the rise in blood urea nitrogen, lactic acid, and corticosterone that occurs during stress and exercise (14). It may also lessen the activation of dopamine receptors in the brain during stressful conditions (16, 23).

The fact that ashwagandha has several active ingredients may explain the different mechanisms of action. These include compounds like anaferine, isopelletierine, steroidal lactones (withaferins and withanolides), and saponins (23–25).

As exercise may be thought of as a kind of acute stress, and the stress response after consumption of ashwagandha results in improvements in human physical performance, the adaptagenic properties of ashwagandha means it could act as an active ergogenic supplement. Therefore, we summarize the studies that have investigated the role of ashwagandha in exercise performance, antioxidant responses, and increased adaptation.

Ashwagandha and antioxidant responses

The antioxidant properties of ashwagandha have been documented using *in vitro* cell culture, *in vivo* animal research, and clinical trials involving healthy individual (24, 26–28). Additionally, researchers have proposed that ashwagandha could be beneficial for those with oxidative stress-related diseases (29). Ashwagandha has been shown to contain high levels of flavonoids, phenolic compounds, and antioxidant compounds (30). Therefore ashwagandha can repair oxidative damage in cells and lipid peroxidation, as well as combat the formation of reactive oxygen species (ROS) as shown in Figure 1. When ROS attack cell membranes, they produce harmful lipid peroxides and malondialdehyde (MDA) (31). Nuclear factor erythroid 2-related factor 2 (Nrf2) controls the gene expression of antioxidant enzymes involved in combating oxidative damage by activating the PI3K/PTEN/Akt pathway. The compound Withaferin A has been found to have beneficial effects on the nervous system, such as preventing cell death and promoting cell growth. This is achieved by inhibiting the protein PTEN and activating the PI3K/AKT/mTOR and PI3K/AKT/GSK3β pathways, as well as preventing the movement of vascular smooth muscle cells (Figure 2) (32, 33). Nrf2 is also strongly induced by withaferin A where it has a cytoprotective effect (34, 35). Antioxidant proteins, including heme oxygenase-1 (HO-1) and heat shock protein 70 (HSP70), as well as antioxidant enzymes like CAT and SOD, glutathione (GSH), GSH reductase, GPx, thioredoxin (Trx), and Trx reductase are all downstream products of Nrf2 activation (36). Since these enzymatic and protein antioxidants do not need to be continuously produced, they show a longer duration of action compared to vitamins and coenzymes, which are depleted during the initial redox reactions (37). In healthy people, the antioxidant function of the bioactive compounds in ashwagandha, support the herb's usage in promoting oxidative equilibrium and possibly preventing disorders linked to oxidative stress.

Ashwagandha and brain function

Extensive research has been conducted on the diverse physiological effects of *Withania somnifera* (WS), with a particular focus on its potential use to treat brain disorders (14, 38). The effects of WS root and WS leaf on the nervous system have been investigated in both preclinical and clinical studies. Two recent reviews have comprehensively compiled and analyzed the findings, providing evidence for the effectiveness of WS in treating neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's (17, 39–41). One of the primary reasons for utilizing ashwagandha products is to alleviate stress, which is a common practice in some populations. There is widespread acknowledgement that stress can lead to both physiological and anatomical alterations within the brain, and stress has been linked to the development of several neuropsychiatric conditions such as anxiety, depression, and insomnia (42). The ways in which stress plays a role in these disorders involve increased activity of the hypothalamic–pituitary–adrenal (HPA) axis and disruption of the normal function of the immune system (43, 44). Based on the strong connection between stress and neuropsychiatric disorders, the anti-stress properties of WS are believed to play a crucial role in its potential benefits for depression, anxiety, and insomnia.

The mechanism of anxiety largely involves GABAergic neurotransmission, as GABA is the primary inhibitory neurotransmitter in the central nervous system (45). The chief location where GABA agonist drugs exert their effects is at GABA type A (GABAA) receptors, which enhance GABAergic function, and these drugs are frequently prescribed for managing anxiety disorders (46). Extensive research in non-human subjects has indicated that substances present in WS actively engage and regulate GABAA receptors, potentially explaining the anxiety-reducing effect of WS. The initial evidence of the ability of WS to mimic GABA was reported by Mehta et al. in 1991. The researchers discovered that a methanolic extract of WS root increased the influx of chloride ions in spinal cord neurons of mammals without the presence of GABA. The extract also hindered the binding of GABA to its receptor similar to how GABAA receptor agonists act (47). Research studies using receptor-binding assays have confirmed that the substances found in methanolic extracts of WS root show a strong binding to GABAA receptors, while they show a noticeably weaker binding to GABAB, glutamatergic, and opioid receptors (48). The GABAA receptor-specific function of WS has been confirmed by various animal experiments. The effects of morphine and ethanol on the dopamine-producing neurons in the ventral tegmental area of the rat brain were

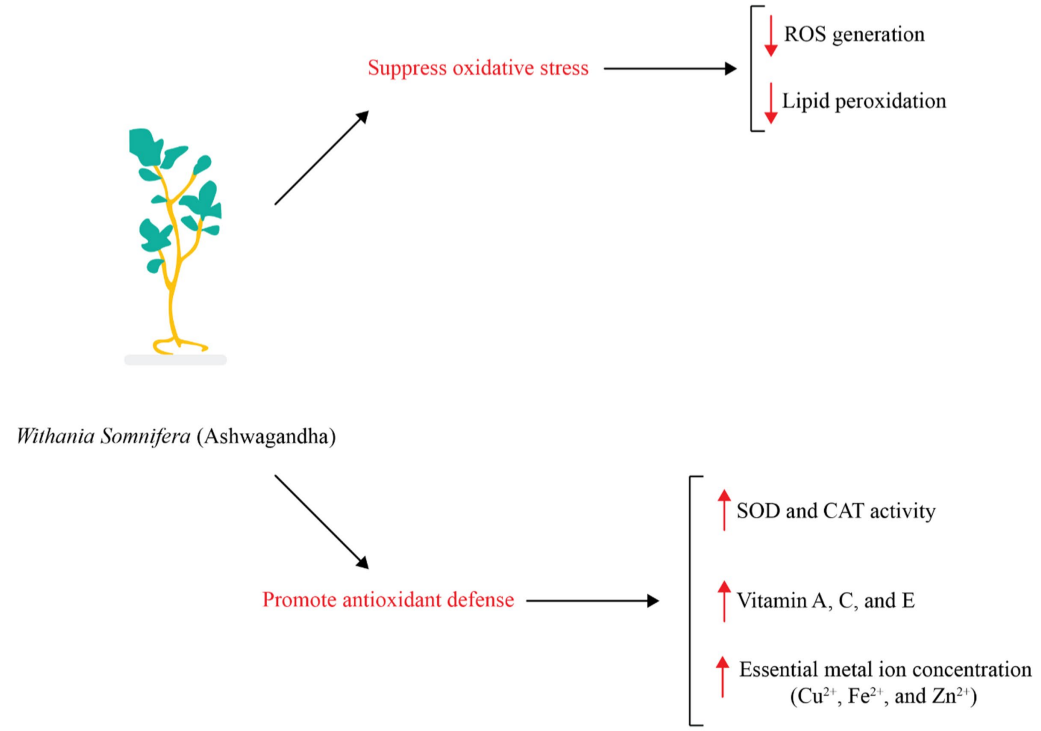

FIGURE 1
The effects of ashwagandha on antioxidant responses.

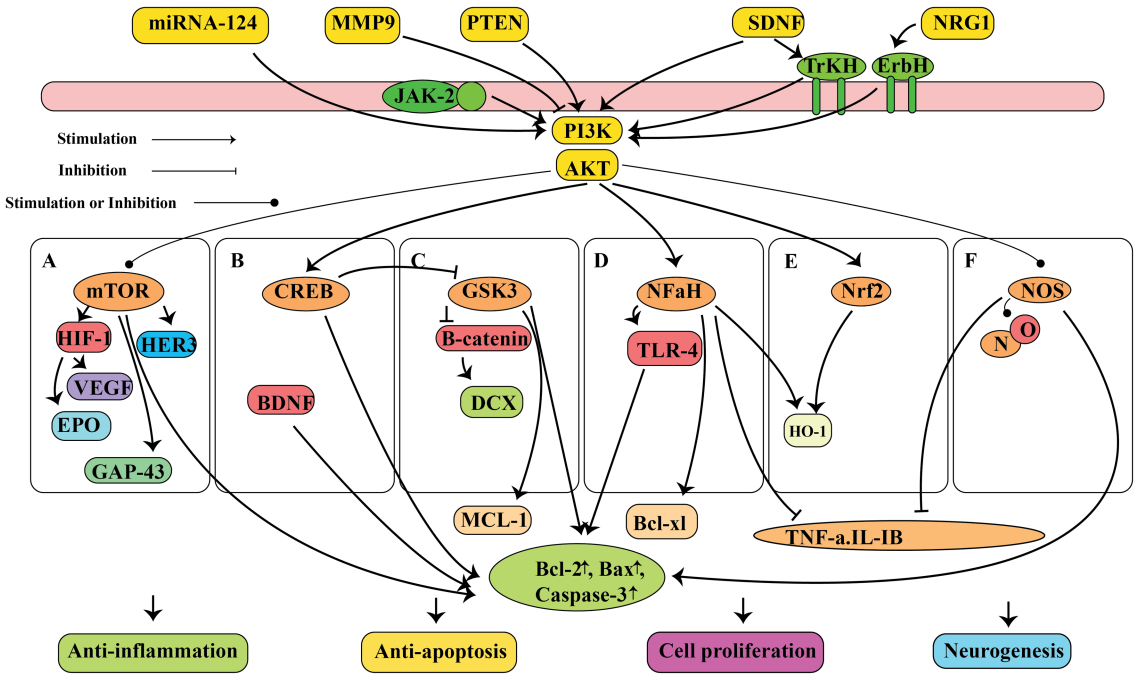

FIGURE 2
The neuroprotective properties of ashwagandha is attributed to the molecular and pathway mechanisms, such as suppression of inflammation, inhibition of cell death, promotion of cell growth, and stimulation of neurogenesis. These effects are mediated by the PI3K/AKT signaling pathway, which activates different pathways including mTOR, CREB, GSK3, NF-κB, Nrf2, and NOS. This figure adapted from Gu et al. (32).

inhibited by a methanolic extract of WS roots. This was attributed to the activation of the GABAA pathway, rather than the GABAB pathway (49). In a mouse experiment, it was observed that a small amount of an unspecified extract from WS roots helped increase the threshold for seizures caused by pentylenetetrazol (PTZ). This effect was enhanced when combined with low doses of GABA to activate

GABA receptors, and diazepam, which alters the function of GABAA receptors (50). In a previous mouse experiment, Kulkarni and colleagues (1993) reported that a methanolic extract of WS (specific plant parts not mentioned) in conjunction with pentobarbital (a GABAA receptor activator) resulted in better defense against PTZ-induced damage, compared to each agent on its own (51). Moreover, when a lower dose of WS was added to GABA, it enhanced the protective properties of the extract.

In a model using rats to simulate sleep disruption, the administration of a specific GABAA activator (muscimol) increased the sedative properties of an unspecified root extract from the WS plant. Conversely, the use of a GABAA receptor blocker (picrotoxin) counteracted this effect (52). Inhibitors of GABAA receptors, specifically picrotoxin and bicuculline, completely abrogated the ability of both methanolic and aqueous extracts from WS roots to cause depolarization in gonadotropin releasing hormone neurons in mice. These inhibitors also prevented the increase of inward ion currents in substantia gelatinosa neurons in mice, as well as in GABAA channels in rat brains (53).

Because the brain is particularly vulnerable to oxidative stress, and anxiety disorders are characterized by a decrease in protective antioxidants as well as an increase in oxidative damage, treatments that protect against oxidative stress are desirable (54, 55). Multiple suggested mechanisms have been suggested to explain how oxidative stress plays a role in brain disorders. Oxidative stress can act as a trigger for these disorders and can also be a result of neuroinflammation, which has also been linked to brain-related disorders (56). Figure 3 shows how the components of ashwagandha can affect cell signaling pathways and the production of inflammatory mediators. Inflammation in peripheral tissues may directly play a role in increasing neuroinflammation and oxidative stress in the brain (56). Although there is evidence linking both oxidative stress and inflammation to brain-related disorders, it has not been conclusively proven which one causes the other or vice versa (57). Studies on animals exploring the effects of WS on anxiety have revealed a strong link between improvements in anxiety-related behavior and the amelioration of oxidative stress and inflammatory indicators.

The root extract and leaf extract of WS, although not clearly described, were able to increase the levels of catalase activity and reduced glutathione (GSH) in the brain, and also lower the levels of lipid peroxidation in a mouse model of sleep deprivation and a zebrafish model of neurotoxicity induced by benzo[a]pyrene (58, 59). Furthermore, WS demonstrated the ability to decrease nitrite levels in the mouse model and also lower protein carbonylation in the zebrafish model (58, 59). In an experiment using rats to simulate an ischemic stroke, a uniform hydroalcoholic extract derived from WS roots effectively decreased the levels of lipid peroxidation and enhanced antioxidant function in the brain (60). ASH-WEX, an aqueous leaf extract, was shown to decrease pro-inflammatory cytokines, specifically TNFα and IL-6, both in the peripheral and central nervous systems, in animal models of neuroinflammation and sleep deprivation (61). ASH-WEX produced a significant reduction in indicators of reactive gliosis, such as GFAP, as well as neuroinflammation, such as NOX2, iNOS, and COX2. Furthermore, it successfully regulated various inflammatory pathways and reduced cellular death in the brain (62). In a rat experiment examining the effects of a high fat diet on obesity, the use of WS dry leaf powder significantly lessened the expression of pro-inflammatory cytokines both in the body and brain, decreased indicators of reactive gliosis and neuroinflammation, modulated the nuclear factor NF-kappa-B (NF-κB) pathway, and lowered cell death (63).

Supplementation with ashwagandha has been shown to lower C-reactive protein (CRP) activity (64), one of the most important indicators of inflammation. Furthermore, other studies have shown that ashwagandha exerts a number of anti-inflammatory effects on chronic inflammation-mediated diseases (65), particularly rheumatoid arthritis (66), inflammatory bowel disorder (67), and systemic lupus erythematosus (68). These studies suggest that ashwagandha may be a useful tool for reducing the cytokine storm. Withanolides, particularly Withaferin A, are responsible for the majority of ashwagandha's anti-inflammatory effects, according to several reports (69, 70). Ashwagandha may work by interacting with components of the proinflammatory cell signaling pathway, such as NF-κB, signaling kinases, HSP90, Nrf2, and the inflammasome complex, even though the mechanisms behind the anti-inflammatory effect of withanolides are not fully understood (71). Since the NF-κB transcription factor family is implicated in a number of chronic disorders caused by inflammation, individuals with high NF-κB levels may benefit from therapeutic targeting of NF-κB. In this situation, ashwagandha has the ability to inhibit and mediate the activity of the NF-κB pathway (72). It is thought that strong protein kinase inhibitor activity is necessary for ashwagandha to function. Ashwagandha has the ability to inhibit the signaling cascades of protein kinases, which are essential in inflammatory pathways (72). Furthermore, it appears that kinase inhibition takes place when nitric oxide synthesis is inhibited, which also benefits the inflammatory process. Another possible explanation for ashwagandha's anti-inflammatory properties is the downregulation or destabilization of HSP activity, which is implicated in regulatory kinase pathways. As previously mentioned, ashwagandha regulates Nrf2 to moderate oxidative stress (34, 73). Nrf2 activation may account for ashwagandha's anti-inflammatory properties, as oxidative stress frequently takes place in sites of inflammation and is thought to be one cause of chronic inflammation (74). Finally, by blocking inflammasomes, cytokines, and other multiprotein pro-inflammatory complexes, ashwagandha may lessen inflammation (72, 75).

According to some reports, ashwagandha is an adaptogen that, due to its antioxidant properties, boosts immunity, helps the body respond to stress more effectively, increases resilience, and fights oxidative stress and cellular damage (76, 77). It has been demonstrated that the bioactive C28-steroidal lactones present in WS leaves possess neuroprotective, anxiolytic, antioxidant, and anti-inflammatory properties. Moreover, Withanoside IV and its metabolite sominone, found in WS roots, have been shown to promote synaptogenesis and neuronal outgrowth. Furthermore, it has been demonstrated that the herb inhibits acetylcholinesterase and protects rats from cognitive decline (78). One study evaluated the effects of ashwagandha root extract 300 mg twice a day in humans with moderate cognitive impairment (79). After 8 weeks, the treatment group outperformed the placebo group in tests measuring immediate and general memory, information-processing speed, executive function, and attention. However, the benefits on working memory and visuospatial processing were not definitive because there was little difference between the two groups' performance on these tasks. Another study examined the effect of ashwagandha extract on cognitive impairment in individuals with bipolar disorder (80). Tests were conducted at baseline and after the intervention, with rats randomly allocated to

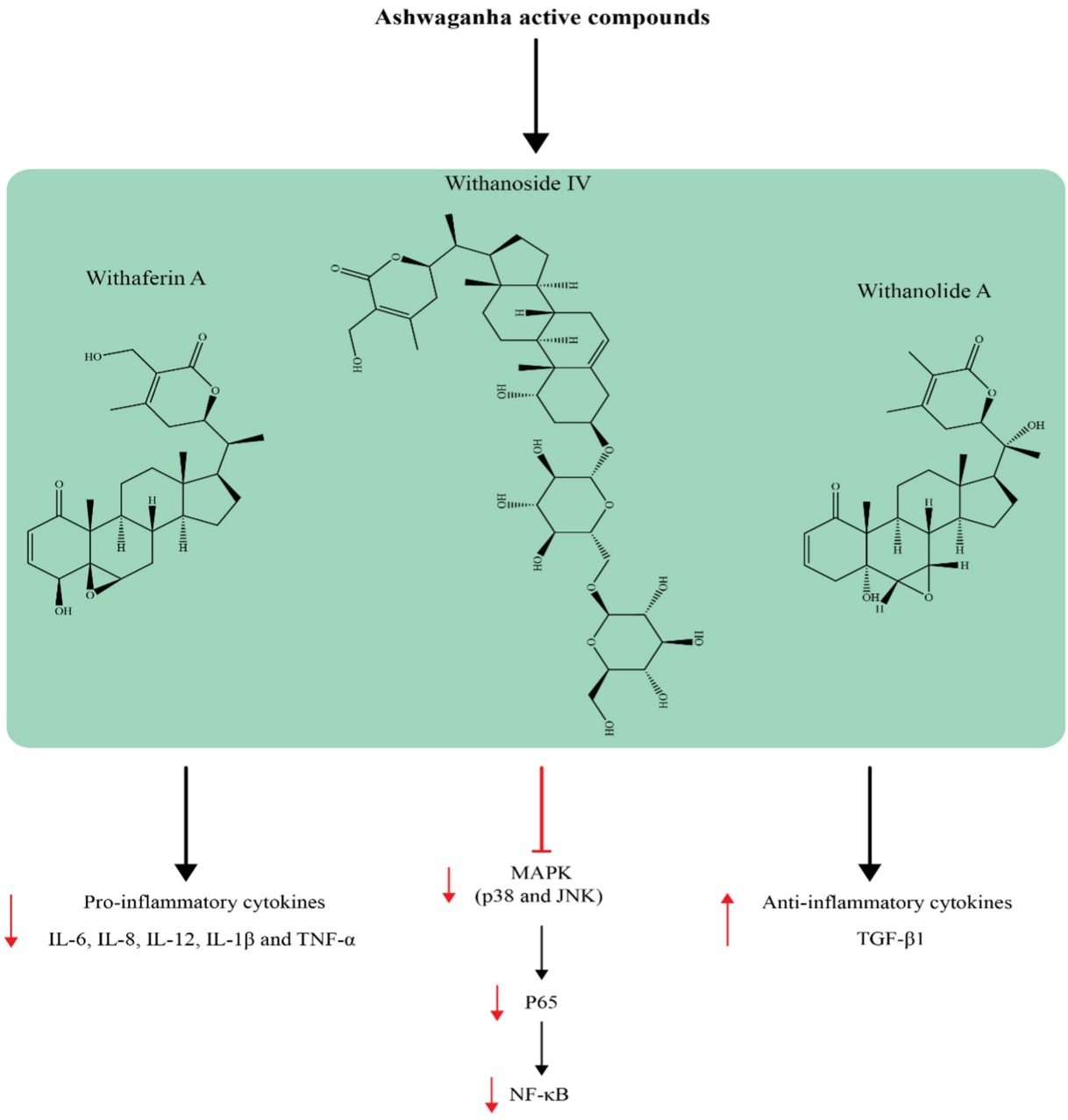

FIGURE 3
The effects of ashwagandha on inflammatory markers.

receive 500 mg/day of ashwagandha or a placebo for 8 weeks. When compared to the placebo, subjects in the treatment group showed significantly better results on the Flanker Test (neutral mean reaction time), the Penn Emotional Acuity Test (mean social cognition response rating), and the Auditory Digit Span (mean digit span backward). These findings suggested that ashwagandha extract may safely enhance cognitive function in bipolar disorder patients, including verbal working memory, response time, and social cognition response. Another study was carried out using ashwagandha root extract on a group of horses. The animals were subjected to a variety of stressors, including loud noises, prolonged physical activity, and separation. Following a 21-day period, the treated group showed a statistically significant reduction in cortisol, glucose, adrenaline, IL-6, lipids, creatinine, aspartate aminotransferase, and alanine aminotransferase (81).

Effects of ashwagandha on sports performance

The physiological measure known as maximum oxygen consumption, or VO2 max, acts as a measure of an individual's aerobic capability. It is a measure of cardiorespiratory fitness that governs both athletic performance and overall health condition (82, 83). With an emphasis on competitive sports, the VO2 max is one of the important variables that control success in endurance activities (84), along with

running efficiency and the anaerobic threshold. It also helps to improve team sports performance by raising the intensity of work, the distance covered, and the quantity of sprints completed (85, 86). In the realm of general health, VO2 max holds particular significance beyond athletic performance. In adults and the elderly, low VO2 max values have been linked to an elevated risk of death and the loss of an independent lifestyle (87), whereas high cardiorespiratory fitness levels have been linked to a lower risk of cardiovascular disease (88, 89). Since a stronger aerobic capacity is linked to a higher quality of life, the VO2 max level is also significant in children (90).

Table 1 lists some clinical trials that have examined the effect of ashwagandha on VO2 max, sports performance and metabolic profiles. The ability of Indian cyclists to sustain cardiorespiratory endurance was assessed in a clinical trial of ashwagandha supplements. Forty top-level Indian cyclists were divided into groups for the intervention and placebo. For 8 weeks, the experimental group was given 500 mg capsules containing aqueous ashwagandha root extract twice a day, while the placebo group received starch capsules for 8 weeks. Compared to the placebo group, which did not exhibit any changes in baseline levels, the experimental group showed a substantial improvement in all parameters, including VO2 max, time to exhaustion on the treadmill, and METS (metabolic equivalents) (91). The conclusions showed that the anaerobic capacity of both males and females had significantly improved (92).

Another study was carried out on healthy subjects undergoing resistance training to investigate the potential benefits of ashwagandha root extract intake on strength and muscle mass. Fifty-seven young male individuals with no prior resistance training experience were divided into treatment and placebo groups for the 8-week trial. The control group was given starch placebo capsules, while the treatment group received 300 mg/twice a day of ashwagandha root extract. After completing baseline assessment, both subject groups engaged in resistance training for 8 weeks, with follow-up measures conducted at the conclusion of the eighth week. The 1-RM load for the bench press and leg extension movements was used to assess muscle strength. Creatine kinase levels, a measure of muscle damage caused by exercise, was used to assess muscle healing. When compared to the group of subjects given a placebo, those treated with ashwagandha saw a significant increase in muscle strength when performing the bench-press exercise (an increase of 46.0 kg compared to 26.4 kg for the placebo group), as well as the leg-extension exercise (an increase of 14.5 kg compared to 9.8 kg for the placebo group). Additionally, the ashwagandha group also had a greater increase in muscle size at the arms (5.3 cm^2 compared to 7.2 cm^2 for the placebo group). These results were statistically significant, with p-values of 0.001 and 0.04 for the bench-press and leg-extension exercises, respectively. According to the study, participants who took ashwagandha had significantly improved muscle strength in their arms (8.6 cm, 95% CI, 6.9, 10.8; $p = 0.01$) and chest (3.3 cm, 95% CI, 2.6, 4.1; $p < 0.001$) compared to those who took the placebo. Additionally, those taking ashwagandha also showed decreased levels of exercise-induced muscle damage, as shown by their stabilized serum creatine kinase levels (1307.5 U/L, 95% CI, 1202.8, 1412.1) compared to the placebo group (1.4 cm, 95% CI, 0.8, 2.0). Ashwagandha supplementation resulted in a significant increase in catalase activity (1462.6 U/L, 95% CI, 1366.2, 1559.1; $p = 0.03$), a significantly higher rise in testosterone levels (18.0 ng/dL, 95% CI, −15.8, 51.8 vs. 96.2 ng/dL, 95% CI, 54.7, 137.5; $p = 0.004$), and a significant decrease in body fat percentage (1.5, 95% CI, 0.4, 2.6% vs. 3.5, 95% CI, 2.0, 4.9%; $p = 0.03$) compared to the placebo group (93).

Ashwagandha and arjuna (*Terminalia arjuna*) were shown to have different effects on the average absolute and relative power, VO2 max, maximum velocity, blood pressure, and balance in humans, both separately and in combination. Forty healthy subjects were assigned to experimental groups, 10 of whom received standardized extracts of

TABLE 1 Clinical trials examining the effects of ashwagandha on VO2 max, sports performance and metabolic profiles.

Participants	Dosage	Duration	Results	Reference
Elite athletes	1,000 mg/day	8 weeks	Improved the cardiorespiratory endurance	(91)
Elite athletes	1,000 mg/day	8 weeks	Improved the anaerobic capacity	(92)
Healthy young men engaged in resistance training	600 mg/day	8 weeks	Significant increases in strength and muscle mass	(93)
Healthy young adults	500 mg/day	8 weeks	Increased velocity, power and VO2 max	(94)
Healthy athletic adults	600 mg/day	8 weeks	Enhanced cardiorespiratory endurance Improved the quality of life	(26)
Young hockey players	500 mg/day	8 weeks	Improved agility levels	(95)
Athletic adults	600 mg/day	12 weeks	Enhanced the cardiorespiratory endurance Improved quality of life	(27)
Hockey Players	1,000 mg/day	8 weeks	Improved VO2 max and hemoglobin concentrations	(96)
Hockey Players	1,000 mg/day	8 weeks	Improved Core Muscle Strength and Stability	(97)
Healthy participants	600 mg/day	8 weeks	Improved muscle strength, growth and endurance	(98)
Male sprinters	2.5–3 g/day	12 weeks	Improved standing broad jump, 50-yard dash, pull-ups, sit-ups, and shuttle run	(99)
Elite cyclists	1,000 mg/day	8 weeks	Improved the aerobic capacity	(100)
Healthy subjects	12 g/day	60 days	Improved hemoglobin and VO2 max	(101)
Healthy subjects	500 mg/day	12 weeks	Improved upper and lower-body strength	(102)
Healthy subjects	20 g/day	4 weeks	Improved Harvard Step Score and VO_2 max.	(103)

ashwagandha root, 10 of whom received standardized extracts of *Terminalia arjuna* bark, 10 of whom received a combination of both herbal extracts, and the remaining 10 received flour-filled capsules as a placebo. Each medication was administered as capsules, with a daily dose of 500 mg. Ashwagandha was shown to improve power, velocity, and VO2 max, whereas arjuna was found to boost VO2 max and decrease resting systolic blood pressure. All indicators displayed improvement when both extracts were administered together, with the exception of diastolic blood pressure and balance (94).

Another study evaluated the effectiveness and safety of ashwagandha in improving cardiorespiratory endurance in fit subjects. Fifty healthy, fit individuals were randomly divided into two groups and given equal amounts of placebo and ashwagandha for 8 weeks. The ashwagandha group was given 300 mg of ashwagandha root extract capsules, twice a day. VO2 max testing and cardiorespiratory endurance was evaluated. The findings showed that the ashwagandha group's VO2 max value was significantly higher than the placebo group. By the conclusion of the trial, the participants in the ashwagandha group showed a statistically significant rise above their baseline. Those in the ashwagandha group showed significantly higher Total Quality Recovery Scores (TQR) than those in the placebo group. When comparing the Ashwagandha group to the placebo group, the Daily Analysis of Life Demands for Athletes (DALDA) questionnaire revealed statistical significance. Better results were also obtained in the Recovery-Stress Questionnaire for Athletes (RESTQ) evaluation, particularly for lack of energy, fatigue recovery, and fitness analysis. In the ashwagandha group, there was also a notable increase in antioxidant levels (26).

An investigation into the effect of ashwagandha supplementation on hockey players' agility level was carried out. A total of 52 male hockey players were randomly separated into two groups. Group II was a placebo control group and Group I was the experimental group receiving WS. For 8 weeks, the experimental group took ashwagandha 500 mg/twice a day, whereas the placebo group took starch capsules. The Illinois Agility Run Test Getchell Test was used to measure the level of agility in the control and experimental groups prior to and following the consumption of placebo or ashwagandha. The results showed that after 4 and 8 weeks, the agility level in the ashwagandha group had significantly improved. However, there was no discernible improvement in agility levels in the placebo group (95). Another report investigated the effects of ashwagandha supplements on hockey players' VO2 max and hemoglobin levels (Hb) in this group of hockey players. Before and after the ashwagandha and placebo were administered, the experimental and control group VO2 max and Hb were monitored. The conclusions showed that the experimental group's VO2 max and Hb had significantly improved (96).

After 4 and 8 weeks, respectively, the experimental group core muscle strength and stability had significantly improved. On the other hand, following 8 weeks of placebo treatment, there was no discernible increase in core muscle strength & stability in the control group (97).

In a different trial, 50 healthy athletes were assessed for their quality of life and how well their cardiorespiratory endurance was improved by ashwagandha root extract. A 20-meter shuttle run test was used to measure VO2 max in order to evaluate cardiorespiratory endurance. The findings showed that after 8 and 12 weeks, ashwagandha supplementation increased mean VO2 max from baseline more than the placebo. At 12 weeks, the quality of life scores in the ashwagandha group considerably improved in comparison to the placebo group in all subdomains (27).

An investigation was conducted on the effects of 600 mg of standardized ashwagandha on strength, muscle mass, and cardiorespiratory endurance after resistance exercise. Eighty healthy male and female volunteers who regularly exercised were divided into two groups and given either 300 mg of ashwagandha supplements twice a day for 8 weeks, or an equivalent placebo. Every participant engaged in resistance training for 8 weeks. VO2 max, muscle mass, and strength were measured at baseline and after 8 weeks. According to the findings, ashwagandha improved endurance, leg press, and bench press performance more than a placebo. Moreover, both male and female ashwagandha users showed higher gains in muscle girth for the chest, arm, and thigh (98).

Another trial examined the impact of ashwagandha consumption on specific physical fitness metrics in male sprinters. The 20 male sprinters were split evenly between experimental and control groups. For 12 weeks, ashwagandha was administered to the experimental group three times a week on alternate days taken with milk. Following a 12-week period, the 20 male sprinters undertook a physical fitness test. According to the results, ashwagandha significantly improved the standing broad jump, number of pull-ups, 50-yard dash, number of sit-ups, and shuttle run performance (99).

Another study was designed to examine the effects of ashwagandha supplements on the physical performance of top Indian cyclists based on gender differences. The study used an identical experimental design with 13 males and 19 females totaling 38 cyclists. METs and VO2 max measures of aerobic capacity were assessed directly using the Bruce technique. For 8 weeks, the experimental group received ashwagandha supplements, whereas the control group received capsules containing starch. The findings showed that whereas average power and peak power greatly improved in females, VO2 max improved significantly in males. After 8 weeks, males using ashwagandha supplements showed improvements in their aerobic and anaerobic capacity (100).

The effectiveness of ashwagandha in increasing VO2 max in healthy participants was assessed in a controlled, single-blind, randomized clinical trial. Two groups each consisted of 54 healthy volunteers, the study group started the day with 12 g of ashwagandha Choorna and 200 mL of milk on an empty stomach, whereas the control group merely had 200 mL of milk. The VO2 max of the study and control groups was assessed using the Rockport fitness walking test on days zero, 60 and 90 of follow-up. It was discovered that the study group VO2 max and Hb had significantly improved (101).

The effect of Sensoril® supplements on strength training adaptation was investigated in another trial. Men who undertook recreational activity were divided into double-blinded groups assigned to receive 500 mg/day of Sensoril® or a placebo. Measurements included 7.5 km cycling time trial, clinical blood chemistry, DEXA measurements of muscle strength, endurance, power, and body composition, at baseline and after 12-weeks of supplementation. The subjects continued to eat normally and adhered to a resistance-training regimen of progressive overload (4 days per week, divided between upper and lower body). The results showed that S500 produced much higher increases in the 1-RM squat and bench press performance. A shift in the android/gynoid ratio determined by DEXA also favored S500. Systemic hemodynamics, visual analog scales for affect and recovery, and body composition did not show any

between-group differences. However, only the S500 group showed significant improvements in peak bench press power, average squat power, 7.5 km time trial performance, and perceived recovery scores. There was only a small polycythemia effect in placebo, according to the clinical chemistry measurement, and no additional statistically or clinically significant alterations were found (102).

Rasayana dravyas is a potential dietary supplement containing granules of ashwagandha, Jeeraka (*Eleusine coracana*), Shatavari (*Asparagus racemosus*), Mudga (*Vigna radiata*), Ragi (finfer millet), and Shunti (*Zingiber officinale*). Twenty-three subjects participated in an open-label, double-arm, controlled clinical trial using a modified wait-listed crossover design. For the duration of the trial, they were given 150 mL of milk and 20 g of ashwagandhadi granules in Rasayana dravyas daily for 1 month. The results revealed a statistically significant difference in the VO_2 max and Harvard Step Score for the treatment group compared to the placebo group (103).

Practical applications

This review has provided useful information that will help sports performance practitioners in real-world scenarios, and to understand how supplements designed to reduce and control ROS damage work. In particular, it focuses on ashwagandha supplementation for improving athletic performance and modulating antioxidant response. In order to boost performance, sports teams may decide to adopt new dietary guidelines and working practices as a result of this evaluation.

Conclusion

Evidence suggests that ROS are key players in physiological signaling networks that control how the body reacts to exercise, and that physical activity-induced oxidative stress and inflammation are necessary for training adaption. The process of physiological adaptation requires a proper balance between antioxidants and free radicals, and it is possible that athletes who take antioxidants could counteract the advantageous effects of ROS in normal cell signaling. According to this theory, ROS produced during exercise are thought to activate PGC1-α and MAPK, two biochemical pathways essential for both muscle growth and aerobic capacity. Some studies have proposed that a brief rise in reactive oxygen species (ROS) brought on by physical activity may have advantageous consequences, including controlling the contraction of muscles, promoting muscle regeneration, and enhancing vasodilation while exercising. On the other hand, oxidative stress and elevated ROS levels can damage tissues and cells and induce inflammation. Many endurance athletes consume diets deficient in antioxidants to maintain their high demands for energy to carry out physical activity, despite their demanding training schedules. Although ashwagandha pills are frequently advised for endurance athletes, the numerous health advantages of exercise alone may make then unnecessary.

Author contributions

SG: Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. MR: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Visualization, 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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Effect of kaempferol ingestion on physical activity and sleep quality: a double-blind, placebo-controlled, randomized, crossover trial

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Background: Kaempferol (KMP), a flavonoid in edible plants, exhibits diverse pharmacological effects. Growing body of evidence associates extended lifespan with physical activity (PA) and sleep, but KMP's impact on these behaviors is unclear. This double-blind, placebo-controlled, crossover trial assessed KMP's effects on PA and sleep.

Methods: A total of 33 city workers (17 males and 16 females) participated in this study. They were randomly assigned to take either 10 mg of KMP or placebo for 2 weeks in the order allocated, with a 7-day washout period in between. All participants wore an accelerometer-based wearable device (Fitbit Charge 4), which monitored daily PA, heart rate (HR), and HR variability during sleep.

Results: The duration of wearing the device was 23.73±0.04h/day. HR decreased in each PA level, and the mean daily step count and distance covered increased significantly during KMP intake compared to placebo. The outing rate, number of trips, number of recreational activities, and time spent in recreation on weekends increased. Sleep quality improved following KMP intake. The decrease in HR and increase in RMSSD may be important in mediating the effects of these KMPs.

Conclusion: KMP leads to behavioral changes that subsequently improve sleep quality and potentially improve long-term quality of life.

Clinical Trial Registration: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000048447, UMIN000042438.

KEYWORDS

behavioral change, daily step count, sleep quality, Fitbit, kaempferol

1 Introduction

Advancements in medicine, technology, and dietary preferences have ushered in an era where people can potentially live up to 100 years (1). The compelling health benefits of physical activity (PA) and quality sleep are undeniable, with increased daily PA substantially lowering the risk of premature death and mitigating over 25 chronic health conditions (2, 3). The

potential of physical inactivity to heighten the risk of various diseases and increase the all-cause mortality rate is widely recognized. Therefore, it is universally acknowledged that moderate exercise is vital for maintaining health and preventing diseases (4–6). An increase of merely 1,500 steps in average daily step count corresponds to an impressive 2.2% reduction in mortality risk (7). Reduced PA is the fourth leading risk factor for premature death, behind hypertension, smoking, and diabetes mellitus (8). Despite the numerous initiatives aimed at promoting PA, there has been little progress in improving these outcomes (9).

PA strongly correlates with maximal oxygen uptake (VO_{2max}) (10, 11), a key metric of aerobic capacity. It plays a fundamental role in mitigating disease risk through diverse mechanisms, including weight loss, inflammation reduction, and promoting mental well-being (12). As individuals age, the decline in mitochondrial function, which is closely associated with PA, leads to a decrease in VO_{2max}, consequently, resulting in reduced daytime activity and compromised sleep quality (13, 14). Strategies to enhance or maintain PA levels typically involve raising awareness through social initiatives, formulating guidelines, and using educational methods. Nutritional approaches are being explored to improve sleep quality, and specific food components such as tryptophan, melatonin, serotonin, and glycine directly enhance sleep quality (15, 16). Saturated fatty acids and proteins can affect sleep quality through the secretion and synthesis of serotonin (15). However, if the decrease in daytime PA is primarily due to mitochondrial dysfunction, which leads to reduced oxygen uptake and energy supply, these strategies might not be effective. In such scenarios, increasing daytime PA emerges as a promising solution. Previous studies predominantly focused on PA's effects on sleep in adults with specific illnesses, health issues, or clinical sleep disorders (17), leaving a gap in understanding how daily PA affects sleep in healthy adults. In recent years, direct-to-consumer wearable devices have become valuable in behavioral research. In particular, the Fitbit activity trackers (Fitbit, San Francisco, California, United States) are highly regarded for their superior accuracy among commercially available wearable devices (18–23), capable of tracking PA, heart rate (HR), global positioning system (GPS) location, and sleep. In particular, this tracker is regarded as a useful device for measuring PA (18) and step counts (22, 23), making it suitable for this trial. This technology, which allows for the continuous monitoring of one's overall daily life and health status, holds promise for scientific research and enables individuals to better understand themselves and naturally heighten their health consciousness.

Kaempferol (KMP), a flavonoid in various edible plants, is renowned for its antioxidant, anti-inflammatory, anticancer, cardioprotective, and neuroprotective properties (24–26). In the *in vitro* assay using C_2C_{12} cells, we found that KMP activates mitochondrial oxidative metabolism and elevates intracellular adenosine triphosphate (ATP) levels under hypoxic conditions by effectively suppressing the stabilization of hypoxia-inducible factor-1α (HIF-1α) (27). It is conceivable that the function of KMP affects specific pathological conditions and broadly influences daily life; however, this aspect has not been investigated. For example, during exercise, the balance between oxygen supply and demand is disrupted in skeletal muscles, resulting in temporary hypoxia and a decrease in mitochondrial function, which reduces exercise capacity (28). HR tends to increase proportionally with accumulating fatigue during exercise (29–31), and improving VO_{2max} can improve sleep quality (14, 32). In this study, we focused on the effect of KMP based on oxygen uptake, mitochondrial function, and energy production. We utilized the abilities of the Fitbit Charge 4 device to investigate how KMP intake influences daily PA levels and sleep quality and its potential to enhance the overall quality of life. Our findings can yield societal benefits in the future. This is the first study investigating the effects of flavonoid ingestion on PA and sleep using a wearable device for comprehensive monitoring and analyses.

2 Materials and methods

2.1 Trial design and participants

Ethical considerations were strictly adhered to during this study, following the principles outlined in the Declaration of Helsinki. All procedures involving human subjects were approved by the Ethics Committee of Otsuka Pharmaceutical Co., Ltd. (approval no. 2003, dated October 30, 2020).

This double-blind, placebo-controlled, randomized, crossover study, was conducted at Saga Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd. between November 2020 and December 2020. Thirty-seven untrained city workers recruited through advertisements using flyers and over email participated in the study. Written informed consent for participation and publication of the data was obtained from each participant after providing a comprehensive explanation of the experimental procedures and associated risks.

Participants visited the laboratory before the supplementation period in the two-week selection period. During this visit, their medical histories were recorded, and they underwent physical examinations, biochemical blood tests, and hematological tests, allowing physicians to assess potential health risks and chronic diseases. Participants consumed 10 mg KMP supplement and a 24 h urine collection was performed to evaluate the urinary excretion of KMP.

Based on these results, we excluded participants with digestive, circulatory, or endocrine diseases, those using medication for treatment, those having food allergies, pregnant women, or those deemed ineligible for this study by a medical doctor. Thirty-four participants proceeded to the double-blind supplementation phase. The random assignment was performed by a person blinded to this study's conduct and analysis. Random allocation to the two groups, placebo start and KMP start, was performed using the permuted block method (block size of four) by entering the described variables (age, sex, and body mass index) into the SAS software (version 9.4; SAS Institute, Cary, NC, United States) in a 1:1 ratio. This facilitated an automatic allocation and generated a unique ID code for each participant. The personnel administering test foods to the participants differed from those performing experiments and analyzing data, ensuring blinding of the staff involved in analyses to the test foods administered to the participants.

During the supplementation period, participants consumed either a granulated formulation containing 10 mg of KMP or a placebo granulate, designed as isocaloric, every day after breakfast for 2 weeks, with a 7-day washout period in between. Throughout this period, various parameters, such as PA levels, heart rate, and sleep indices were monitored using the Fitbit Charge 4 device. Participants

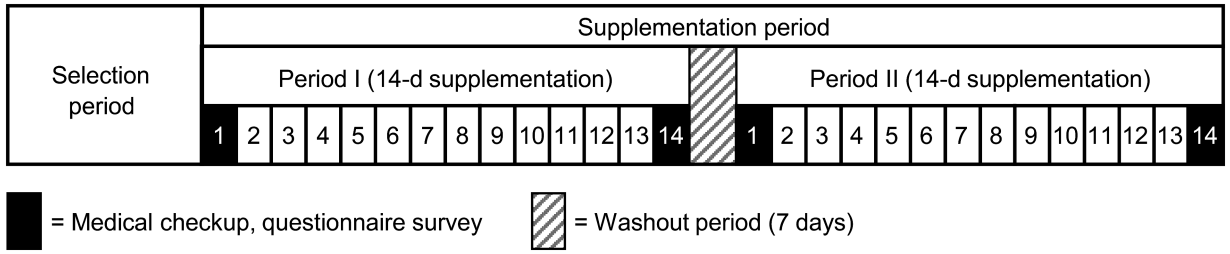

FIGURE 1
Schematic illustration of the crossover design of the study.

maintained a diary detailing their physical condition, PA, and specifics of supplement intake. A schematic of the study design is shown in Figure 1. Participants were instructed to avoid making significant changes to their lifestyle habits during the study period or disclose any use of nutritional supplements or medication. They were directed to avoid consuming foods and beverages high in KMP content throughout the selection period.

2.2 Data collection using Fitbit

Each participant received a Fitbit Charge 4 tracker to wear on their non-dominant hand for the study. Detailed instructions on proper device use and mobile application installation according to the manufacturer's specifications were provided. Additionally, participants were trained on the periodic synchronization of their Fitbit tracker to ensure accurate data recording and monitoring throughout the study.

Parameters related to PA (step count, walking and running distance, metabolic equivalent of task [MET], GPS position) and HR measurements were directly downloaded from the web server using a developer's application programming interface (API) provided by Fitbit. This API facilitated the direct extraction of data from the Fitbit web server. The non-wear time was defined as 120 or more consecutive minutes with zero steps counted. All other time intervals were categorized as wear time (33). Minutes with over 200 steps were flagged as missing data points (34, 35). Fitbit data were considered adequate when there were at least 10 h of Fitbit wear time during a given day. This strategy was applied to guarantee the consistency and accuracy of the data collected throughout the study.

The PA levels were categorized as rest (<1.5 METs), light (1.5–2.9 METs), moderate (3–5.9 METs), and vigorous (>6 METs) from the data obtained from Fitbit. PA level and HR at each intensity were computed, and HR-MET curves were generated for each supplement intake using Microsoft Excel (Microsoft Corporation, Redmond, WA, United States). The minute-by-minute GPS data downloaded from the Fitbit application was imported into QGIS (QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.org). Walking speed was calculated using the distance traveled derived from GPS coordinates during walking and was expressed as distance traveled per minute. A 5-m square grid was created around the Tomi city office and Tomi welfare center where the participants worked, and heat maps were generated based on the number of steps taken per minute. Fitbit data provided sleep information in 30-s intervals based on movement and HR data, including sleep score and stage data (sleep time, wake, light sleep, deep sleep, and rapid eye movement (REM) sleep). The HR data during sleep was used to extract the respiratory rate at 5-s intervals via the API. The root mean square of the successive differences (RMSSD) and low frequency/high frequency (LF/HF) ratio during sleep were calculated using a PC program (HRVanalysis, Université Jean Monnet Saint-Etienne, France).

2.3 Time-use and activity diary

The participants recorded their in-home and out-of-home daily activities (such as shopping, recreation, exercise, jog-related activities, housework, rest, and sleep) in a logbook. The accuracy of the information recorded in the logbook was cross-referenced with the GPS coordinates of the activity locations at the end of the study. The percentage of participants making at least one trip per day (outing rate), number of trips, number of recreational activities, and time spent on recreation on weekends were calculated.

2.4 KMP analysis in urine

Urine analysis involved mixing 100 µL of each sample with 100 µL (50 units) of β-glucuronidase solution (Sigma Aldrich, St. Louis, MO, United States) in 0.2 M sodium acetate buffer (Wako Pure Chemicals Industries Ltd., Tokyo, Japan), pH 5.0. The mixture was incubated at 37°C for 30 min, followed by the addition of 200 µL of 4% phosphate buffer containing internal standard, apigenin-d5 (Toronto Research Chemicals, Inc., North York, ON, Canada).

The prepared urine samples were transferred to a conditioned 96-well Oasis MCX µElution plate (Waters Corporation, Milford, MA, United States), passed through the sorbent bed, and washed with 200 µL of 2% formic acid (Wako Pure Chemicals Industries Ltd., Tokyo, Japan) in water, followed by 200 µL of 40% methanol (Fisher Scientific, Hampton, NH, United States). KMP and the internal standard were eluted with 150 µL methanol/acetonitrile (Fisher Scientific, Hampton, NH, United States) in another 96-well elution plate for final analysis. Chromatographic separation involved injecting 20 µL of the prepared sample into a reverse-phased C18 analytical column (50 mm × 2 mm, 3 µm particle size, Cadenza CD-C18, Imtakt Co., Kyoto, Japan). We achieved high-performance liquid chromatographic (HPLC) separation on a Shiseido Nanospace HPLC system (Tokyo, Japan). The mobile phase comprised 0.1% formic acid with water and acetonitrile, and operated at a flow rate of 0.35 mL/min. The chromatographic conditions were held constant at the initial

mobile phase composition (20% acetonitrile) for 0.5 min, followed by a linear gradient to 95% acetonitrile for 2 min, maintained at 95% acetonitrile for 3.5 min, followed by a linear gradient to 20% acetonitrile for 3.6 min, and maintained a 20% acetonitrile for 5.5 min. The HPLC-separated samples were analyzed using a Sciex API-3000 tandem mass spectrometer (Sciex, Framingham, MA, United States) equipped with a Turbo Ion Spray interface.

2.5 Statistical analyses, PCA, and correlational analysis

All data are presented as the mean ± standard error of the mean. All statistical analyses were performed using SAS software (version 9.4), R (version 4.3.3) with packages "beeswarm" and "corrplot," and Python (version 3.10). A p-value < 0.05 was considered statistically significant. The data were analyzed using a linear mixed-effects model. Sequence, period, and treatment were the fixed factors, while subjects within the period and within the subject were the random factors. We did not input the missing data into the primary model considering the mixed model of repeated measures approach.

PCA (principal component analysis) was performed using standardized data on 18 indicators, including daily step count; moving distance; total METs; total-, interrupted-, rem-. light-, and deep-sleep times; sleep score; daily, rest, light, middle, and vigorous activities; sleep-HR; RMSSD; and LF/HF. The missing data were complemented by the values calculated by applying default parameters with the KNNImputer of sci-kit-learn (v1.2.2). The differences in principal component scores (PC1 and PC2) between placebo and KMP were calculated, and the cosine similarity with the principal component loadings of each variable was calculated.

Spearman correlation analysis for effects due to KMP intake was performed between the change in the 18 indicators (Δ = KMP – Placebo) and KMP absorbability.

Spearman correlation analysis for each indicator was performed using data for the 19 parameters, including 18 indicators and KMP absorbability for placebo and KMP groups. KMP absorbability in the placebo group was set to 0.

3 Results

3.1 Participants' information

This trial comprised a selection period, 14-day food intake period, 7-day washout period, and 14-day food intake period (Figure 1). Figure 2 illustrates the CONSORT flow diagram describing the participant allocation, follow-up, and analysis process. The CONSORT checklist is provided as a Supplementary material. Participant eligibility was assessed during the recruitment period. Initially, 37 participants were screened; however, two withdrew their consent to participate and one was excluded because of medication. Thus, 34 participants were enrolled and randomly assigned to two groups. Of these, 33 completed all trial procedures. One participant suffered from bacterial enteritis during the washout phase and discontinued participation in the study.

The mean wearing time of the device was 23.73 ± 0.04 h/day. One participant did not intake the supplement for 1 day, while the remaining participants had a 100% intake rate. Per-protocol analysis (PPS) was the primary analysis of efficacy. Table 1 shows the background information of the participants whose data were included in the PPS analysis.

3.2 KMP intake resulted in a decrease in HR across all activity levels, leading to an increase in step count and moving distance

The intake of KMP-containing supplements decreased HR during light, middle, and vigorous activities by 4 bpm, 3 bpm, and 2 bpm, respectively. Compared with the placebo, KMP intake decreased the HR during sleep and at rest by 6 bpm each (Figure 3A). The physical load index represented the approximated curve obtained by plotting HR on the vertical axis and the exercise intensity (METs) on the horizontal axis, significantly decreased with the intake of KMP-containing supplement (Figure 3B).

The intake of KMP-containing supplements significantly increased the step count and moving distance by 624 steps/day and 0.4 km/day, respectively, compared to the placebo, with no change in walking speed (Figure 3C). The movement of participant in the area around their office increased (Figure 3D).

3.3 KMP ingestion led to an increase in weekend activities and recreation

The intake of KMP-containing supplements significantly increased the outing rate and the number of trips on weekends from 91.7 ± 2.6% to 97.7 ± 1.3% and from 3.7 ± 0.2 trips/day to 4.1 ± 0.2 trips/day, respectively (Table 2). Additionally, the intake of KMP significantly increased the number of weekend recreational activities from 0.37 ± 0.05 times/day to 0.53 ± 0.06 times/day, and the weekend time spent on recreation from 1.5 ± 0.3 h/day to 2.2 ± 0.3 h/day, respectively.

3.4 KMP ingestion improved sleep quality without affecting sleep time and interruption

No significant changes were observed in sleep time, sleep interruption time, REM sleep time, light sleep time, and deep sleep time with the intake of KMP-containing supplements (Figure 4A). On the other hand, sleep score and RMSSD significantly increased by 3.9 points and 3.6 ms, respectively, compared to the placebo (Figure 4B). Furthermore, after the intake of KMP-containing supplement, the LF/HF ratio significantly decreased by 0.40 compared to the placebo.

When comparing the trends in sleep scores during the trial period, a significant difference was observed between the placebo and KMP periods, regardless of the test food initiated in the trial. Interestingly, this significant difference disappeared during the washout period (Figure 4C).

In PCA, patterns of change from placebo to KMP were observed across participants with different backgrounds (Figure 4D). While the magnitude of the changes did not significantly surpass the individual differences at baseline, the direction of change was similar. When comparing the direction of change in each participant and the

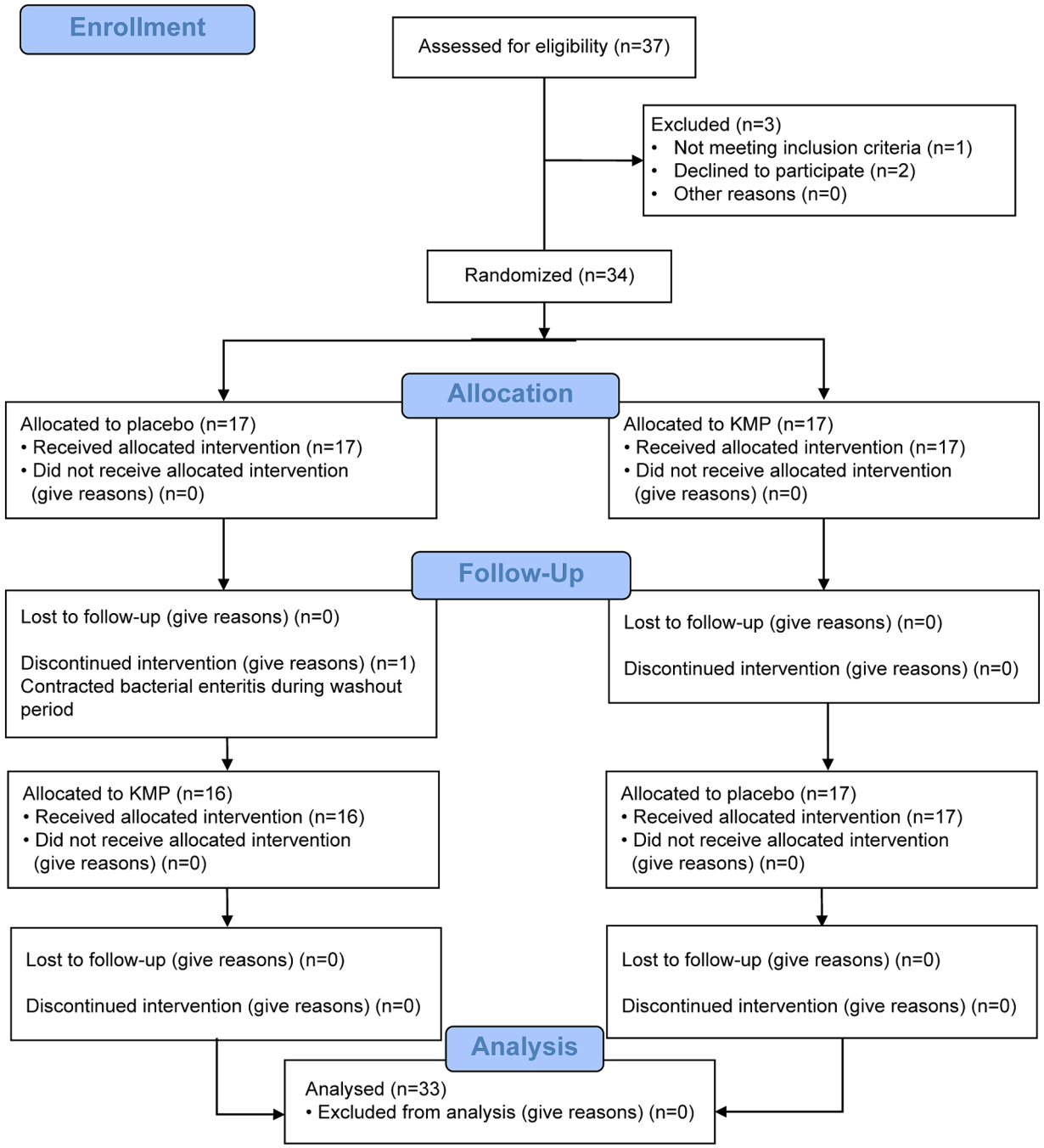

FIGURE 2
Flowchart for participant enrolment in the study. PPS, per-protocol set.

direction of the contribution of each indicator, RMSSD was the most similar index (Supplementary Figure S1).

3.5 KMP ingestion improved sleep quality without affecting sleep time and interruption

Figure 5A shows the correlation between the amount of changes of each indicator due to KMP intake. The KMP excretion rate showed a positive correlation with Δdaily step count (Figure 5B), Δmoving distance ($r = 0.407$, $p = 0.0187$), Δtotal METs ($r = 0.401$, $p = 0.0208$) and Δmiddle activity ($r = 0.416$, $p = 0.0161$), and a weak negative correlation with Δlight sleep time ($r = -0.382$, $p = 0.0282$); no correlation with Δsleep score (Figure 5B) and ΔRMSDD (Figure 5B) was found. Δdaily step count correlated positively with Δdeep sleep time (Figure 5B), and Δdaily HR (Figure 5B), Δsleep HR (Figure 5B), and Δresting HR ($r = -0.417$, $p = 0.0158$) correlated negatively with ΔRMSSD.

Supplementary Figure S2A shows the correlation between indices combining data from the placebo and KMP groups. The KMP excretion rate showed a positive correlation with sleep score

TABLE 1 Participants' characteristics.

Characteristic	$n = 33$
Sex	Male, 17; Female, 16
Age (years)	42.1 ± 1.9
Height (cm)	164.6 ± 1.6
Weight (kg)	64.5 ± 2.1
Body mass index (kg/m^2)	23.7 ± 0.6
KMP excretion (mg)	1.02 ± 0.12
Wearing time of the Fitbit (h/day)	23.73 ± 0.04

Data are presented as the mean ± standard error of the mean.

(Supplementary Figure S2B) and a weak correlation with RMSSD ($r = 0.382$, $p = 0.0015$) and LF/HF ($r = -0.357$, $p = 0.0033$). Sleep HR, daily HR, and LF/HF negatively correlated with RMSSD (Supplementary Figure S2B). Light activity HR ($r = -0.434$, $p = 0.0003$), middle activity HR ($r = -0.403$, $p = 0.0008$), and resting HR ($r = -0.398$, $p = 0.0009$) showed significant or weak negative correlation with RMSSD.

4 Discussion

Daily intake of KMP improved both PA and sleep quality. Regular PA is associated with a reduction in mortality and the risk of chronic diseases (36–38). PCA and correlational analysis suggested that the effects of KMP were based on a decrease in HR and an increase in RMSSD. The increase in PA levels depends on KMP absorbability, suggesting that increased blood concentration is directly important. By contrast, sleep quality does not depend on the absorbability of KMP, suggesting that it is effective at low blood concentrations or operates through an indirect mechanism. KMP, a single ingredient, has multiple health benefits through multiple mechanisms, which is intriguing and strongly indicates its usefulness. Our results suggest that KMP, by mediating effects on such as oxygen supply and energy production and improving overall quality of life rather than treating specific diseases, has the potential to offer broad value to individuals and society.

4.1 KMP ingestion led to a decrease in HR and an increase in PA

By incorporating KMP intake into daily life without restricting everyday activities, we observed a decrease in HR across all PA levels and an increase in step counts and visual behavioral volumes as shown by the heatmaps. These results are similar to previous survey findings that daily PA increases email and web-based interventions (39–41), suggesting the potential of daily KMP intake as a new tool for enhancing daily PA. Recreation serves as a tool for diversion and stress relief, as well as promoting health (42). Additionally, it contributes to mental well-being.

Even light exercises such as walking or bicycling, when continued, prevent diseases and reduce all-cause mortality rates (43, 44). There is a difference of approximately 1,000 steps per day between individuals with and without fatigue (31). Our previous study has revealed that KMP intake improves VO_{2max}, muscle strength, and sleep quality, all of which have a negative correlation with fatigue (45–47). Therefore, the increase in PA observed in this study is likely due to the fatigue-reducing effect of KMP. If this hypothesis is correct, it is noteworthy that HR decreased in all aspects of daily life. HR increases with the accumulation of fatigue and reflects the physical load of daily activities (29), but it has been difficult to evaluate HR and PA levels simultaneously. In this regard, the physical load index evaluation conducted in this study holds potential as a new index for conveniently measuring daily fatigue and the physical load of daily activities.

4.2 KMP intake led to sleep quality improvements

HR variability affects PA levels and sleep quality. For example, a lower HR during sleep is associated with improved autonomic function and better sleep quality (48). In fact, the LF/HF ratio decreases with the depth of sleep (49), and lower RMSSD is associated with difficulty falling asleep and frequent awakenings during the night (50). Our findings revealed that KMP intake reduced HR and LF/HF ratio and increased RMSSD during sleep, thereby increasing sleep score, suggesting the potential for KMP intake to provide healthy sleep. On the other hand, self-evaluation of sleep may be more important than objective evaluation of the relationship (51, 52), and we are currently evaluating the relationship between the results of the 3DSS (three-dimensional sleep scale), which can calculate subjective sleep quality, and Fitbit Charge 4 in a separate clinical trial.

There are several potential mechanisms that could lead to improved sleep quality. Dworak et al. reported a positive correlation between deep sleep and brain ATP content (53). In our previous animal studies, in the brains of rats exposed to a low-oxygen environment (12% oxygen), ATP content decreases, but this decrease is mitigated by oral administration of KMP (27). Additionally, a meta-analysis has shown the impact of increased PA on sleep quality (54). Taking these pieces of information together, it can be hypothesized that the improvement in sleep indices due to KMP intake could be due to increased PA, potentially forming a positive feedback loop between oxygen supply, decreasing HR, promoting PA level, regulating the autonomic nervous system, and improving sleep quality.

PCA showed that KMP intake exerts similar effects in participants with variable backgrounds, reinforcing the potential for KMP's effects to be generalized and widely applied. The changes from placebo to KMP were characterized by RMSSD. However, as mentioned below, the action of KMP on RMSSD may be indirect owing to the increase in PA. Therefore, the effects of food ingredients such as KMP, unlike those of highly selective drugs, are non-selective actions that broadly affect our bodies, yielding various health benefits.

4.3 Mechanisms underlying multiple effects of KMP

We conducted a correlation analysis between the change in indicators due to KMP intake to understand the overall effects of KMP and predict its mechanism. The ΔPA level correlated with the absorbability of KMP, suggesting that the absorbability of KMP is

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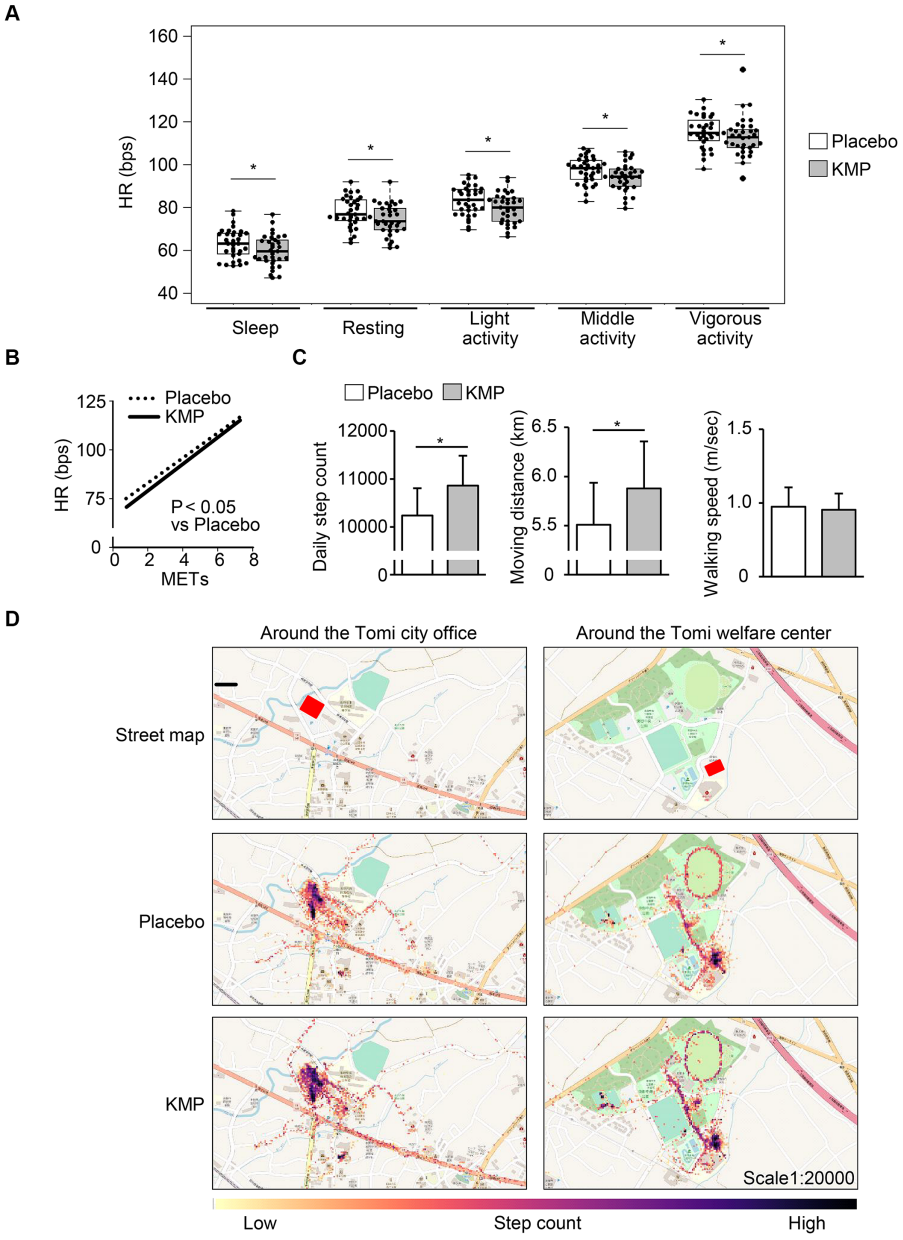

FIGURE 3
Effects of KMP intake on HR and activity levels. **(A)** HR at each activity level. Data are presented as box plots (median ± interquartile range), and dots represent the data for each participant. *$p < 0.05$ KMP vs. placebo, mixed model for repeated measures for crossover design. **(B)** Approximate curves obtained by plotting the heart rate during activity for each metabolic equivalent of task (METs) during each supplement intake. Statistical analyses were performed using a mixed model for repeated measures for cross-over design. **(C)** Daily step count, moving distance and walking speed. Data are presented as the mean in the box, and standard error is represented by the error bar for each individual. *$p < 0.05$ KMP vs. placebo, mixed model for repeated measures for crossover design. **(D)** Heat map created by the number of steps taken per minute.

TABLE 2 **Information on weekend outings and recreation.**

	Placebo	KMP
Outing rate on weekends (%)	91.7 ± 2.6	97.7 ± 1.3*
Number of trips on weekends (trips/day)	3.7 ± 0.2	4.1 ± 0.2*
Number of recreations on weekends (times/day)	0.37 ± 0.05	0.53 ± 0.06*
Time spent for recreations on weekends (h/day)	1.5 ± 0.2	2.2 ± 0.3*

Data are presented as the mean ± standard error of the mean. *$p < 0.05$ vs. placebo, mixed model for repeated measures for cross-over design.

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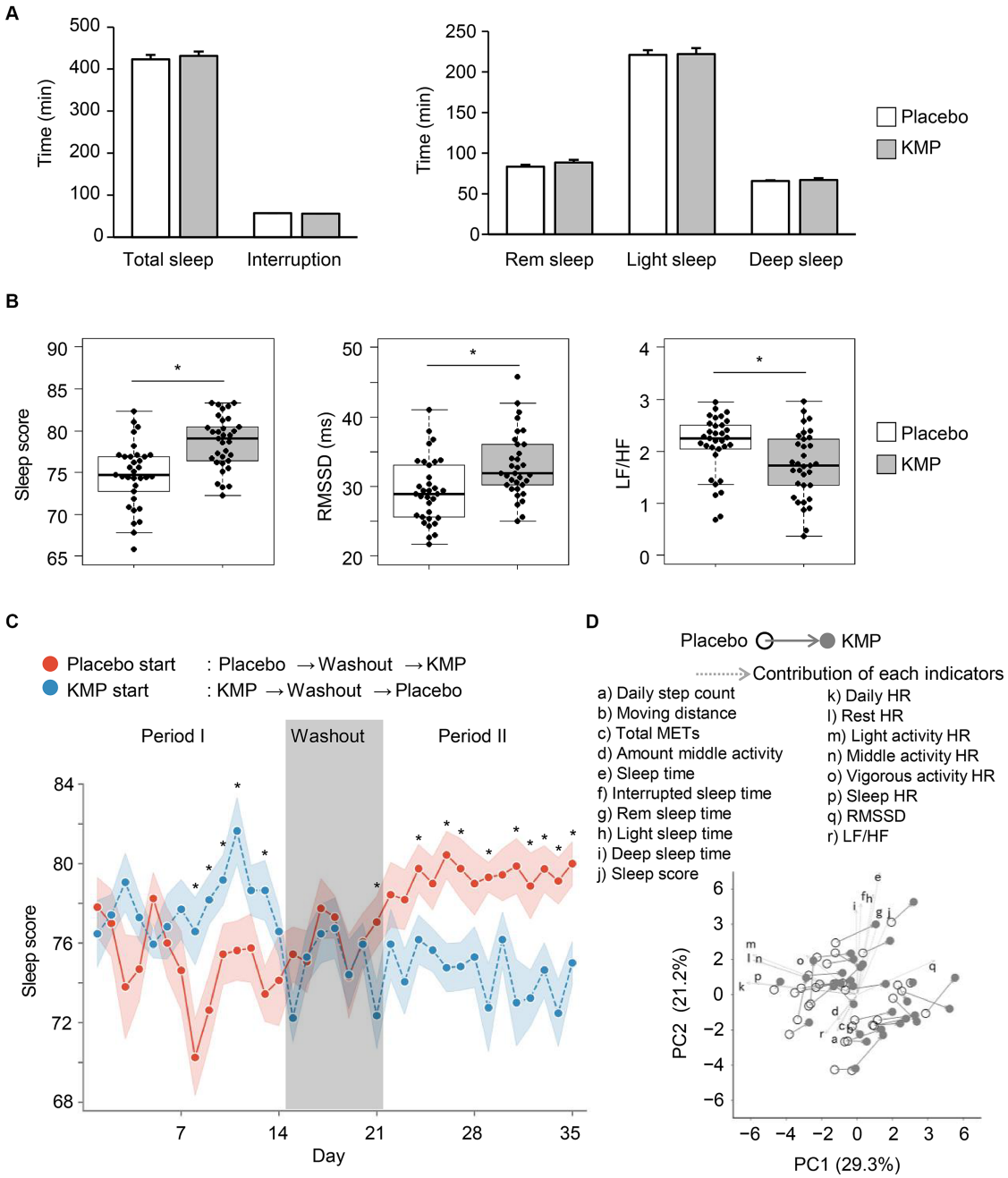

FIGURE 4
Effects of KMP intake on sleep and regulation of autonomic nervous system. **(A)** Mean time of each sleep stage. Error bars indicate standard error. **(B)** Sleep-related indicators. Data are presented as box plots (median ± interquartile range), and dots represent the data for each participant. *$p < 0.05$ KMP vs. placebo, mixed model for repeated measures for crossover design. **(C)** Transition of sleep scores throughout the trial period. Circle indicates the mean value, and ribbon represents the range of standard error. *$p < 0.05$ placebo start group vs. KMP start group, *t*-test. **(D)** PCA using the dataset of the placebo group and KMP group of each participant. Solid arrow indicates a change from placebo to KMP, and dotted arrows represent the contribution of each indicator.

likely to be directly involved in the mechanism that improved the PA levels. Therefore, enhancing the bioavailability of KMP could lead to an increase in daily PA levels.

While the sleep score and RMSSD in the KMP group were significantly higher than those in the placebo group, there was no correlation between Δsleep score and ΔRMSSD and absorbability of KMP, suggesting that the effects of KMP on sleep score and RMSSD could be exerted "regardless of the level of absorbability," or through a "pathway driven by low blood concentration," or "indirectly such as through metabolically converted components," or through a "change in PA levels." The Δdaily step count correlated positively with Δdeep-sleep time. KMP intake did not affect the average sleep time of the whole group but affected the deep-sleep time in individuals who showed changes in PA levels, i.e., those with high absorbability of KMP. These factors show a complex relationship and are presumed to affect sleep quality. Interestingly, ΔRMSSD, which

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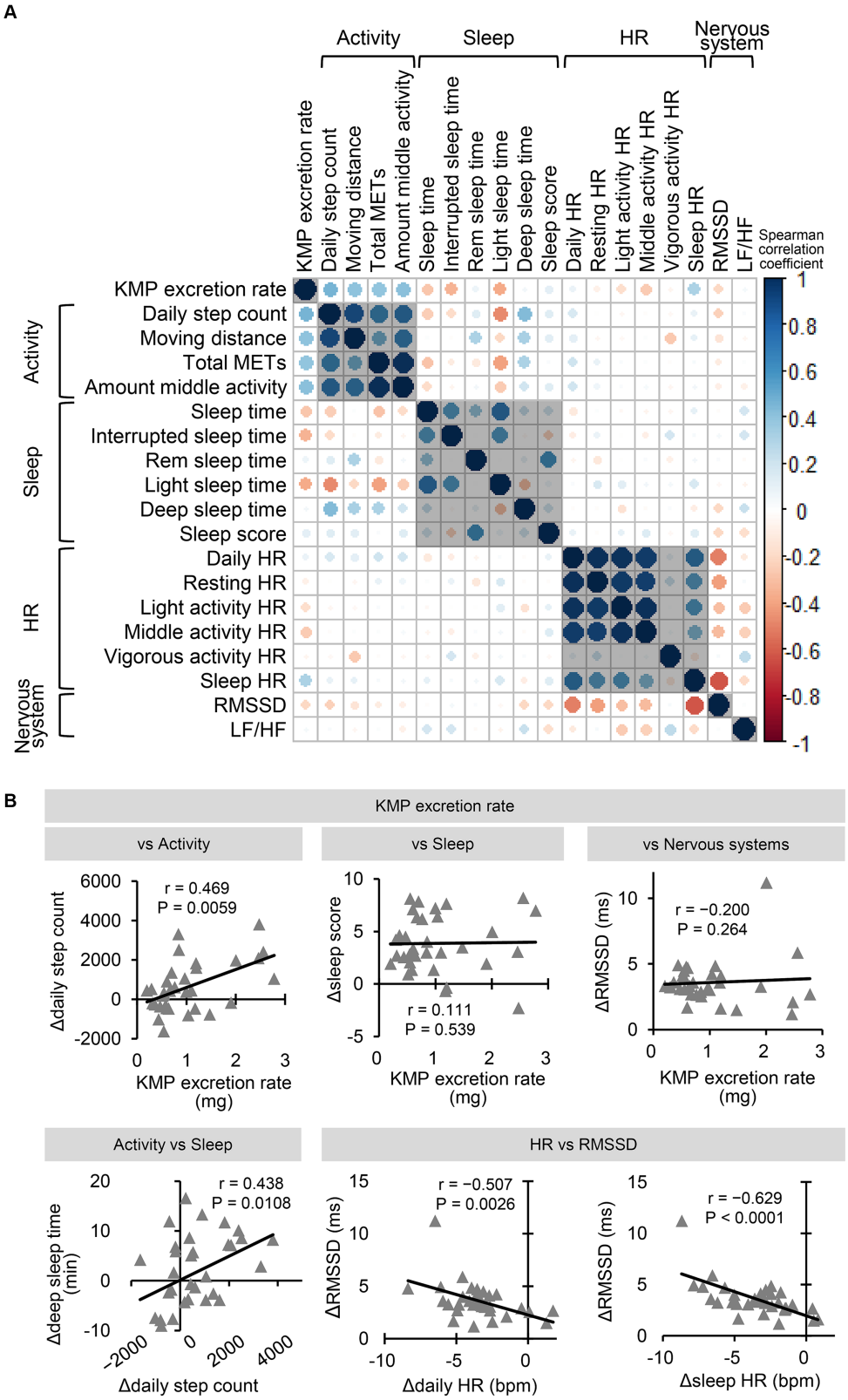

FIGURE 5
Correlation between changing indicators due to KMP intake. **(A)** Correlation of change amount of each indicator (Δ = KMP − Placebo). The color and size of the circle represent the value of the Spearman correlation coefficient. **(B)** Correlation relationship between change in the levels of the two indicators. Solid line represents the approximation curve.

characterizes the change from placebo to KMP, showed a sufficient correlation with ΔHR, suggesting the importance of the relationship between these two indicators. In the correlational analysis combining data from the placebo and KMP groups, a strong inverse correlation was found between RMSSD and HR, suggesting that the decrease in HR and increase in RMSSD are core factors underlying the effects of KMP.

4.4 Potential for KMP to solve health problems caused by hypoxia

Hypoxia is a lethal risk to the survival of living organisms and causes energy deficiency. To address hypoxia, the body increases respiratory rate and HR, along with pulmonary artery constriction and HIF activation (55). Among high-altitude residents, some develop chronic mountain sickness or pulmonary hypertension (56, 57) but most are known to adapt to hypoxic environments (58). Their daily diet may underlie adaptation mechanisms.

Environmental stresses, including ultraviolet exposure, increase the content of KMP in plants (59). Plants at high altitudes contain more KMP than those at low altitudes (60), suggesting that the daily intake of KMP-containing plants by high-altitude residents may contribute to their adaptation to the hypoxic environment. Our basic experiments revealed that KMP suppresses HIF-1α stabilization under hypoxia conditions and increases oxygen utilization efficiency in cells with reduced oxygen supply (27).

Flavonoids, including KMPs, generally found in plants, exist as glycosides, and they might not be absorbed even when ingested. The test food we used in this study is easily absorbable, having converted KMP glycoside into aglycone using a unique method. A positive correlation was observed between ΔPA levels and the absorbability of KMP, indicating that providing food containing easily absorbable KMP is important for increasing PA levels and, ultimately, promoting health.

Nagano Prefecture, the site of our study, has a large population of long-lived individuals and an average altitude of over 1,000 m. During the placebo intake period, participants' mean number of steps per day exceeded 10,000. These participants may contribute to their daily PA and enhance oxygen utilization efficiency by regularly consuming vegetables cultivated at high altitudes.

4.5 Limitations and future directions

This study has some limitations that must be addressed. First, the city workers who participated in this study were a group without exercise habits but achieved an average of 10,000 steps per day. The effects of KMP in people with average or below-average exercise habits need further investigation to confirm these results in individuals with low PA levels. Second, this study investigated whether PA changes occurred only when KMP was ingested, without limiting daily activities. Although PA levels increased, future studies need to confirm that this was not correlated with the ingestion of other supplements that may increase PA. Third, subjective assessments may be more important than objective assessments for sleep (48, 49). Polysomnography is commonly used to simultaneously measure electromyography and electro-oculography results (61), and there may have been limitations in these measurements using a simple wearable device. Future studies need to investigate whether KMP ingestion improves objective and subjective sleep indices while elucidating the underlying mechanism of action. Finally, there was a significant correlation between HR and RMSSD, and is a core relationship mediating the effect of KMP. KMP may exert its effects through a direct mechanism in PA or secondary metabolites or indirect mechanisms involving sleep quality. Therefore, while increasing the absorption of KMP is crucial for enhancing PA levels, the improvement in sleep quality is not related to the amount of KMP absorbed, suggesting that it can benefit everyone. At this point, we interpret that the improvement in sleep quality was owing to the increase in PA. In the future, clarifying the relationship between KMP intake and sleep quality will be necessary to better understand the phenomena occurring throughout the body.

There is a positive correlation between VO_{2max} and frontal lobe function (57), as well as mental health that controls emotions (41). Our findings suggested that HR and RMSSD values are involved. Therefore, fluctuations in the oxygen supply-energy supply-HR axis may be important for performance during activity and regulating the autonomic nervous system at rest. KMP affected these control systems but the causal relationship and molecular mechanism were not examined and need to be clarified in future studies.

5 Conclusion

The daily intake of KMP leads to behavioral changes, including an increase in PA and the number of outings on weekends, which subsequently improve sleep quality. Furthermore, we have elucidated the relationship between PA and sleep. The associated decrease in HR and regulation of the autonomic nervous system may have played important roles in these changes. Generally, the effects of food are not as potent as those of pharmaceuticals; however, KMP could potentially be used as a food ingredient that can help maintain the quality of life for people living in the era of a 100-year lifespan, considering that it can be ingested daily. Future research should focus on elucidating the detailed molecular mechanisms of KMP, and its potential contribution to the lives of people with diverse backgrounds.

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 Otsuka Pharmaceutical Co., Ltd. (approval no. 2003, dated October 30, 2020). 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

YI: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. AG-K: Formal analysis, Visualization, Writing – review & editing. SO: Investigation, Methodology, Validation, Writing – review & editing. SH: Investigation, Methodology, Validation, Writing – review & editing. TS: Investigation, Methodology, Validation, Writing – review & editing. TM: Data curation, Investigation, Methodology, Validation, Writing – review & editing. BS: Supervision, Writing – review & editing.

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Conflict of interest

YI, AG-K and TM were employed by Otsuka Pharmaceutical Co. Ltd.

The remaining 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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1386389/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Contribution degree of each indicator in the changes owing to KMP intake. The similarity of the vector direction from placebo to KMP with each indicator's vector was calculated. Cosine similarity is shown in the box plots (median ± interquartile range), and dots represent the data for each participant.

SUPPLEMENTARY FIGURE S2
Correlation relationship between each indicator. **(A)** Correlation of each indicator throughout placebo and KMP periods. The color and size of the circle represent the value of the Spearman correlation coefficient. **(B)** Correlation relationship between two indicators. Solid line represents the approximation curve.

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Association between changes in lean mass, muscle strength, endurance, and power following resistance or concurrent training with differing high protein diets in resistance-trained young males

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Background: We assessed the relationship of changes in upper and lower body lean mass with muscle strength, endurance and power responses following two high protein diets (1.6 or 3.2 $g \cdot kg^{-1} \cdot d^{-1}$) during 16 weeks of either concurrent training (CT) or resistance training (RT) in resistance-trained young males.

Methods: Forty-eight resistance-trained young males (age: 26 ± 6 yr., body mass index: 25.6 ± 2.9 $kg.m^{-2}$) performed 16 weeks (four sessions·$wk.^{-1}$) of CT or RT with either 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ protein (CT + 1.6; $n = 12$; RT + 1.6; $n = 12$) or 3.2 $g \cdot kg^{-1} \cdot d^{-1}$ protein (CT + 3.2; $n = 12$; RT + 3.2; $n = 12$). Relationships between upper (left arm + right arm + trunk lean mass) and lower body (left leg + right leg lean mass) lean mass changes with changes in muscle performance were assessed using Pearson's correlation coefficients.

Results: For upper body, non-significant weak positive relationships were observed between change in upper body lean mass and change in pull-up ($r = 0.183$, $p = 0.234$), absolute chest press strength ($r = 0.159$, $p = 0.302$), chest press endurance ($r = 0.041$, $p = 0.792$), and relative chest press strength ($r = 0.097$, $p = 0.529$) while non-significant weak negative relationships were observed for changes in absolute upper body power ($r = -0.236$, $p = 0.123$) and relative upper body power ($r = -0.203$, $p = 0.185$). For lower body, non-significant weak positive relationships were observed between the change in lower body lean mass with change in vertical jump ($r = 0.145$, $p = 0.346$), absolute lower body power ($r = 0.109$, $p = 0.480$), absolute leg press strength ($r = 0.073$, $p = 0.638$), leg press endurance ($r < 0.001$, $p = 0.998$), relative leg press strength ($r = 0.089$, $p = 0.564$), and relative lower body power ($r = 0.150$, $p = 0.332$).

Conclusion: Changes in muscle strength, endurance and power adaptation responses following 16 weeks of either CT or RT with different high protein

intakes were not associated with changes in lean mass in resistance-trained young males. These findings indicate that muscle hypertrophy has a small, or negligible, contributory role in promoting functional adaptations with RT or CT, at least over a 16-week period.

KEYWORDS

exercise training, body composition, nutrition, nutritional supplements, muscular adaptations

Introduction

Resistance training (RT) is considered the most effective approach to promote anabolic-related adaptations, including increases in muscle strength, and power in trained adults (1). On the other hand, endurance training (ET) may lead to improvements in VO_{2max}, resulting in enhanced cardiovascular health and function, and an increase in skeletal muscle oxidative capacity (2). Considering the distinct training adaptations of ET and RT, which can be influenced by various factors including training type, intensity, and volume, integrating both modalities into a cohesive training regimen to maximize concurrent anabolic, metabolic, and oxidative adaptation responses is often required for exercise/sport performance and overall health and wellbeing. Concurrent training (CT) involves the integration of RT and ET into a unified training protocol (3) and has been shown to augment muscle strength, anaerobic power, aerobic capacity, and maximum velocity contractions (4–8). Despite the favorable adaptations observed with CT, some studies have demonstrated diminished enhancements in muscle strength, power, and hypertrophy when compared to RT conducted independently, which is often referred to as the "interference effect" (9–14). The literature presents contradictory findings about dampened anabolic training responses within this paradigm, which may be influenced by factors such as participants' training experience, the order in which training sessions are conducted, and the specific forms of exercise implemented (15–18).

Various proposed methods for overcoming the interference effect have focused on implementing longer periods of recovery (i.e., 6–24 h) between training sessions, substituting cycling for running as ET, and integrating post-exercise dietary strategies (19). Various studies have shown that protein ingestion, either via food or supplements, in combination with RT, leads to improved muscle adaptations such as increased strength, lean mass, or power (1, 20–27). Greater gains in lean mass (3.2 vs. 2.2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) have been reported with higher protein intake (~2.2–2.4 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) compared to ~1.0 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ following three, but not six, months of CT in recreationally trained males (28). This finding suggests higher daily protein availability can significantly impact adaptation responses with CT. Moreover, results of a recent systematic analysis support the use of protein supplementation to improve the growth of skeletal muscle mass and increase strength and power when combined with CT. Specifically, consuming approximately 0.49 g/kg after exercise is believed to optimize the rate of myofibrillar protein synthesis in this particular context (29). Morton and colleagues conducted a systematic review and meta-analysis, which resulted in a recommendation. A protein intake of 1.6 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ was enough to provide the maximum increase in fat-free mass (FFM) after RT (26). On the other hand, others have suggested that trained individuals should consume about 2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ to meet their daily requirements (20). It is important to note that these recommended daily protein amounts are derived only from research that includes RT. Due to the larger training volumes associated with CT compared to single-mode exercise training, it is probable that the necessary dietary protein intake is greater for CT (3). Regardless, increasing muscle/lean mass is highly important for promoting physical performance and health among all populations (30) with the quantity of lean mass shown to be associated with performance indicators, including aerobic and anaerobic capacities (31–34). Thus, dietary strategies that can help facilitate increases in muscle mass, strength and functional adaptations with CT represents a highly important consideration for individuals undertaking this form of exercise training.

There is a paucity of information regarding whether potential associations between changes in muscle mass and strength exist with CT. Moreover, much less is known about the correlation between increases in lean mass resulting from RT or CT following high-protein diets and muscle performance in resistance-trained males. Dietary protein supplementation has been shown to significantly enhance changes in muscle strength and size with RT in healthy adults (26) and thus represents an important variable in maximizing skeletal muscle adaptation responses to exercise training. We have previously reported that RT and CT in combination with higher-protein diets (i.e., 1.6 [1.6] or 3.2 [3.2] $g.kg^{-1}.d^{-1}$) similarly improved lean mass and selected muscle performance measures (35). In light of this, the current study aimed to explore the relationship between changes in lean mass and muscle strength and power following 16 weeks of RT and CT in conjunction with consumption of with 1.6 or 3.2 $g.kg^{-1}.d^{-1}$ of protein in resistance-trained young males. It was primarily hypothesized that post-intervention increases in muscle growth responses will correlate with changes in muscle strength. Additionally, as there were no differences between the magnitude of gains in lean mass among RT and CT groups in our previous study findings, it was also hypothesized that there will no differences between RT and CT groups regarding the association between lean mass and muscle strength, endurance, and power adaptations.

Methods

Participants

The current investigation recruited 48 resistance-trained young males. These individuals were between the ages of 18 and 36 and were

recruited via the use of advertising on various social media platforms. The research and testing protocols were communicated to interested participants via telephone or in-person sessions held at nearby fitness facilities. Participants were instructed to complete a health and fitness history questionnaire, providing information about their previous training background, specifically engaging in three sessions per week for a minimum of 1 year of RT experience (with three to four sessions per week). Additionally, participants were required to report sleeping for a duration of seven to 8 h within a 24-h day, abstaining from the use of steroids or any illegal substances known to enhance muscle size for the past year, consuming less than ~1.6 g·kg^{-1}·d^{-1}, and being free from any musculoskeletal disorders. Participants who met the aforementioned criteria supplied a written and verbal agreement to participate in the study. Furthermore, as part of the permitting process, participants were provided with a medical history questionnaire and revisited the research site to complete the study procedures. The procedure underwent a thorough evaluation by the Institutional Human Subject Committee and the Ethics Committee of the University of Isfahan (IR.UI.REC.1400.098) and was conducted strictly adhering to the principles outlined in the Declaration of Helsinki. The present research has been duly filed with the Iranian Registry of Clinical Trials with the registration number IRCT20191204045612N2.

Study design

Following the collection of baseline measures, as detailed in the following section, participants were familiarized with the research tests and procedures. Subsequently, they were randomized to one of four groups using the use of an online resource:[1] CT + 1.6 g protein·kg^{-1}·d^{-1} (CT + 1.6; $n = 12$), CT + 3.2 g protein·kg^{-1}·d^{-1} of protein (CT + 3.2; $n = 12$), RT + 1.6 g protein·kg^{-1}·d^{-1} (RT + 1.6; $n = 12$) or RT + 3.2 g protein·kg^{-1}·d^{-1} (RT + 3.2; $n = 12$). The initial planned duration of this research was 6 months; however, in response to the global COVID-19 pandemic, we made a voluntary decision to conclude the study after 16 weeks. Consequently, data were gathered at the first assessment and the 18th week, specifically 2 weeks after the implementation of the training intervention, at the same time of day, with a time difference of ±1 h. After completing these assessments, study participants engaged in an initial consultation with the research's dietician. This meeting served as an opportunity to discuss their individual dietary preferences and establish specific protein and calorie intake goals in preparation for the commencement of their respective training programs. To assess sleep quality and health status, the Pittsburgh Sleep Quality Index (PSQI) and the General Health Questionnaire-28 (GHQ-28) were used, respectively (36).

Anthropometry and body composition

Participants were asked to report to the laboratory after an overnight fast, with a 24-h dietary recall collected before testing. Participants were instructed to void completely within 30 min of the test to minimize hydration status errors and advised to refrain from caffeinated beverages, alcohol, and other diuretics 12 h before measurements. Participants' body mass and height were measured with a digital scale (Lumbar, China) to the nearest 0.1 kg and a stadiometer (Race industrialization, China) to the nearest 0.1 cm. Total lean mass was assessed using whole-body dual-energy x-ray absorptiometry (DXA; Hologic, Discovery, Wi [S/N 93045 M]). For DXA measurements, previous test re-test reliability in our laboratory are as follows: BFP intraclass correlation coefficient (ICC) = 0.998; coefficient of variation (CV): <1%; lean mass: ICC = 1.00; CV: <1%. All DXA scans were conducted as described previously (37). In the present study, we assessed the relationships between upper (left arm + right arm + trunk lean mass) and lower body (left leg + right leg lean mass) lean mass and muscle performance changes.

Resistance training

The participants in the two RT groups engaged in a structured exercise regimen consisting of four weekly sessions as described previously. Prior to each RT session, participants performed 10 min of general (5 min slow running on a treadmill; 3–5 km speed, or elliptical; with 5–10 level) and specific warm-up activities (5 min, e.g., medicine ball twist 1 × 10, medicine ball wood chops 1 × 10, straddled toe touch 2 × 5, dynamic quadriceps stretch 1 × 5, medicine ball squat 1 × 5–8). Participants then completed an upper-body RT program consisting of seven exercises (chest press, lateral pulldown, standing barbell shoulder press, standing shoulder shrugs, bicep curl, triceps press down, and abdominal crunch) 2x/wk. and six exercises of lower-body RT program (45-degree leg press, back squats, seated leg curl, Barbell hip thrusts, back extension, and calf raises) performed for 2x/wk. Participants performed 3 sets of 12 repetitions with 75% of 1-RM for weeks 1–4, 3 sets of 10 repetitions with 80% of 1-RM for weeks 5–8, 4 sets of 8 repetitions with 85% of 1-RM for weeks 9–12, and 4 sets of 6 repetitions with 90% of 1-RM for weeks 13–16. Rest intervals between exercises and sets lasted no longer than 2 min (38). The periodized RT program was based on our previous work (38) and following recommendations by the National Strength and Conditioning Association (39). Verbal encouragement and comments were provided to the participants both during and after each set. The training data for each participant was recorded, ensuring that the training intensity was optimized throughout each session and that participants effectively adopted progressive overload in a personalized manner. In addition, study personnel supervised all training throughout the study. A detailed outline can be found in Supplementary Table 1.

Concurrent training

The participants in both CT groups engaged in a total of four sessions each week, namely on Saturday, Monday, Wednesday, and Thursday. Each session consisted of RT done at the start, followed by ET, as per the prescribed exercise order sequence (40) to minimize possible interferences in muscle anabolism. Prior to each CT session, participants performed 10 min of general (5 min slow running on a treadmill; 3–5 km speed, or elliptical; with 5–10 level) and specific warm-up activities (5 min, e.g., medicine ball twist 1 × 10, medicine ball wood chops 1 × 10, straddled toe touch 2 × 5, dynamic

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quadriceps stretch 1 × 5, medicine ball squat 1 × 5–8). The participants then engaged in the same RT program as previously stated. Immediately following the completion of RT, participants then performed endurance cycle training on ergometers that consisted of a mixture of hill simulation rides of varying intensities (25–110 of maximum aerobic power [MAP]), moderate-intensity continuous training at 50% MAP, moderate-intensity continuous training (MICT) at 70% MAP, and high-intensity interval training (HIIT) at 100% MAP. Moderate-intensity intervals were separated by a 60-s recovery period at ~40% MAP to establish a 2.5:1 or 5:1 work-to-rest ratio. High-intensity intervals were separated by 20- to 60-s recovery periods, completed at ~40% MAP, to establish a 1:5, 1:2, or 1:1 work-to-rest ratio. All cycling sessions were preceded by 3–5 min of cycling at ≤50 W. Progressive overload was applied by manipulating the number of intervals and relative intensity of load throughout. A detailed outline can be found in Supplementary Tables 2A,B.

Training volume

RT volume was calculated using the following formula in each session and was reported weekly (41):

1- RT volume = [repetitions (n) × sets (n) × load or selected weight (kg)].
2- ET volume was calculated using the following formula: Total ET volume: [work + rest].
3- Work: [Intensity × maximum aerobic power (MAP) × (set × duration [as noted in training protocol] × 0.06)].
4- Rest: [Intensity × MAP × (set × duration [as noted in training protocol] × 0.06)].
5- Intensity: percent of MAP; Set: number of repetitions of each session; Duration: spent time (minutes); 0.06: Convert watts to kilojoules.

Maximal strength testing

Maximal strength was determined (first upper and then lower limb) using 1-RM for chest press and plate-loaded leg press in the morning. This testing (1-RM) also was performed to determine training intensity for RT protocols. Participants performed a general 10 min warm-up (5 min slow running on a treadmill; 3–5 km speed, or elliptical; with 5–10 level) and specific warm-up activities (5 min, e.g., medicine ball twist 1 × 10, medicine ball wood chops 1 × 10, straddled toe touch 2 × 5, dynamic quadriceps stretch 1 × 5, medicine ball squat 1 × 5–8) before the test. The participants then performed two attempts, recording their highest lifted weight and number of repetitions. The number of repetitions to fatigue did not exceed 10. Participants were allowed 3 to 5 min rest periods between attempts, and there was no arousing stimulus during testing. After the testing session, participant's maximal strength was predicted using the formula: 1-RM = weight / (1.0278–0.0278 × reps) (19). Chest and leg press exercises were used as upper and lower body strength measures, and 1-RM was used to determine individualized RT prescription.

Muscle endurance

The participants rested for 5 min after the 1-RM testing prior to completing the muscle endurance test (first upper and then lower limb) in the morning (9:00–10 a.m.). Participants were instructed to perform leg- and chest press exercises at 75% of the 1-RM to test muscle endurance, denoted as the number of successful repetitions completed prior to technical failure (37).

Muscle power

Upper- and lower-body anaerobic power was assessed via Monark Wingate cycle ergometry (Monark model 894e, Vansbro, Sweden) as previously described in the evening (8, 42). Briefly, participants were acquainted with the test and instructed to stay seated in the saddle for the test duration. Participants cycled or cranked against a pre-determined resistance (7.5% of the body mass for the lower body test and 5.5% for the upper body test) as fast as possible for 30 s. Participants were verbally encouraged to pedal as hard and fast as possible throughout the whole 30-s test. There was a time gap of roughly 1 h between the upper and lower tests, with the upper test being conducted first. Therefore, we can be certain that fatigue did not impact the performance of the alternative limb. Peak power output was documented in real-time during the test using Monark Anaerobic test software (3.3.0.0).

Muscle performance and power testing

Maximal vertical jump height and total pull-ups (1 set) were assessed. Each participant generally performed the following warm-up: a 5-min run/bike on a treadmill or cycle ergometer at a self-directed leisurely pace followed by a dynamic warm-up consisting of 10 yards each of high knees, butt kicks, side shuffles, and karaoke running drill, and finally 10 pushups and 10 bodyweight squats. Participants then rested for 2–3 min before commencing the muscle strength and power tests. Subsequently, the following tests were performed in the order given: vertical jump—highest value with a maximum number of three attempts; pull-ups—highest repetitions with a maximum number of three attempts. For both tests, there was a rest interval of approximately 60–180 s.

Diet

The study participants were instructed to record their food intake for a total of six consecutive 24-h periods. These periods included four weekdays that were not consecutive and two non-consecutive weekend days. The purpose of this data collection was to assess the participants' typical protein intake patterns. To assist in achieving their targeted protein intake (i.e., 1.6 or 3.2 $g \cdot kg^{-1} \cdot d^{-1}$), participants consumed a 40 g of isolated whey protein (Wisser nutrition, Iran) beverage upon cessation of every training session that comprised the following nutrition profile per scoop (28 g): calories,110; total fat, 0.5 g; saturated and trans-fat, sugars and dietary fiber, 0 g; sodium, 50 mg; potassium, 112 mg; total carbohydrate, 2 g; protein, 24 g. The remaining amounts of protein were received from dietary sources, and the habitual intake of protein remained consistent across all

groups during the intervention. The decision to include the protein group with a daily intake of 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ was based on findings by Morton et al. (26) that reported this quantity of protein intake can optimize improvements in fat-free mass after RT (26). To create a clear disparity between dietary protein interventions, we chose to double the 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ amount for the comparison high protein group (i.e., 3.2 $g \cdot kg^{-1} \cdot d^{-1}$) while also ensuring this amount can be safely tolerated. In support, previous work by Antonio and colleagues demonstrated this amount (~2.51–3.32 $g \cdot kg^{-1} \cdot d^{-1}$) to exert no harmful effects on liver and kidney function markers (43).

The participants engaged in regular consultations with a certified dietitian every fortnight. During these consultations, they received instructions on how to meet their protein and energy requirements. Specifically, they were advised to distribute their protein intake throughout the day across 4–7 meals, with each meal containing 20–40 g of protein. This approach aimed to optimize muscle protein synthesis (MPS) (44, 45). The research included monitoring the macronutrient composition, with particular emphasis on total energy intake (TEI) and protein intake. It has been recommended that individuals maintain their carbohydrate and fat intake within the Acceptable Macronutrient Distribution Range, which suggests a range of 45–65% of total energy intake for carbohydrates and 20–35% of total energy intake for fats. The participants were instructed to maintain a state of positive energy balance to mitigate any possible disruptions to anabolic adaptations caused by energetic stress (46, 47). Food records were kept daily by participants throughout the study using mobile phone applications Easy Diet Diary (Xyris Software Pty Ltd., AUS, for those with iPhones, Apple Inc., United States; $n = 18$) and My Fitness Pal (MyFitnessPal Inc., United States) for those with Android-based devices; $n = 26$. All dietary intake data were analyzed using (Diet Analysis Plus, version 10; Cengage) to ensure the same food database was used for all analyses.

Statistical analysis

As this study represents a secondary analysis to the original primary aim of comparing muscle effects of different training and protein supplementation protocols (35), no specific sample size calculations were performed for the current work. Nevertheless, prior research on the topic utilized sample sizes that were approximately comparable to the sample size used in this current study (33, 34). The normality of the distribution of all variables was evaluated before performing statistical analyses using the Shapiro–Wilk test; there were no missing values at any time point. Baseline characteristics (at PRE) between groups were reported using mean (SD). Effects of training and nutritional interventions on dependent variables were analyzed using a two × four analysis of variance (ANOVA) with repeated measures (time [pre-test vs. post-test] × group [CT + 1.6 vs. CT + 3.2 vs. RT + 1.6 vs. RT + 3.2]) to determine the differences between the treatments over time. When the group-by-time interaction was significant, we used Sidak *post-hoc* analysis to determine between-group differences. Pearson's simple linear regressions were performed with a 95% confidence interval (CI) as well as Pearson correlation coefficients. Values between 0 and 0.3 (0 and −0.3) indicate a weak positive (negative) linear relationship through a shaky linear rule. Values between 0.3 and 0.7 (−0.3 and −0.7) indicate a moderate positive (negative). Values between 0.7 and 1.0 (−0.7 and −1.0) indicate a strong positive (negative) (48). Figures with only one curve indicates that it adequately fits all the data sets. All analyses and figure production were performed using GraphPad Prism (version 8.4.3).

Results

Participant characteristics

A total of 112 individuals underwent assessment to determine their eligibility. Twenty-eight of them failed to satisfy the established criteria for inclusion, while 36 individuals expressed a lack of interest in participating after the first interview (Supplementary Figure 1). One participant from each group withdrew from the research, citing reasons such as scheduling constraints, lack of interest, COVID-19, or musculoskeletal injury. There were no statistically significant differences seen between the groups in terms of baseline characteristics (Table 1).

Lean mass

All four intervention groups demonstrated significant increases in upper [CT + 1.6 = 1.15 kg (95% CI = 0.34 to 1.96; $p = 0.0026$), CT + 3.2 = 1.26 kg (95% CI = 0.45 to 2; $p = 0.0009$), RT + 1.6 = 1.2 kg (95% CI = 0.39 to 2; $p = 0.0015$)], and RT + 3.2 = 1 kg (95% CI = 0.20 to 1.82; $p = 0.0088$) and lower body lean mass [CT + 1.6 = 0.68 kg (95% CI = 0.11 to 1.25; $p = 0.0120$), CT + 3.2 = 0.66 kg (95% CI = 0.09 to 1.22; $p = 0.0019$), RT + 1.6 = 0.82 kg (95% CI = 0.25 to 1.39; $p = 0.0019$)], and RT + 3.2 = 1 kg (95% CI = 0.52 to 1.65; $p < 0.0001$) from baseline to post-test with no differences between groups ($p > 0.05$).

Muscle strength

All four intervention groups had significant increases in absolute chest press strength [CT + 1.6 = 10.09 kg (95% CI = 6.77 to 13.41; $p < 0.0001$), CT + 3.2 = 10.36 kg (95% CI = 7.048 to 13.68; $p < 0.0001$), RT + 1.6 = 12.55 kg (95% CI = 9.23 to 15.86; $p < 0.0001$)], and RT + 3.2 = 12.91 kg (95% CI = 9.59 to 16.22; $p < 0.0001$), relative chest press strength [CT + 1.6 = 0.10 kg. kg BM^{-1} (95% CI = 0.052 to 0.162; $p < 0.0001$), CT + 3.2 = 0.11 kg. kg BM^{-1} (95% CI = 0.06 to 0.17; $p < 0.0001$), RT + 1.6 = 0.12 kg. kg BM^{-1} (95% CI = 0.06 to 0.17; $p < 0.0001$)], and RT + 3.2 = 0.13 kg. kg BM^{-1} (95% CI = 0.07 to 0.18; $p < 0.0001$), absolute leg press strength [CT + 1.6 = 72.64 kg (95% CI = 44.07 to 101.2; $p < 0.0001$), CT + 3.2 = 74.82 kg (95% CI = 46.26 to 103.4; $p < 0.0001$), RT + 1.6 = 82.36 kg (95% CI = 53.80 to 110.9; $p < 0.0001$)], and RT + 3.2 = 76.82 kg (95% CI = 48.26 to 105.4; $p < 0.0001$), and relative leg press strength [CT + 1.6 = 0.81 kg. kg BM^{-1} (95% CI = 0.47 to 1.15; $p < 0.0001$)], CT + 3.2 = 0.89 kg. kg BM^{-1} (95% CI = 0.55 to 1.23; $p < 0.0001$), RT + 1.6 = 0.87 kg. kg BM^{-1} (95% CI = 0.53 to 1.21; $p < 0.0001$), and RT + 3.2 = 0.78 kg. kg BM^{-1} (95% CI = 0.44 to 1.11; $p < 0.0001$) from baseline to post-test with no differences between groups ($p > 0.05$).

Muscle endurance

There was no significant change from baseline to post-test for chest and leg press endurance ($p > 0.05$).

TABLE 1 Baseline characteristics of the participants.

	CT + 1.6	CT + 3.2	RT + 1.6	RT + 3.2
Measure				
Anthropometry, body composition, and training experience				
Age (y)	27 ± 6	25 ± 7	26 ± 6	28 ± 5
Height (cm)	178 ± 5	179 ± 8	180 ± 7	182 ± 6
Body mass (kg)	83.8 ± 10.6	81.6 ± 10.7	82.1 ± 9.1	85.2 ± 10.9
BMI ($kg.m^{-2}$)	26.3 ± 3.4	25.2 ± 3.1	25.1 ± 2.3	25.7 ± 2.9
Training experience (yr)	3.7 ± 2.2	4.6 ± 2.6	3.5 ± 1.7	4.8 ± 2.4
Upper body lean mass (kg)	36.3 ± 4.1	35.8 ± 4	35.7 ± 3.9	39.6 ± 7.3
Lower body lean mass (kg)	20 ± 2.4	20.3 ± 1.9	20.6 ± 2.9	21.6 ± 4.1
Muscle strength, power and endurance				
Absolute chest press strength (kg)	97 ± 22	101 ± 19	98 ± 18	108 ± 16
Relative chest press strength (kg. kg BM^{-1})	1.16 ± 0.24	1.25 ± 0.32	1.22 ± 0.31	1.28 ± 0.22
Chest press endurance (rep)	12 ± 2	13 ± 2	11 ± 2	12 ± 2
Absolute leg press strength (kg)	412 ± 77	388 ± 74	390 ± 71	408.7 ± 69.5
Relative leg press strength (kg. kg BM^{-1})	4.97 ± 1.12	4.85 ± 1.23	4.83 ± 1.17	4.87 ± 1.08
Lower body endurance (r)	14.1 ± 2.8	15.3 ± 3.3	14.8 ± 3.1	15.2 ± 2.2
Absolute upper body power (W)	496 ± 62.4	505 ± 92.6	456.9 ± 61.3	509 ± 70.9
Relative upper body power (W. kg BM^{-1})	5.63 ± 0.80	6.32 ± 1.66	5.63 ± 1.07	6.05 ± 1.10
Absolute lower body power (W)	696.2 ± 91.8	738.8 ± 83	695.8 ± 53	752.1 ± 68.8
Relative lower body power (W. kg BM^{-1})	8.38 ± 1.36	9.21 ± 1.81	8.60 ± 1.40	8.96 ± 1.44
Vertical jump (cm)	50.6 ± 4.5	47.8 ± 8.2	43 ± 6.9	44.9 ± 7.1
Pull-up (rep)	11.3 ± 2.8	13.2 ± 3	12.7 ± 2	14.3 ± 4.3

Values are presented as mean ± standard deviation. BMI, body mass index; VO_{2max}, maximum rate of oxygen consumption; y, year; cm, centimeter; kg, kilogram; $kg.m^{-2}$, kilogram-meter $^{-2}$; g, gram; rep, repetition; CT + 1.6, concurrent training + 1.6 $g\,kg^{-1}.d^{-1}$; CT + 3.2, concurrent training + 3.2 $g\,kg^{-1}.d^{-1}$; RT + 1.6, resistance training + 1.6 $g\,kg^{-1}.d^{-1}$; RT + 3.2, resistance training + 3.2 $g\,kg^{-1}.d^{-1}$.

Muscle power

All four intervention groups noted significant increases in absolute upper body power CT + 1.6 = 29.36 w (95% CI = 18.44 to 40.29; $p < 0.0001$), CT + 3.2 = 31.91 w (95% CI = 20.98 to 42.84; $p < 0.0001$), RT + 1.6 = 44.18 w (95% CI = 33.25 to 55.11; $p < 0.0001$), and RT + 3.2 = 49.45 w (95% CI = 38.53 to 60.38; $p < 0.0001$), absolute lower body power CT + 1.6 = 35.82 w (95% CI = 22.27 to 49.37; $p < 0.0001$), CT + 3.2 = 41.91 w (95% CI = 28.36 to 55.46; $p < 0.0001$), RT + 1.6 = 69 w (95% CI = 55.45 to 82.55; $p < 0.0001$), and RT + 3.2 = 65.64 w (95% CI = 52.08 to 79.19; $p < 0.0001$), relative upper body power CT + 1.6 = 0.32 kg. kg BM^{-1} (95% CI = 0.10 to 0.54; $p = 0.0013$), CT + 3.2 = 0.41 kg. kg BM^{-1} (95% CI = 0.19 to 0.62; $p = 0.0081$), RT + 1.6 = 0.41 kg. kg BM^{-1} (95% CI = 0.19 to 0.62; $p < 0.0001$), and RT + 3.2 = 0.46 kg. kg BM^{-1} (95% CI = 0.24 to 0.67; $p < 0.0001$), and relative lower body power CT + 1.6 = 0.33 kg. kg BM^{-1} (95% CI = 0.09 to 0.56; $p = 0.0030$), CT + 3.2 = 0.44 kg. kg BM^{-1} (95% CI = 0.21 to 0.68; $p < 0.0001$), RT + 1.6 = 0.63 kg. kg BM^{-1} (95% CI = 0.40 to 0.87; $p < 0.0001$), and RT + 3.2 = 0.60 kg. kg BM^{-1} (95% CI = 0.36 to 0.83; $p < 0.0001$) from baseline to post-test with no differences between groups ($p > 0.05$) except for absolute upper and lower body power gains. The increases of absolute upper body power in RT + 1.6 were significantly lower than in RT + 3.2 ($p = 0.044$). Also, regarding absolute lower body power, the increases in RT + 3.2 was significantly greater than in CT + 1.6 ($p = 0.034$).

Muscle performance

All four intervention groups noted significant increases in vertical jump CT + 1.6 = 2.90 cm (95% CI = 0.88 to 4.93; $p = 0.0022$), CT + 3.2 = 4.72 cm (95% CI = 2.70 to 6.75; $p < 0.0001$), RT + 1.6 = 3.54 cm (95% CI = 1.52 to 5.56; $p = 0.0002$), and RT + 3.2 = 3.45 cm (95% CI = 1.43 to 5.47; $p = 0.0003$) and pull-up CT + 1.6 = 3.54 reps (95% CI = 2.05 to 5.04; $p < 0.0001$), CT + 3.2 = 3.81 reps (95% CI = 2.32 to 5.31; $p < 0.0001$), RT + 1.6 = 4.72 reps (95% CI = 3.23 to 6.22; $p < 0.0001$), and RT + 3.2 = 5 reps (95% CI = 3.50 to 6.49; $p < 0.0001$) from baseline to post-test with no differences between groups ($p > 0.05$).

Associations between changes in lean mass and muscle performance

Upper body

Weak non-significant positive relationships were observed between change in upper body lean mass and changes in pull-up (Figure 1A), chest press strength (Figure 1B), chest press endurance (Figure 1C), and relative chest press strength (Figure 1D) while weak non-significant negative relationship for upper body power (Figure 1E) and relative upper body power (Figure 1F). Data are shown in Table 2 and Figure 1.

Lower body

Weak non-significant positive relationships were observed between the change in lower body lean mass with vertical jump (Figure 2A), lower body power (Figure 2B), leg press strength (Figure 2C), leg press endurance (Figure 2D), relative leg press strength (Figure 2E), and relative lower body power (Figure 2F). Data are shown in Table 3 and Figure 2.

Discussion

The purpose of this study was to investigate the relationship between changes in lean mass with muscle strength, endurance, and power adaptation responses in resistance-trained males following CT or RT with two different daily high protein doses (1.6 or 3.2 g.kg^{-1}.d^{-1}). Despite previously observing significant increases in exercise-training

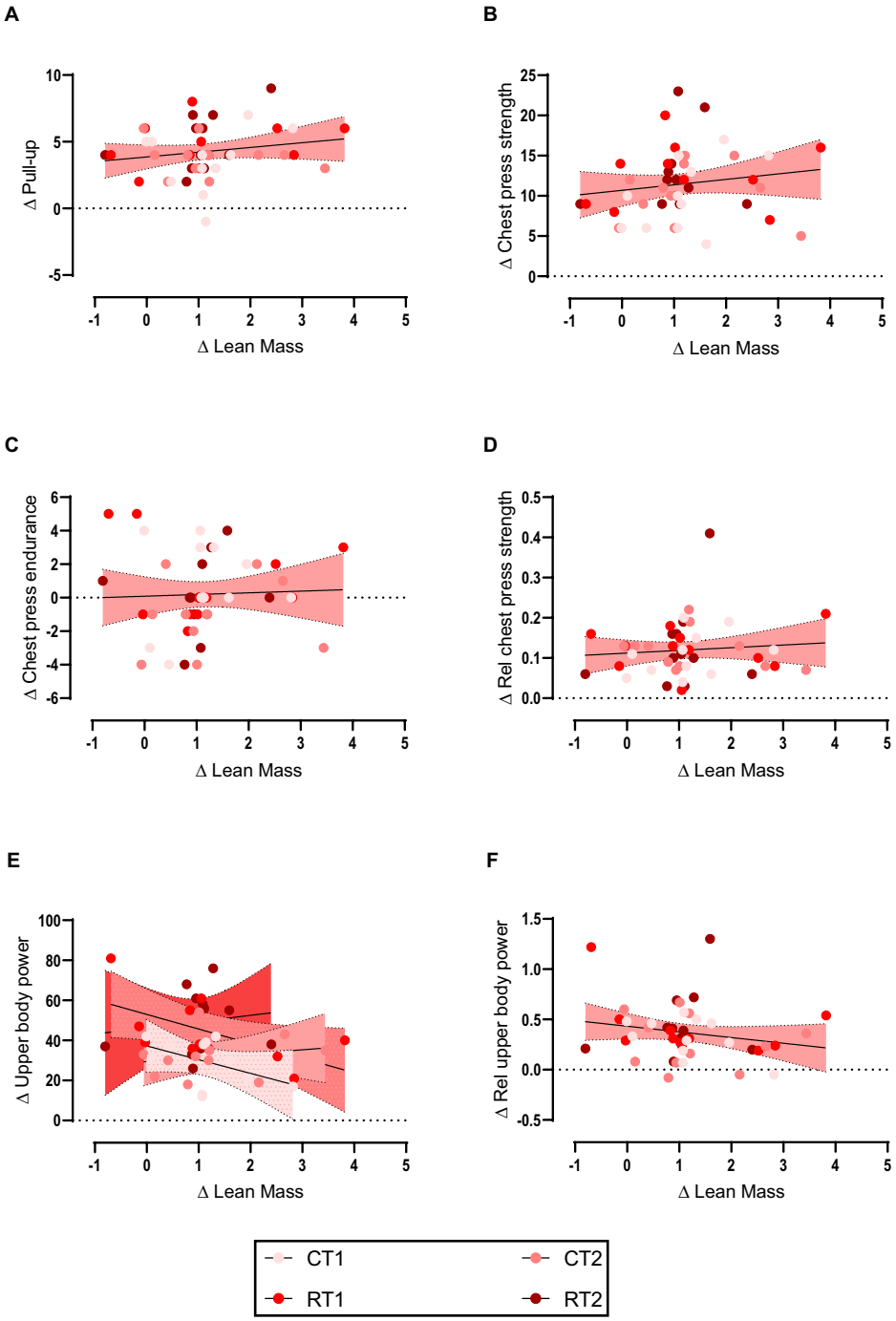

FIGURE 1
(A–F) linear regression (Pearson's) of Δ (performance) as a function of Δ upper body lean mass (kg). Linear regression is indicated by solid black lines for single models and different lines for others, 95% confidence intervals are indicated by different colors as legends show.

TABLE 2 The relationship between the upper body lean mass change and performance.

		Δ Lean Mass for CT + 1.6	Δ Lean Mass for CT + 3.2	Δ Lean Mass for RT + 1.6	Δ Lean Mass for RT + 3.2
Pull-up	*r*	0.270	−0.223	0.298	0.484
	95% CI	−0.39 to 0.74	−0.72 to 0.43	−0.36 to 0.76	−0.16 to 0.84
	p	0.421	0.509	0.372	0.130
Chest press strength	*r*	0.535	−0.028	0.127	0.193
	95% CI	−0.09 to 0.85	−0.61 to 0.58	−0.51 to 0.67	−0.45 to 0.71
	p	0.089	0.933	0.708	0.568
Chest press endurance	*r*	0.129	0.158	−0.098	0.129
	95% CI	−0.51 to 0.67	−0.48 to 0.69	−0.65 to 0.53	−0.50 to 0.67
	p	0.704	0.641	0.773	0.703
Relative chest press strength	*r*	0.364	−0.299	0.130	0.244
	95% CI	−0.30 to 0.79	−0.76 to 0.36	−0.50 to 0.67	−0.41 to 0.73
	p	0.270	0.371	0.701	0.468
Upper body power	*r*	−0.480	0.202	−0.607	0.145
	95% CI	−0.83 to 0.16	−0.45 to 0.71	−0.88 to −0.01	−0.49 to 0.68
	p	0.134	0.551	0.047	0.669
Relative upper body power	*r*	−0.527	−0.135	−0.416	0.262
	95% CI	−0.85 to 0.10	−0.68 to 0.50	−0.81 to 0.24	−0.40 to 0.74
	p	0.095	0.690	0.202	0.435

CT + 1.6, concurrent training + 1.6 g·kg^{-1}·d^{-1}; CT + 3.2, concurrent training + 3.2 g·kg^{-1}·d^{-1}; RT + 1.6, resistance training + 1.6 g·kg^{-1}·d^{-1}; RT + 3.2, resistance training + 3.2 g·kg^{-1}·d^{-1}.

induced muscle hypertrophy, strength and selected power responses in both RT and CT modalities, here we found no significant associations between changes in lean mass and performance. The magnitude of non-significant associations was generally similar regardless of exercise training and protein amount, indicating that these factors do not appear to influence associations between lean mass changes and functional adaptation responses.

RT or CT-induced changes in muscle fiber cross-sectional area can be influenced by various factors, including nutrition, genetics, and mechanical factors (49, 50). Such factors can subsequently impact the magnitude of training-induced responses and are also implicated in exercise 'responder' and 'non responder' paradigm (51). Using resistance-trained participants, our study placed significant focus on the role of training history as an influential variable in the observed results. While this may intuitively lower the possibility of observing correlations between changes in lean mass and strength given there may have been a 'lower ceiling' for adaptive responses compared to untrained participants, it also allowed us to utilize a more technically developed and regimented training program to maximize anabolic adaptations. Several studies have compared changes in lean mass and selected measures of muscle performance following diverse exercise protocols. Raymond-Pope et al. (33) observed a positive association between changes in lean mass and strength in NCAA Division I college athletes (33). Others have similarly reported associations between muscle mass and explosive power in young adults (52, 53) while a higher level of muscle strength has also been demonstrated in athletes with higher lean mass (54). In contrast, several studies have not observed any correlations between changes in muscle strength or power and muscle hypertrophy (55–57). The basis with this variability in the literature is likely to be a result of several different including variations in the methods used to conduct tests on subjects, differences in how changes are measured, and variations in the composition of subject groups. For instance, factors such as age, gender, physical condition, and training history can significantly impact study outcomes. Researchers may encounter differences when comparing professional athletes to non-athletes. Another key factor in our study was the inclusion of the CT groups. To our knowledge, this is the first study to examine such correlations in muscle adaptation measures with this training modality. Many individual and team-based sports such as football, rugby and basketball utilize CT as part of their performance as they require a diverse combination of muscle strength, explosive power and aerobic capacity in addition to match-specific movements (e.g., jumping, tackling, changing directions, rapid acceleration, etc.) for successful performance. Thus, investigating the correlation in adaptive responses with CT as we have undertaken in this current study can provide important information as to their specific exercise training variables that may be more beneficial for optimizing outcome measures.

No significant protein-by-group differences were found in associations between lean mass and muscle strength and power adaptation responses in this investigation, demonstrating that alterations occur independently of protein intake. The combination of adequate protein intake and regular physical activity is crucial for enhancing gains in lean mass and muscle strength, but the effects are small (26, 58). A protein intake of 1.6 g·kg^{-1}·d^{-1} seems adequate to maximize lean mass, muscle strength and power adaptation responses in both RT and CT groups, and aerobic capacity in CT groups, as we demonstrated in our earlier work, except for peak power (35). Notably, recent evidence suggests that there may actually be no upper limit in magnitude and duration of MPS responses to protein ingestion during recovery from exercise *in vivo* in humans (59). Specifically, using a comprehensive quadruple isotope tracer feeding-infusion approach, this study showed that the ingestion of 100 g protein results in a greater and more prolonged (>12 h) anabolic response when compared to the ingestion of 25 g protein. However, collective findings from a number of studies posit that daily protein intakes up to 2.2 g·kg^{-1}·d^{-1} are adequate for maximizing muscle protein accretion with resistance exercise (60). Moreover, ingestion of 5.5 times the recommended dietary allowance (RDA) of protein (4.4 g.kg^{-1}.d^{-1}) resulted in similar gains in FFM compared to 1.8 g.kg^{-1}.d^{-1} in resistance-trained individuals who otherwise maintain the same training regimen (61). Another study assessed protein requirements during the early stages of training in 12 detrained (for at least 1 year) novice male bodybuilders who received 2.62 g.kg^{-1}.d^{-1}or 1.35 g.kg^{-1}.d^{-1}of protein while following an isoenergetic diet for 4 weeks (62). Although the increased protein intake resulted in somewhat larger improvements in certain measurements, the disparities between the conditions were not statistically significant. These results show that during the early stages of reinitiating an RT

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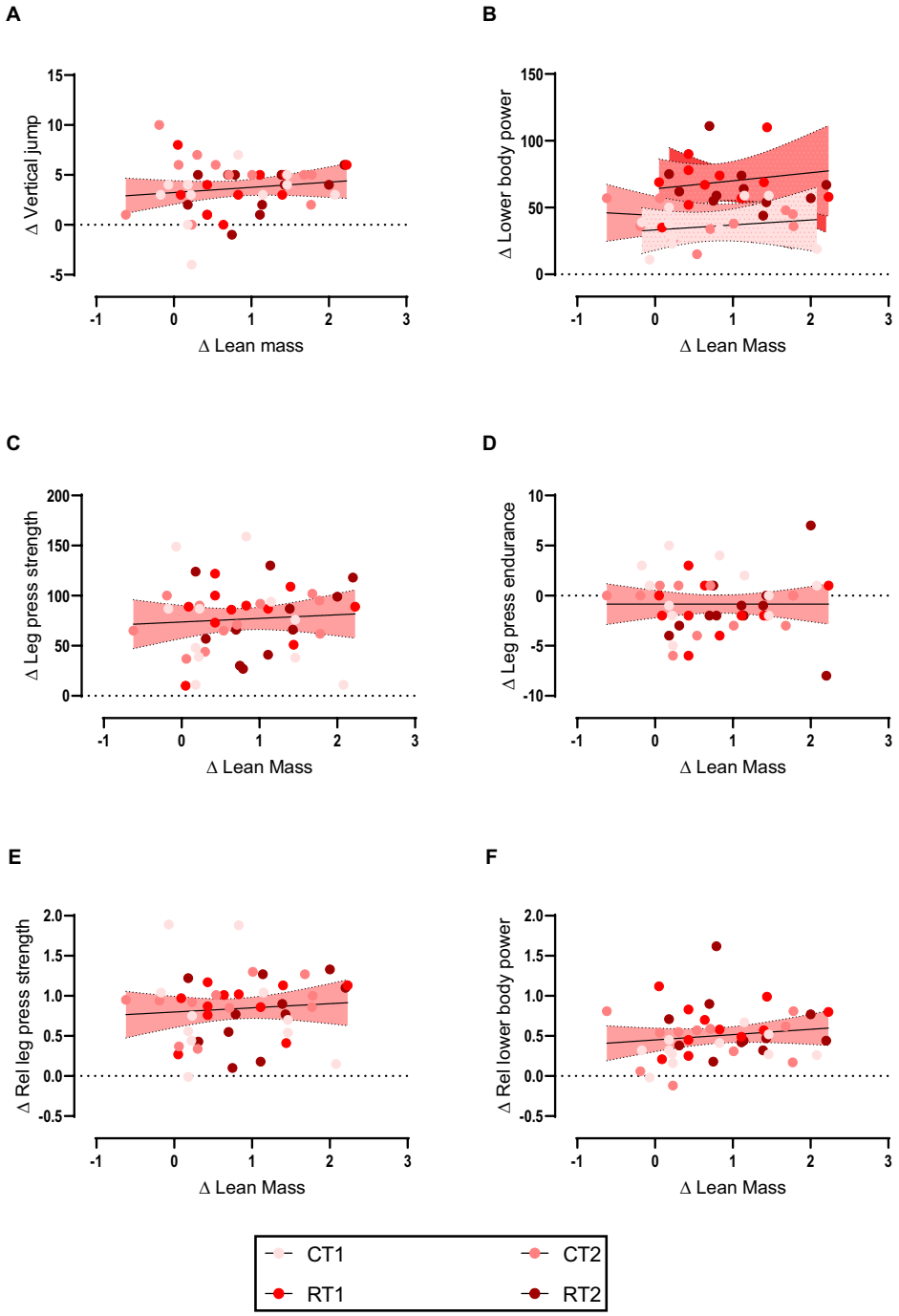

FIGURE 2
(A–F) linear regression (Pearson's) of Δ (performance) as a function of Δ lean mass (kg). Linear regression is indicated by solid black lines for single models and different lines for others, 95% confidence intervals are indicated by different colors as legends show.

program, there is no benefit to consuming very high amounts of protein. Our findings thus support a seemingly growing consensus that an optimal daily protein intake for maximizing muscle mass gain with exercise, whether it be RT or now with CT, is typically between 1.6 and 2.2 $g.kg^{-1}.d^{-1}$ (60). The human body is capable of assimilating substantial quantities of dietary protein. Nevertheless, the protein translational machinery does not employ all of the constituent amino acids to produce new proteins. Thus, when protein intake exceeds levels of 1.6 and 2.2 $g.kg^{-1}.d^{-1}$, MPS becomes saturated. This leads to an increase in the breakdown of amino acids via oxidation and urea formation, resulting in fewer amino acids being available for MPS (60). These explanations may explain as to why 3.2 $g.kg^{-1}.d^{-1}$ of protein intake in the present study did not result in further gains in comparison to 1.6 $g.kg^{-1}.d^{-1}$. Given that the gains in lean mass did not show significant differences between protein doses, one could argue that the similarity in strength and power adaptation responses is not solely dependent on lean mass. Further investigation is required to examine these associations across various participants (e.g., trained,

TABLE 3 The relationship between the lower body lean mass change and performance.

		Δ Lean Mass for CT + 1.6	Δ Lean Mass for CT + 3.2	Δ Lean Mass for RT + 1.6	Δ Lean Mass for RT + 3.2
Vertical jump	*r*	0.297	−0.075	0.245	0.320
	95% CI	−0.36 to 0.76	−0.64 to 0.54	−0.41 to 0.73	−0.34 to 0.77
	p	0.374	0.825	0.467	0.336
Lower body power	*r*	0.179	−0.169	0.201	−0.308
	95% CI	−0.47 to 0.70	−0.69 to 0.47	−0.45 to 0.71	−0.76 to 0.35
	p	0.598	0.618	0.552	0.355
Leg press strength	*r*	−0.281	0.332	0.191	0.314
	95% CI	−0.75 to 0.38	−0.33 to 0.77	−0.46 to 0.70	−0.35 to 0.76
	p	0.401	0.318	0.572	0.346
Leg press endurance	*r*	−0.035	−0.099	0.102	0.176
	95% CI	−0.62 to 0.57	−0.65 to 0.53	−0.53 to 0.66	−0.47 to 0.70
	p	0.917	0.771	0.764	0.603
Relative leg press strength	*r*	−0.264	0.395	0.267	0.400
	95% CI	−0.74 to 0.39	−0.26 to 0.80	−0.39 to 0.74	−0.26 to 0.80
	p	0.431	0.228	0.427	0.222
Relative lower body power	*r*	0.357	0.094	0.233	−0.146
	95% CI	−0.30 to 0.78	−0.53 to 0.65	−0.42 to 0.73	−0.68 to 0.49
	p	0.280	0.781	0.490	0.666

CT + 1.6, concurrent training + 1.6 g·kg^{-1}·d^{-1}; CT + 3.2, concurrent training + 3.2 g·kg^{-1}·d^{-1}; RT + 1.6, resistance training + 1.6 g·kg^{-1}·d^{-1}; RT + 3.2, resistance training + 3.2 g·kg^{-1}·d^{-1}.

untrained, etc.) and to get a more profound comprehension of the underlying processes involved.

Another notable finding from the current study was that there we found no significant improvements in endurance adaptations in either of the exercise and high protein interventions. This finding supports findings from a recent systematic review showing long-term protein supplementation to further enhances CT-mediated increases in skeletal muscle mass and strength/power, but not whole-body aerobic capacity (i.e., VO_{2max}/peak) (29). Protein intake following ET has been shown to increase myofibrillar protein synthesis (63) and augment the remodeling of muscle and whole-body proteins (64). Such protein remodeling is theorized to be an important aspect of the acute recovery process after exercise that ultimately underpins the adaptations (e.g., greater muscle power, aerobic capacity) that can accrue with ET (64). In addition to the RT component, participants in the CT group in the current study performed stationary cycling that incorporated a mixture of hill simulation rides of varying intensities that was primarily intermittent (i.e., 'work' and 'rest' periods) in nature. Considering both the selective improvements in muscle strength and power outcomes, and contractile nature of this ET stimulus of the CT program, this finding of no improvements in ET adaptations may likely relate to the 'specificity of adaptation'. Indeed, one of the central proponents of exercise physiology is the principle of training specificity that proposes an exercise that training responses/adaptations are tightly coupled to the mode, frequency and duration of exercise performed (65). This would imply that training-induced adaptations mostly occur in muscle fibers that have been recruited during exercise regimen, with little or no adaptive changes occurring in untrained musculature. Indeed, ET adaptations may require higher repetitions with lower loads (66). Thus, irrespective of the high protein availability with both diets in the current study, it is likely that the ET component within the CT group (and lack of in the RT group), was not of the appropriate volume, intensity and frequency of exercise sessions to promote ET adaptations. Whether the incorporation of more ET (i.e., high duration and low-to-moderate intensity) within a CT program can significantly improve endurance performances when combined with high protein availability remains an area of future investigation.

A potential limitation of our current work is the small sample size which may restrict the applicability of the findings to a wider population. Additionally, we did not measure protein excretion. Given that the participants consumed a high-protein diet, it would be prudent for future research to include this significant component. Specifically, the study focused on resistance-trained males, making it less relevant for females or individuals with diverse training backgrounds. It also cannot be ruled out that the 'one off' nature of 1-RM testing may have resulted in true maximum strength results not being measured (67) although this was relative for all participants. In summary, the relationship between changes in lean mass and muscle strength and power adaptation responses in resistance-trained males generally did not differ according to protein intake (1.6 or 3.2 g·kg^{-1}·d^{-1}) or training mode (RT or CT). The findings demonstrated that, regardless of the protein intake or the type of training, increases in lean mass exhibited weak positive associations with most upper and lower body muscle strength, endurance, and power adaptation response measures. The study proposes that functional enhancements could be associated with neural adaptations rather than muscle hypertrophy, but this assertion warrants careful interpretation. Furthermore, the duration of the study period might influence the observed associations, and more extensive, long-term investigations could yield further insights.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the protocol was reviewed by the Institutional Human Subject Committee and the Ethics Committee of the University of Isfahan (IR.UI.REC.1400.098) and carried out in accordance with the Declaration of Helsinki. This study has been registered with the Iranian Registry of Clinical Trials (IRCT20191204045612N2). 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

RB: Conceptualization, Formal analysis, Writing – original draft. ZK: Writing – original draft. DC: Writing – original draft, Writing – review & editing. DS: Writing – review & editing. MB: Writing – review & editing. RS: Writing – review & editing. MK: Writing – review & editing. FD: 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

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1439037/full#supplementary-material

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Effects of nano-*Rhodiola rosea* combined with treadmill exercise on anti-exercise fatigue in rats

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Objective: To explore the potential strategies and mechanisms for enhancing the bioavailability of *Rhodiola rosea*.

Methods: 36 Sprague–Dawley rats (8-weeks-old) were randomly assigned to six groups ($n = 6$ per group). Groups I and II received nano-dose forms of *R. rosea*, groups III and IV received normal dose form of *R. rosea*, and groups V and VI served as distilled water control groups. Groups II, IV, and VI were combined with moderate -intensity treadmill exercise. Each group received a daily gavage with 0.5 mL of nano -*R. rosea* solution (0.01 mg/mL), normal *R. rosea* solution, and distilled water. All rats were subjected to exhaustive swimming after 4 weeks. Outcome measures include GSH-px activity, T-AOC activity, MDA content, hepatic glycogen content, and T-SOD activity.

Results: For plasma MDA content, group I was lower than group III ($p < 0.01$) and group V ($p < 0.01$), group II was lower than group III ($p < 0.01$), group VI was higher than group II ($p < 0.05$) and group IV ($p < 0.05$). For plasma T-AOC activity, group II was higher than group VI ($p < 0.01$). For plasma GSH-px activity, group I was lower than group IV ($p < 0.05$), groups II, III, and IV were higher than group V ($p < 0.05$), and group V was lower than that of group VI ($p < 0.05$). For T-SOD activity of quadriceps muscle, groups I and III were higher than that in group V ($p < 0.05$).

Conclusion: *R. rosea* has a positive effect on anti-exercise fatigue in rats, with the nano-dosage form of *R. rosea* showing more significant efficacy than the normal form especially combined with aerobic exercise.

KEYWORDS

***Rhodiola rosea*, nano, anti-exercise fatigue, free radical, antioxidant enzyme**

1 Introduction

Sports nutrition supplements play an important role in relieving exercise-induced fatigue, and traditional Chinese medicines (*Panax ginseng*, *Lycium Chinense* Miller, *acanthopanax*, *astragalus*, *Panax notoginseng*, *Carthamus tinctorius*, and *Cordyceps sinensis*, etc.) have been considered to be an excellent material for the research and development of nutritional supplements for athletes (1–3). *Rhodiola rosea* is a perennial herb or subshrub in the genus Sedum (4, 5), which has anti-fatigue, anti-aging, anti-oxidation, anti-tumor and other bioactive functions (4–9). It also has a certain protective effect on the cardiovascular system (4, 10). *R. rosea* is a potent antioxidant, exerting a positive effect on anti-exercise fatigue and prolonging the duration

of exhaustive exercise (11–14) through multiple mechanisms including energy reserves (15, 16) metabolites (17–21) free radicals (22–24) and antioxidants (12, 16, 20, 25–27). Importantly, *R. rosea* contains no ingredients prohibited by the International Olympic Committee (IOC) and exhibits no side effects in long-term clinical studies. Thus, it fully meets the requirements as a sports nutrition supplement (28).

Most of the studies have used the normal dosage form of *R. rosea* in studies for exercise-induced fatigue (29–31). Some scholars have also proposed to make *R. rosea* into nano dosage form so as to improve its bioavailability and enhance the effect of anti-exercise fatigue (30, 32, 33). Theoretically, the nano-dosage form of *R. rosea* can significantly increase the surface area of the particles, enhance the adherence performance, and prolong the retention time at the site of absorption. Moreover, cell walls are entirely disrupted, increasing the content of active ingredients and further stimulating previously concealed ones. So the nano-dosage form of *R. rosea* is conducive to improving the bioavailability and maximum biological activity, but there is limited research on the anti-exercise fatigue of the nano-dosage form of *R. rosea*.

Accordingly, this study focused on exploring the effects of nano-dosage and normal dosage forms of *R. rosea* combined with treadmill exercise on exercise-induced fatigue in rats, searching for possible pathways and specific mechanisms of action to enhance the anti-exercise fatigue of *R. rosea*, and providing a basis for the further development of *R. rosea* as a sports nutrition supplement.

2 Materials and methods

2.1 Preparation of *Rhodiola rosea*

R. rosea, produced in Tibet, was purchased by Beijing Tong Ren Tang and then made into nano-dosage and normal dosage forms of *R. rosea* by Qinhuangdao Taiji Huan Nano-Products Co, Ltd. The nano-dosage form of *R. rosea* was processed using a high-energy nano-impact grinding device in a closed physical milling system. Through rapid multidirectional motion of the grinding chamber, the material was pulverized to the nanoscale, with an average particle size of 260–280 nm. During the formal experiments, 0.01 mg/mL of *R. rosea* in nano-form and normal form were dispensed daily.

2.2 Animals

SpF-grade male SD rats, 8 weeks old, 36 animals, average weight 345.0 ± 29.7 g, were provided by Shanghai Sipulpikai Laboratory Animal Co. Ltd. During the whole experiment, the animals were housed in the Laboratory Animal Center of Tongji University. The temperature in the laboratory was maintained at 22 ± 2°C, the relative humidity was about 55%, and the light time was from 7:00 to 19:00. The rats were acclimatized for one week after entering the laboratory. During this period, all experimental rats were routinely fed basal feed (provided by Jiangsu Synergistic Pharmaceutical and Biological Engineering Co. Ltd.) and distilled water and were fed *ad libitum*. After the experiment, the animals were euthanized by cervical dislocation. The personnel involved in the operation, surgery, and euthanasia of animals have been trained internally by the Laboratory Animal Center of Tongji University.

2.3 Group

The rats were randomly divided into 6 groups, with 6 rats in each group, and the specific groupings are shown in Table 1.

2.4 Procedure

After the end of adaptive feeding, the experiment was conducted on an adaptive treadmill. Throughout this adaptive phase, group II, group IV, and group VI were acclimatized to treadmill training for one week. The intensity of exercise was set at a platform inclination angle of 5°, a speed of 15 m/min, a duration of 45 min, an exercise frequency of 3 days/week, and an interval of 1 day between two consecutive exercise sessions. Concurrently, the rest of the groups were reared normally without any interventions. After this week, the rats were basically adapted to the treadmill exercise, and there was no obvious adverse reaction.

During the formal experiment, rats in each group were free to ingest food and water every day. Every morning, from 9:00 a.m. to 10:00 a.m., each group was gavaged with 0.5 mL of nano-dose *R. rosea* solution, normal-dose *R. rosea* solution, and distilled water, respectively, once a day for 4 weeks. The daily dose of nano and normal forms of *R. rosea* supplemented in groups I, II, III, and IV was 0.005 mg.

On the 29th day, all rats underwent an acclimatization swim lasting 15 min in a swimming pool maintained at a constant temperature. The size of the swimming box was 60*60*60 cm, with a water depth of 40 cm and a temperature of 28°C. On day 30, all rats were given a day of rest.

On the 31st day, each rat was subjected to an exhaustive swimming exercise wherein a load equivalent to 2% of its body weight was applied to its tail. Each group, comprising six rats, constituted one round of exhaustive swimming exercise, and the water was changed after every two rounds. The exhaustion criterion was defined as the rats' nostrils remaining under the water surface for more than 6 s. The duration of exhaustive swimming was recorded for all rats. After exhaustion, the rats were dried with a hair dryer and returned to the cage.

TABLE 1 Rats in each group.

Groups	Information for each group	
	Supplementation	Treadmill exercise
Group I	Nano *R. rosea*	
Group II	Nano *R. rosea*	+
Group III	Normal *R. rosea*	
Group IV	Normal *R. rosea*	+
Group V	Distilled water	
Group VI	Distilled water	+

Abbreviations: CAT, catalase; CK, creatine kinase; DINB, dithio-bis-nitrobenzoic acid; EDTA, ethylenediaminetetraacetic acid; FFA, free fatty acid; GPT, glutamic-pyruvic transaminase;; GSH-Px, glutathione peroxidase; GSH, reduced glutat; T-AOC, total antioxidative capacity; T-SOD, total superoxide dismutase.

On day 32, all rats were given a day of rest, and at 7:00 p.m. All rats were removed from all fodder except water. In the morning of day 33, all rats were anesthetized with 2% pentobarbital sodium at a dose of 0.025 mL/g.

Blood samples were taken from the abdominal aorta and placed in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. Subsequently, the samples were centrifuged for 10 min at 3,000 rpm, and the upper layer of plasma was extracted. The liver and quadriceps femoris were harvested, rinsed with saline, blotted with filter paper, wrapped in foil, and labeled with a marker pen. All samples were then stored at −80°C for subsequent testing. Figure 1 depicts the whole procedure and exercise program below.

2.5 Aerobic exercise

The training program for moderate-intensity treadmill exercise was modified based on the classical exercise load model established by Bedford (34). This exercise intervention was implemented specifically for groups II, IV, and VI (Table 2). The training time was daily, from 1:00 p.m. to 5:00 p.m.

To guide the rats during exercise, sound stimulation and small sticks were used to stimulate their tails, prompting them to remain in the front half of the runway. When necessary, a controlled amount of electrical stimulation was applied to ensure the smooth execution of the moderate-intensity running exercise.

2.6 Detection of biochemical indicators

The experimental samples included plasma, liver, and quadriceps muscle. Plasma served as the sample for detecting MDA content, T-AOC activity, and GSH-px activity. The liver was used as the sample to detect hepatic glycogen content, and quadriceps muscle was used as the sample to detect total superoxide dismutase (T-SOD) activity. All kits were purchased from the Nanjing Jianjian Bioengineering Institute. The test procedures were strictly in accordance with the instructions on the kits.

The test methods are as follows: hepatic glycogen content was tested using the anthrone method (35); plasma MDA was tested using the flow-credited barbiturate (TBA, Thiobarbituric AIC) method (36); plasma T-AOC activity was measured using the phenanthroline method (37); plasma GSH-px activity was determined by the direct method of dithio-bis-nitrobenzoic acid (DINB) (38); quadriceps T-SOD activity was tested by the xanthine oxidase method (39).

2.7 Data analysis

All results are presented as mean ± standard deviation (X ± S), with independent samples t-test between two groups and ANOVA between multiple groups, with $p < 0.05$ as a significant difference and $p < 0.01$ as a highly significant difference. Statistical analyses were performed using GraphPad Prism 9.5 and SPSS 18.0 software.

3 Results

3.1 The duration exhaustive swimming exercise

As shown in Figure 2, the duration of exhaustive swimming of rats in group II was significantly longer than that in group V ($p < 0.05$).

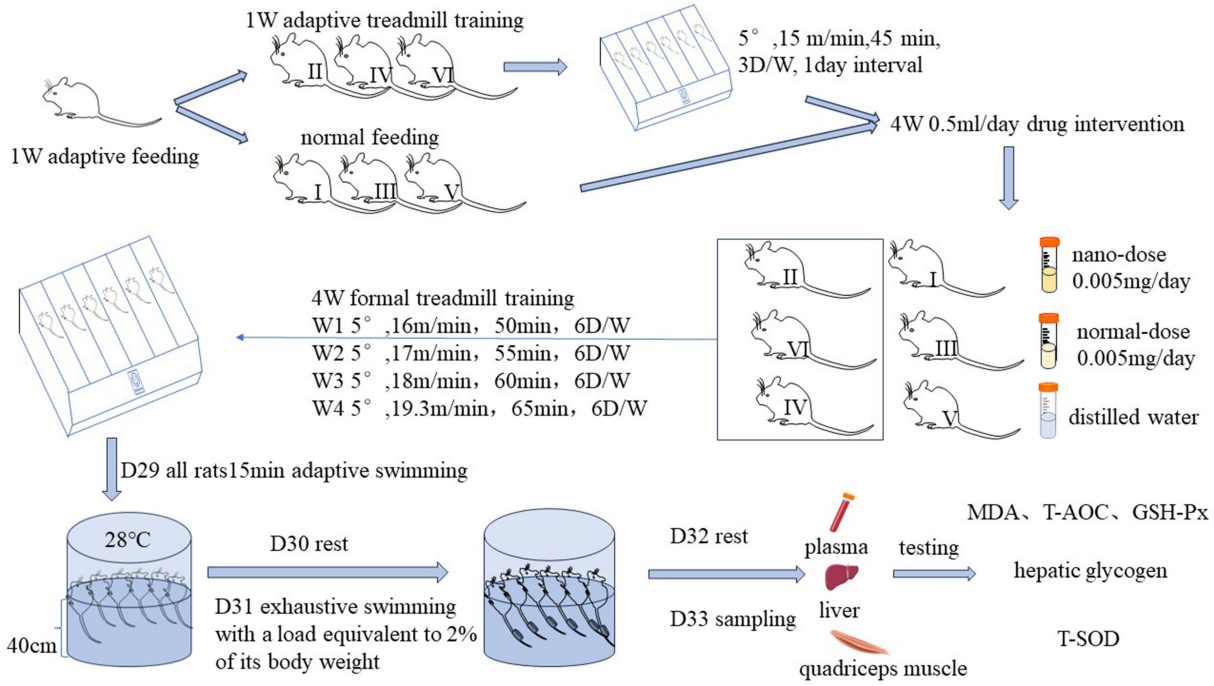

FIGURE 1
Experimental flow.

TABLE 2 Exercise schedule for moderate load intensity running in rats.

Timing	Training programs			
	Running platform inclination	Speed (m/min)	Duration (minutes/times)	Frequency (days/week)
Week 1	5°	16	50	6
Week 2	5°	17	55	6
Week 3	5°	18	60	6
Week 4	5°	19.3	65	6

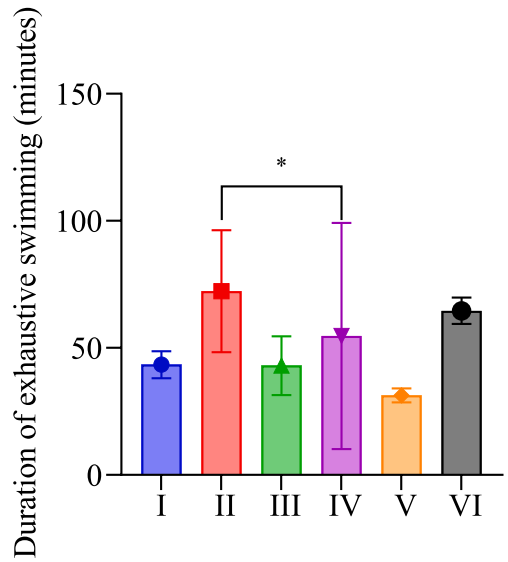

FIGURE 2
Duration of exhaustive swimming in rats of each group.

Ranking the groups based on the duration of exhaustive swimming resulted in the following order: groups II, VI, IV, I, III, and V.

3.2 Hepatic glycogen content

As shown in Figure 3, group V had the highest hepatic glycogen content, followed by group I, and group VI was the lowest. There was no significant difference between the groups.

3.3 MDA content, T-AOC activity, GSH-px activity of plasma

As shown in Figure 4, the plasma MDA content (Figure 4A) of rats in group II was the lowest; group I was significantly lower than group III ($p < 0.01$); group I was significantly lower than group V ($p < 0.01$); group I was significantly lower than group VI ($p < 0.05$); group II was significantly lower than group III ($p < 0.01$); group II was significantly lower than group V ($p < 0.01$); group II was significantly lower than group VI ($p < 0.05$). The plasma MDA content was ranked as group III, group VI, group V, group IV, group I, and group II.

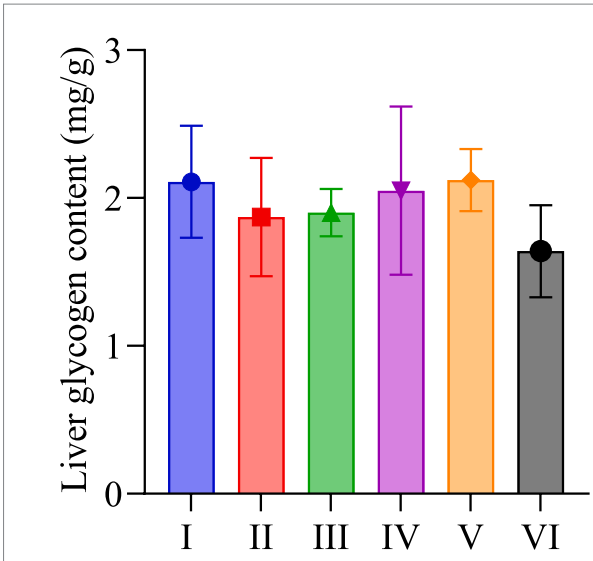

FIGURE 3
Hepatic glycogen content of rats in each group.

The plasma T-AOC activity (Figure 4B) of rats in group II was significantly higher than that of group V ($p < 0.05$); group II was significantly higher than group VI ($p < 0.01$); group III was higher than group V and lower than group I. The rats of each group were ranked according to their plasma T-AOC activity as group II, group IV, group I, group III, group V, and group VI.

Plasma GSH-px activity (Figure 4C) of rats in group IV was the highest, followed by group II, and group V was the lowest. The plasma GSH-px activity of rats in group I was significantly lower than that in group IV ($p < 0.05$); group II was significantly higher than group V ($p < 0.05$); group III and group IV were significantly higher than group V ($p < 0.05$); and group V was significantly lower than group VI ($p < 0.05$).

3.4 T-SOD activity in quadriceps femoris muscle

As shown in Figure 5, group I had the highest T-SOD activity, followed by group III, and group V had the lowest. The T-SOD activity of quadriceps in group I was significantly higher than that of group V ($p < 0.05$); group II was significantly higher than group V ($p < 0.05$); group III was significantly higher than group V ($p < 0.05$); and group III was significantly higher than group V ($p < 0.05$).

4 Discussion

The duration of exhaustive swimming is an important indicator for evaluating the degree of aerobic endurance (40). The results of the study were statistically significant only for nano-formulated *R. rosea* combined with running exercise compared with the distilled water group, which is not consistent with the results of the particles smaller than 50 nm used in the related study (32). The particle size may affect the release, absorption, and utilization of active ingredients in *R. rosea* by the organism. Furthermore, the individual variations among rats

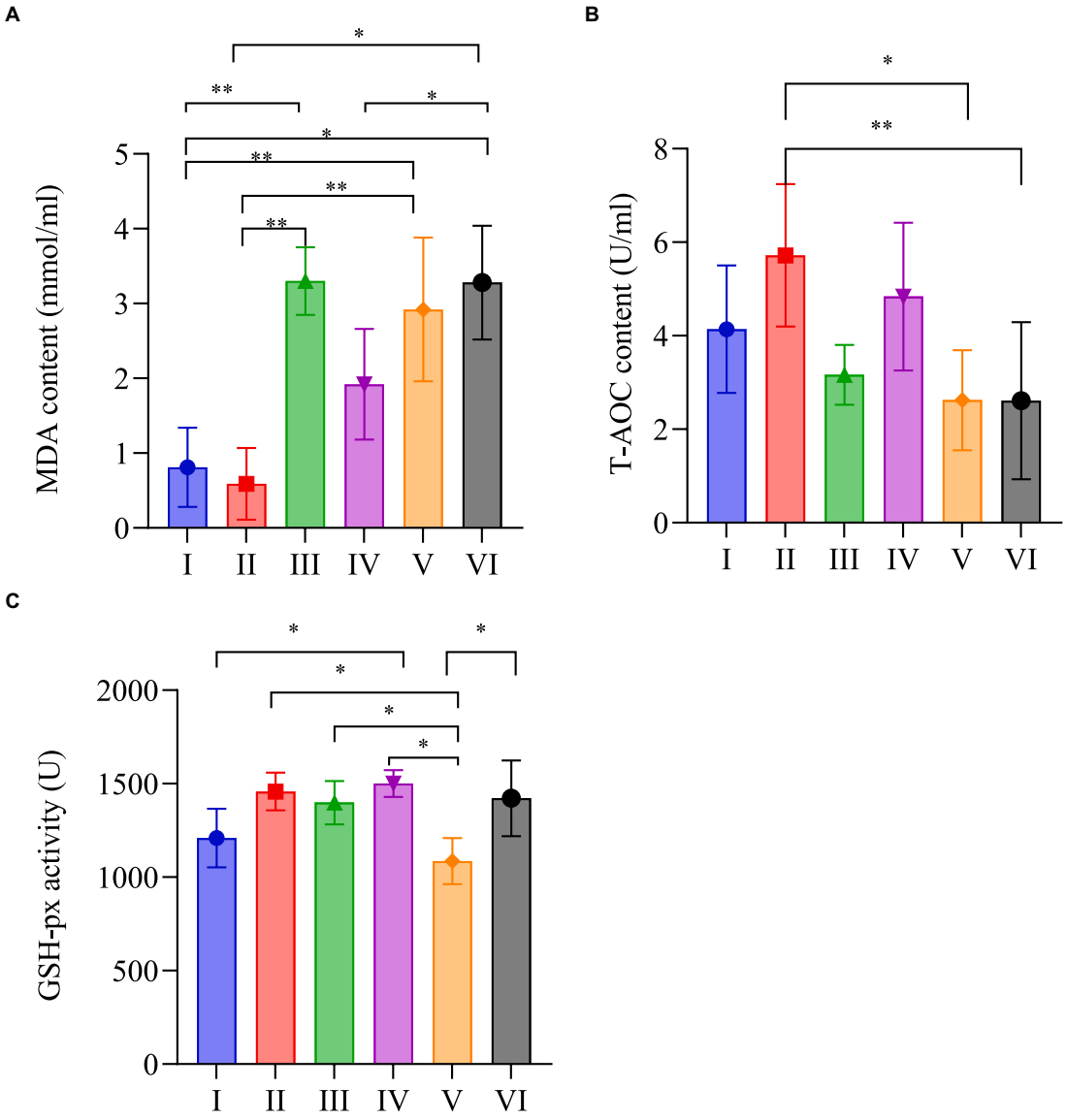

FIGURE 4
MDA content **(A)**, T-AOC activity **(B)**, GSH-px activity **(C)** of plasma in each group.

may have contributed to substantial result variation caused by exercise or *R. rosea* consumption, which could have influenced the experimental outcomes. Additionally, the rats with exercise intervention performed better in exhaustive swimming, suggesting that moderate-intensity treadmill exercise intervention can promote the anti-exercise fatigue effect of *R. rosea*, especially the nano dosage form of *R. rosea*. The nano dose is potentially more conducive to enhancing the efficacy of active ingredients in *R. rosea* within the organism compared to ordinary particle diameters, particularly in an exercise environment.

Hepatic glycogen content after exhaustive swimming exercise is an important indicator of the body's energy recovery, which reflects the level of energy supply during exercise and its ability to resist exercise fatigue (41). In this study, the hepatic glycogen content of rats was all at a lower level. After exhaustive exercise, the hepatic glycogen has been depleted, resulting in a reduction in the original reserve. Additionally, the short rest time and inadequate food supply affect the rate of hepatic glycogen recovery in the organism. The rats in the *R. rosea* group exercised for a longer time and consumed more hepatic glycogen. After the same resting time, the content of hepatic glycogen in the R rosea group was slightly higher than that in the distilled water group. Therefore, supplementation with *R. rosea* may be more conducive to promoting the recovery of hepatic glycogen after exhaustive exercise. At a similar exhaustive and resting time, the hepatic glycogen content was higher in the nano dosage form of *R. rosea* group than in the normal dosage group. So the nano dosage form of *R. rosea* may be more favorable to promote the recovery of hepatic glycogen in the organism, which was more obvious after combined with the moderate-intensity of the treadmill exercise.

MDA is recognized as a sensitive indicator of the metabolism of free radicals, reflecting the level of lipid peroxidation and the degree of cellular damage (42). Exhaustive exercise can result in the substantial generation of reactive oxygen species by the mitochondria, leading to a significant increase in MDA content (43). Compared with

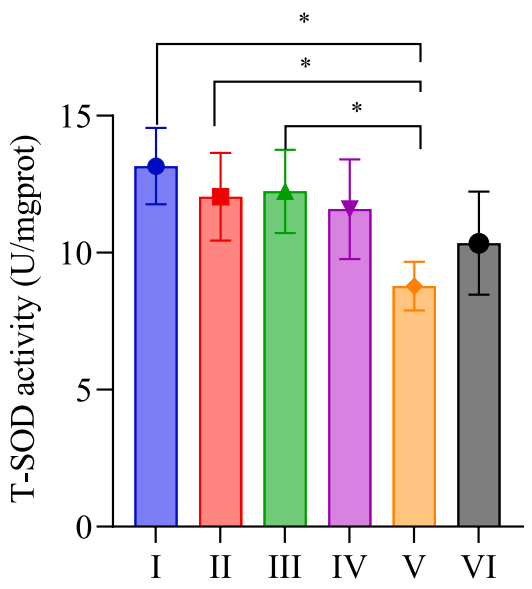

FIGURE 5
T-SOD activity of quadriceps femoris muscle in each group.

the control group, supplementation with *R. rosea* demonstrated a notable impact on enhancing the elimination of MDA following exhaustive exercise. Moreover, the use of the nano dosage form of *R. rosea* exhibited more significant efficacy in reducing excess MDA than the normal dosage form after exhaustive exercise. Meanwhile, the level of plasma MDA content for nano dosage form of *R. rosea* combined with moderate-intensity treadmill exercise is higher than that of the normal dosage form of *R. rosea* combined with the same exercise regimen. This implies that nano dosage form of *R. rosea* may be more effective in mitigating the extent of lipid peroxidation following exhaustive exercise and heightening the capacity to shield the body from damage caused by free radicals.

The cellular metabolic process is always accompanied by the generation of free radicals. A large number of free radicals are generated under the state of exhaustive exercise, and the organism defends itself through the internal antioxidant defense system. Antioxidant enzymes are an important part of the antioxidant defense system, especially GSH-px and SOD. T-AOC is an important indicator for evaluating the antioxidant capacity of biologically active substances, reflecting the overall antioxidant capacity of various antioxidant macromolecules, small molecules, and enzymes in the system. The activity of GSH-px reflects the level of selenium in the organism, and it also specifically catalyzes the reduction reaction of GSH to hydrogen peroxide, which serves to protect the structural and functional integrity of cell membranes (44).

Supplementation with *R. rosea* significantly enhanced the activities of plasma T-AOC and GSH-px after exhaustive exercise, suggesting that *R. rosea* can effectively improve the level of anti-lipid peroxidation in the organism. The nano dosage form was more obvious to enhance T-AOC activity after exhaustive exercise, especially when combined with treadmill intervention. It was consistent with the changes in plasma MDA content after exhaustive exercise in the corresponding groups of rats, probably due to the fact that the small diameter of nano dosage form of *R. rosea* particles was more conducive to the release of active ingredients in *R. rosea* and the process of absorption and utilization by the organism. However, the plasma GSH-px content showed the opposite result, and the normal dosage form of *R. rosea* was more able to stimulate the activity of plasma GSH-px, which may be caused by the overall antioxidant capacity of T-AOC. And MDA focuses on reflecting the body's overall level of lipid peroxidation and the degree of cellular damage, whereas the main target of GSH-px is hydrogen peroxide, which only represents a certain aspect of the body's antioxidant capacity. Meanwhile, the activity of plasma GSH-px increased after the two dosage forms of *R. rosea* combined with moderate-intensity treadmill exercise. Group IV only increased by 7.2%, while group II increased by 20%. This discrepancy suggests that moderate-intensity running exercise can enhance the impact of *R. rosea*, particularly nano dosage form of *R. rosea*, on the activity of GSH-px after exhaustive exercise in rats.

SOD functions as a natural antagonist to oxygen free radicals, scavenging the superoxide anion (O^{2-}). This role is integral to maintaining a balanced oxidative and antioxidant milieu in the body, thereby shielding cells from damage (45). The results showed that *R. rosea* significantly increased the T-SOD activity of the quadriceps muscle in the organism after exhaustive exercise in rats. Unlike other indexes (e.g., MDA, T-AOC, and GSH-px), the T-SOD activity in the quadriceps femoris muscle did not exhibit a clear upward trajectory and, in fact, demonstrated a slight decrease when two dosage forms of *R. rosea* were combined with moderate-intensity platform running exercise. It is conjectured that the mechanism of action on T-SOD activity in quadriceps muscle may be through one or more SOD fractions. The combination of moderate-intensity treadmill running exercise with *R. rosea* may not have achieved the expected benign cycle, as the moderate-intensity running exercise may have disrupted the signaling pathway associated with a specific fraction of SOD activity targeted by *R. rosea*. This interference likely led to disturbances in the pathway, contributing to an overall decline in the T-SOD activity of quadriceps. Alternatively, the mechanism may be diametrically opposed, wherein medium-intensity running exercise diminishes the fraction of SOD activity that *R. rosea* can enhance, and reciprocally, *R. rosea* reduces the portion of SOD activity that can be augmented by medium-intensity running exercise. The mutual weakening effect between the two decreased the overall T-SOD activity of the quadriceps muscle. To validate and elucidate this potential interaction, further studies should be undertaken.

In summary, nano-dose showed a stronger anti-fatigue effect compared with normal-dose, and the underlying mechanism may involve multiple molecular pathways. The application of nanotechnology may have enhanced the solubility and bioavailability of the active ingredients in *Rhodiola rosea*, enabling controlled release and thus promoting more efficient absorption and distribution (46, 47). Active ingredients within the nano-*R. rosea*, such as salidroside and rosavin, may modulate intracellular signaling pathways like AMPK and mTOR (48), enhancing cellular energy metabolism and antioxidant capacity, thereby improving anti-fatigue capabilities. In addition, nano-*rhodiola rosea* may regulate mitochondrial function (49), improve the activity of antioxidant enzymes in cells, reduce the generation and accumulation of free radicals, and thus reduce the oxidative stress damage caused by exercise.

Other anti-fatigue drugs have also shown significant results in improving athletic performance and reducing fatigue. Chen et al.

(50), evaluated the potential beneficial effect of nano-bubble curcumin extract in reducing exercise-related injuries and improving performance. Results indicated that nano-bubble curcumin extract supplementation alters the gut microbiota composition and aids in overcoming physical fatigue. Liu et al. (51) and Yun et al. (52), examined the synergistic effects of combining *R. rosea* and caffeine supplementation on muscle endurance and explosiveness in SD rats and human subjects. In both the rat model and human subjects, the *R. rosea* + caffeine group demonstrated significantly greater physical performances compared to the use of *R. rosea* or caffeine supplements individually. When used alone, *R. rosea* as an adaptogen herb may have a milder and longer-lasting anti-fatigue effect. Caffeine is a central nervous system stimulant that can temporarily increase alertness and physical performance, but its long-term use can lead to dependence and side effects such as heart palpitations and insomnia.

Although *Rhodiola rosea* is considered safe for traditional use, the application of nanotechnology may present new safety concerns. The high surface area and surface activity of nanoparticles may increase their bioreactivity *in vivo*, leading to potential toxic reactions. For example, nano *R. rosea* may trigger cell damage or inflammatory responses by affecting the integrity and function of cell membranes. In addition, nanoparticles may accumulate in the body, and long-term accumulation may have negative effects on organs such as the liver and kidneys (53). Further toxicological studies are needed to assess its safety.

5 Highlights and limitations

This study innovatively combines nano-Chinese medicine technology with exercise intervention to conduct an in-depth investigation into the anti-fatigue effects of *Rhodiola rosea*. It reveals the significant advantages of nano-dosage of *R. rosea* over the normal formula in terms of enhancing bioavailability and antioxidant capacity. A comprehensive assessment of its efficacy was made through a series of biochemical indicators, providing a new perspective for the modern application of traditional Chinese medicine. The advent of nanotechnology has introduced a novel approach to the processing of traditional Chinese medicine, with the physical nanotechnology used in this study also offering a new attempt for further processing of other Chinese medicine-based sports nutrition supplements.

However, there are limitations, including insufficient exploration of dose effects, long-term effects, and individual differences. No detailed analysis was performed of changes in body weight in rats, which may affect the accuracy of drug dosage and metabolic rate. In addition, the potential side effects of *Rhodiola rosea* have been poorly discussed, and direct human research validation is lacking. Although nanotechnology has been applied to improve bioavailability, the safety and long-term accumulation effects of nanoparticles still require further toxicological studies. Future research could consider the use of nano-biotechnology, such as improving the targeting of traditional Chinese medicine effects through nano-drug delivery technology. This could more specifically reveal the mechanism of resistance to exercise fatigue of *R. rosea* and could also be more conducive to enhancing the efficacy of *Rhodiola rosea* in combating exercise fatigue.

6 Conclusion

R. rosea has a positive effect on anti-exercise fatigue in rats. Notably, the anti-fatigue effect of nano-formulations is more significant than that of normal formulations when combined with aerobic exercise. The probable mechanism is to reduce the generation of free radicals or scavenging of free radicals by regulating the oxidative stress system, which is stronger than that of the common formulations.

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

The animal study was approved by Science and Technology Ethics Committee of Tongji University. License numbers: TJAG01924201. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JW: Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. GZ: Formal analysis, Visualization, Writing – original draft, Writing – review & editing. DW: Conceptualization, Project administration, Writing – original draft, Writing – review & editing. YY: Data curation, Writing – original draft, Writing – review & editing. QY: Supervision, 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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Dietary intake, energy availability, and power in men collegiate gymnasts

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Introduction: The purpose was to examine the prevalence of low energy availability (LEA), explore dietary behaviors in men collegiate gymnasts ($n = 14$), and investigate the relationships between energy availability (EA), body composition, and plyometric performance.
Methods: Body composition was measured using air displacement plethysmography. Lower- and upper-body peak power (PWRpeak) and modified reactive strength index (RSI_{mod}) were calculated from countermovement jump (CMJ) and plyometric push-up (PP) assessments. Energy expenditure was tracked over 3 days, while daily energy and macronutrient intake were recorded. EA was calculated and used to categorize athletes into LEA and non-LEA groups. Pearson correlation coefficients were used to examine relationships between EA, body composition, and performance metrics.
Results: 85.7% of athletes ($n = 12$) exhibited LEA (20.98 ± 5.2 kcals/kg FFM), with non-LEA athletes ($n = 2$) marginally surpassing the <30 kcal/kg of fat-free mass (FFM) threshold (30.58 ± 0.2 kcals/kg FFM). The cohort ($n = 14$) consumed insufficient energy (30.5 ± 4.5 kcal/kg/day) and carbohydrates (3.7 ± 1.1 g/kg/day), resulting in LEA (22.36 ± 5.9 kcal/kg/FFM). EA was not correlated with body composition or performance metrics.
Discussion: A high prevalence of LEA may exist in men gymnasts, largely due to a low relative energy and carbohydrate intake.

KEYWORDS

low energy availability, body composition, reactive strength index, power output, gymnastics

1 Introduction

Gymnastics is a physically demanding sport that requires the combination of strength, muscular endurance, flexibility, coordination, and technical skill to execute intricate routines with precision and control (1). The sport of men's gymnastics includes six events: floor exercise, pommel horse, rings, vault, parallel bars, and high bar (2). Many routines involve a combination of twists, jumps, inversions, and other high-velocity movements resulting in a unique variety of physiological demands and concomitant adaptations (3). Durations of routines vary substantially with event, ranging from approximately 5 s in the vault to 70–90 s on the floor exercise, further highlighting the sport's diverse bioenergetic demands (1). To develop physiological capabilities, gymnasts adhere to rigorous training schedules from a young age, maintaining weekly

training loads ranging from 21 to 37 h year-round, which presents long-term challenges to weight management practices (4, 5).

Low body fat to high fat free mass ratios are thought to enhance performance by improving movement efficiency, aesthetic presentation, strength-to-mass ratio, and providing various biomechanical advantages (3, 6). Specifically, leanness is considered a critical body composition characteristic for success, with elite female and male gymnasts maintaining body fat levels ranging from 13%–16% to 8%–12%, respectively (3, 7). Consequently, athletes engaged in aesthetic sports like gymnastics have been found to be at a higher risk of developing behaviors of disordered eating and excessive activity levels in an attempt to minimize fat mass (6). While research on disordered eating in men's gymnastics is limited, reports indicate that nearly 30% of male athletes engaged in sports emphasizing leanness exhibit harmful weight control behaviors (8, 9), marked by restricted energy and carbohydrate intake (10). Additionally, evidence suggests a curvilinear relationship exists between body mass index (BMI) and gymnastics performance, suggesting that excessively low BMI may adversely affect both performance and health (3, 11). Therefore, gymnasts' pursuit of a lean physique, beyond the point of performance optimization, increases the risk of disordered eating patterns, potentially leading to relative energy deficiencies (5).

Low energy availability (LEA) is a metric used to quantify the residual energy available to support the body's physiological functions after accounting for the energy expenditure from exercise (12–14). LEA is used to identify athletes at risk of Relative Energy Deficiency in Sport (RED-S), which represents a multifactorial physiological dysfunction resulting from chronic energy deficiency, stemming from insufficient energy intake, excessive energy expenditure from training, or a combination of both (15, 16). Calculated energy availability (EA) values for males are: <30 kcal/kg FFM/day, clinical LEA; 30–45 kcal/kg FFM/day, subclinical LEA; ≥40 kcal/kg FFM/day, optimal EA (17). The majority of studies investigating the consequences of LEA have focused predominantly on female athletes (9). Research within women's gymnastics has highlighted the increased risk of LEA among pre-teenage (5), teenage (5), and adult (18, 19) gymnasts. Emerging evidence suggests that male athletes, particularly those in aesthetic-based competitions (18) or weight-category sports (20), are vulnerable to the detrimental effects of LEA (9). Indeed, the 2014 International Olympic Committee's (IOC) consensus statement (21), along with subsequent updates in 2018 (22) and 2023 (23), on RED-S, has broadened the understanding of LEA to include male athletes. While LEA is frequently used to identify risk factors or diagnose RED-S, there is a lack of research investigating the prevalence of LEA among men collegiate gymnasts. Moreover, uncertainties exist regarding the potential impact of LEA on various indices of physical performance, particularly in terms of plyometric performance.

The rapid muscle contractions required during gymnastics routines on apparatuses such as the vault, rings, and floor exercise demand explosive lower- and upper-body power (24). These contractions are driven by the stretch-shortening cycle (SSC), marked by a swift transition between the initial eccentric "stretch" and the subsequent concentric "recoil" (25). The modified reactive strength index (RSI_{mod}), which is calculated from jump height, ground contact time, and body mass, serves as a useful indicator of successful technical execution (24), especially for movements requiring muscle re-contraction after landing. While RSI_{mod} focuses on the ability to produce force during the SSC, the measurement of peak power (PWR_{peak}) provides distinct yet related information. PWR_{peak} represents the maximum instantaneous power and is crucial for generating the height, rotation, and momentum necessary for movements such as vaulting, tumbling, and dismounting from apparatuses (24). Previous research has examined aspects of upper- and lower-body power in gymnasts including girls (26), women collegiate (27), and boys (28). However, research among men collegiate gymnasts, the metrics of RSI_{mod} and PWR_{peak}, and their relationship with EA and body composition remain understudied.

Therefore, the purpose of the current study was to examine the prevalence of LEA and explore dietary behaviors among a convenience sample of National Collegiate Athletic Association (NCAA) Division III collegiate men's gymnastics athletes. A secondary aim was to investigate the relationships between EA, body composition, and upper- and lower-body plyometric performance metrics.

2 Materials and methods

2.1 Experimental design

This observational study began in August prior to the academic term and gymnastics season. Athletes underwent assessments of body composition, reactive strength index (RSI), and power output during one day of testing. Additionally, a 3-day monitoring period was conducted to evaluate EA. Athletes recorded their dietary intake and were provided with team-based heart rate monitoring systems equipped with inertial sensors to measure activity energy expenditure during practices.

2.2 Participants

NCAA Division III men gymnastics athletes ($n = 14$) participated in the current study (age: 21.0 ± 1.2 years; height: 1.66 ± 4.68 m; body mass: 67.6 ± 5.1 kg). Players were medically cleared for intercollegiate athletic participation, had the risks and benefits explained beforehand, signed an institutionally approved written consent form, and completed a medical history form.

2.3 Procedures

2.3.1 Body composition

Athletes were instructed to refrain from exercise, eating, and drinking for ≥2 h before testing. Testing was conducted in the early morning after an overnight fast. Upon arrival to the laboratory, height and body mass were recorded to the nearest 0.01 cm and 0.02 kg, respectively, using a stadiometer (Detecto,

Webb City, MO, USA) and digital scale (BOD POD model 2000A; BOD POD; Cosmed USA, Concord, CA, USA), with each subject barefoot. Body composition variables (i.e., percent body fat [BF%], fat-free mass [FFM], and fat mass) were assessed using air displacement plethysmography (BOD POD model 2000A; BOD POD; Cosmed USA, Concord, CA, USA) according to standard operating procedures. Fat-free mass index (FFMI) was calculated using the following equation (29): FFM (kg)/Height^2 (m^2).

Before each testing session, calibration procedures were completed according to the manufacturer guidelines using an empty chamber and a calibrating cylinder of a standard volume (49.55 L). Once all tests passed, researchers proceeded with testing. A trained technician performed Bod Pod testing. Participants were instructed to enter the Bod Pod and sit in an erect position with their hands folded in their laps to obtain body volume. Athletes were instructed to wear unpadded compression shorts or spandex and, when applicable, a swim cap with the hair tucked in. They were asked to remove all jewelry to reduce air displacement. Athletes entered the Bod Pod and sat in an upright position with hands folded on their laps. Lung gas volume was estimated using manufacturer guidelines.Two tests were performed to ensure reliability of the assessment. If the tests results were not within 150 ml of each other, 2 more tests were executed to achieve reliable data.

2.3.2 Plyometric performance variables

Lower- and upper-body peak power (PWR_{peak}, w) and modified RSI (RSI_{mod}, AU) were calculated from countermovement jump (CMJ) and plyometric push-up (PP) assessments, using a commercially available force plate (AccuPower Force Platform, AMTI: Watertown, MA, USA). Athletes were instructed to keep their hands on their hips during the jump and were instructed to maintain straight legs during the flight, before landing on both feet with flexion of the hips, knees, and ankles. For the PP, athletes were positioned with their hands right below their shoulders and their feet shoulder-width apart. When instructed, athletes lowered their elbows to the height of their shoulders and pushed up as fast and as forcefully as they could. A trial was discarded and repeated when a participant did not lower their elbows to their shoulder height as visually inspected by the same trained researchers. Three attempts of CMJ and PP were conducted per assessment, with a minimum of 2-min rest between each trials to minimize fatigue effects.

PWR_{peak} was automatically yielded from AMTI's data acquisition software (NetForce, Watertown, MA, USA). RSI_{mod} was subsequently computed by normalizing force plate-derived jump height and ground contact time with respect to each athlete's body mass:

$$RSI_{mod} = \frac{\text{Jump Height}}{\text{Ground Contact Time X } \sqrt{\text{BodyMass}}}$$

2.3.3 Energy availability

Athletes were asked to record dietary intake using an online commercially available nutrition analysis program (MyFitnessPal, Under Armour, Baltimore, MD, USA). Prior to this period, participants attended an informational session where they were educated on methods to estimate portion sizes accurately. This session included detailed descriptions and demonstrations of portion size estimatation techniques. Additionally, they were provided with informational packets and instructional videos to reinforce accurate self-reporting. Daily energy and macronutrient intakes were averaged across the 3-day monitoring period. Absolute energy and macronutrient intakes (kcal/day or g/day) were recorded and were expressed relative to body weight (kcal/kg/day or g/kg/day) to enable comparison between individuals. During the same 3-day period, activity energy expenditure was monitored via wearable monitoring devices (Polar H9, Polar Electro, Kempele, Finland) and calculated from proprietary algorithms. These devices have been used in previous studies to measure activity energy expenditure in athletic populations (30). EA was then calculated by subtracting the activity energy expenditure from energy intake and expressed as kcals per kilogram of FFM. A threshold of <30 kcal/kg of FFM was used to classify athletes as having LEA.

2.4 Statistical analysis

SPSS version 29.0 (IBM Corp., Armonk, NY, USA) was used for summary statistics. Athletes were categorized into LEA vs. non-LEA groups to observe differences in body composition, EA, and plyometric performance variables. Absolute and relative carbohydrate, protein, and fat intake were also analyzed. However, due to the small sample size of athletes in the non-LEA group ($n = 2$), inferential statistics were de-emphasized and a descriptive analysis (mean ± SD, range) was prioritized. Pearson correlation coefficients were used to examine relationships between EA, body mass, fat-free mass, fat mass, body fat percentage, CMJ-height, CMJ-RSI_{mod}, CMJ-PWR_{peak}, PP-height, PP-RSI_{mod}, and PP-PWR_{peak}. The following criteria were used for interpreting the correlation coefficients: very weak: <0.20; weak: 0.20–0.39; moderate: 0.40–0.59; strong: 0.60–0.79; and very strong: >0.80 ($p < 0.05$) (31).

3 Results

A summary of anthropometric, body composition, and demographic data stratified by energy status are presented as mean ± SD in Table 1. All athletes exhibited body fat levels below 10% (9.2 ± 3.5%; range: 5.7–19.1), with a body mass of 67.6 ± 5.1 kg (range: 57.5–76.0), FFM of 61.3 ± 5.7 kg (range: 52.1–69.9), FFMI of 22.12 ± 1.7 kg m^2 (range: 18.93–24.76), and FM of 6.15 ± 2.2 kg (range: 3.5–12.3).

A summary of dietary intake (energy, carbohydrates, protein, fat), activity energy expenditure, and EA, stratified by LEA status is provided in Table 2. A total of 85.7% of athletes ($n = 12$) exhibited LEA (20.98 ± 5.2 kcals/kg FFM), with non-LEA athletes ($n = 2$) marginally surpassing the <30 kcal/kg of FFM threshold (30.58 ± 0.2 kcals/kg FFM), thus qualifying for subclinical LEA

(30–40 kcal/kg/FFM) (9, 17). The entire cohort ($n = 14$) consumed insufficient energy (30.5 ± 4.5 kcal/kg/day; range: 23.5–39.0), resulting in LEA (22.36 ± 5.9 kcal/kg/FFM; range: 12.0–30.7). Carbohydrate intake was low (3.7 ± 1.1 g/kg/day; range: 2.6–7.0), likely contributing to low energy intake. Relative protein and fat intakes for the cohort were 1.6 ± 0.4 kg (range: 90.3–113.6) and 0.9 ± 0.3 kg (range: 53.0–88.3), respectively. Average activity energy expenditure for the 3-day monitoring period was 685.8 ± 170.4 kcal (range: 400.0–1,003.0).

A summary of average movement height, RSI_{mod}, and PWR_{peak} for CMJ and PP, stratified by LEA status is included in Table 3. For lower body, athletes had a CMJ-height of 0.38 ± 0.05 m (range: 0.29–0.46), RSI_{mod} of 0.48 ± 0.10 AU/kg (range: 0.31–0.64), and a PWR_{peak} of 3,663.9 ± 563.6 w (range: 2,362.6–4,662.6). For upper body, athletes had a PP-height of 0.25 ± 0.09 m (range: 0.12–0.42), RSI_{mod} of 0.19 ± 0.06 AU/kg (range: 0.11–0.29), and PWR_{peak} of 1,678.9 ± 295.3 w (range: 1,233.8–2,301.9).

Relationships between EA, body composition metrics, and upper- and lower-body plyometric metrics are presented in Table 4. EA was not correlated with body composition metrics nor upper- or lower-body performance metrics. BF% was correlated with FFM ($p = 0.025$; 95% CI: −0.855, 0.093), FM [$p < 0.001$; 95% CI: (0.95, 0.995)], CMJ-height [$p = 0.012$; 95% CI: (−0.877, −0.177)], and CMJ-RSI_{mod} [$p = 0.43$; 95% CI: (−0.835, −0.024)]. FM was correlated with CMJ-height [$p = 0.008$; 95% CI: (−0.888, −0.226)] and CMJ-RSI_{mod} [$p = 0.037$; 95% CI: (−0.841, −0.043)]. CMJ-height was correlated with CMJ-RSI_{mod} [$p < 0.01$; 95% CI: (0.401, 0.923)] and CMJ-PWR_{peak} [$p = 0.04$; 95% CI: (0.031, 0.838)]. CMJ-RSI_{mod} was corelated with CMJ-PWR_{peak} [$p = 0.025$; 95% CI: (0.091, 0.855)]; PP-height was correlated with PP-PWR_{peak} [$p < 0.001$; 95% CI: (0.537, 0.945)].

TABLE 1 Summary of subject anthropometric, body composition and demographic data, stratified by energy status ($n = 14$).

	LEA ($n = 12$)	Non-LEA ($n = 2$)	All ($n = 14$)
Height (m)	1.66 ± 4.66	1.66 ± 6.8	1.66 ± 4.68
Body mass (kg)	67.9 ± 5.4	64.4 ± 2.2	67.6 ± 5.1 [1.36]
Fat-free mass (kg)	61.7 ± 6.1	58.7 ± 0.6	61.3 ± 5.7 [1.52]
FFMI ($kg \cdot m^2$)	22.78 ± 1.7	21.29 ± 1.9	22.12 ± 1.7
Fat mass (kg)	6.23 ± 2.37	5.74 ± 1.59	6.15 ± 2.2 [0.59]
Body fat (%)	9.2 ± 3.8	8.9 ± 2.2	9.2 ± 3.5 [0.94]
Age (years)	21.1 ± 1.3	20.5 ± 0.7	21.0 ± 1.2

Values are mean ± standard deviation; LEA, low energy availability; FFMI, fat-free mass index. Values within brackets represent the standard error of the estimate (SEE) for BodPod variables.

4 Discussion

The primary aim of the current study was to examine the prevalence of LEA and to explore dietary behaviors among a convenience sample of men collegiate gymnasts. Of note, 85.7% of the athletes within the cohort experienced LEA, as determined through direct assessment of dietary intake and activity energy expenditure. Additionally, the cohort consumed inadequate energy (30.5 ± 4.5 kcal/kg/day), largely influenced by a low carbohydrate intake (3.7 ± 1.1 g/kg/day), leading to the observed state of LEA (22.36 ± 5.9 kcals/kg FFM/day). No significant correlation existed between EA and either body composition or upper- and lower-body plyometric performance metrics. This study is novel as it marks the first instance of LEA prevalence reported in a sample of male gymnasts.

Previous reports in women's gymnastics indicate LEA prevalence rates range from 35%–56% for adult recreational and elite athletes (18), and 75% for teenage athletes (5), which are all lower than the prevalance of LEA observed in the current study. Moreover, the high prevalence of LEA observed in men gymnasts surpasses previous findings in men's dancing (29%) (32), a similar aesthetic sport emphasizing weight-control behavior. However, it is important to consider that LEA assessment in the study of men's dancing was conducted using the Dance-Specific

TABLE 3 A summary of average upper and lower body plyometric performance variables.

		LEA ($n = 12$)	Non-LEA ($n = 2$)	All ($n = 14$)
CMJ	Height (m)	0.37 ± 0.05	0.39 ± 0.09	0.38 ± 0.05
	RSI_{mod} (AU)	0.47 ± 0.09	0.54 ± 0.14	0.48 ± 0.10
	PWR_{peak} (w)	3,656.4 ± 612.3	3,708.6 ± 36.4	3,663.9 ± 563.6
PP	Height (m)	0.24 ± 0.09	0.31 ± 3.4	0.25 ± 0.09
	RSI_{mod} (AU)	0.19 ± 0.06	0.22 ± 0.09	0.19 ± 0.06
	PWR_{peak} (w)	1,644.6 ± 303.9	1,885.1 ± 133.7	1,678.9 ± 295.3

Values represented as mean ± standard deviation; CMJ, countermovement jump; PP, plyo push-up; RSI_{mod}, modified reactive strength index; PWR_{peak}, peak power; LEA, low energy availability.

TABLE 2 A summary of average daily activity energy expenditure and macronutrient intake by energy status.

		LEA ($n = 12$)	Non-LEA ($n = 2$)	All ($n = 14$)
Intake	Energy Intake (kcal/day)	1,987.4 ± 268.3	2,051.8 ± 300.8	2,051.8 ± 300.8
	Relative energy intake (kcal/kg/day)	29.3 ± 3.5	37.8 ± 1.7	30.5 ± 4.5
	Carbohydrate intake (g/day)	246.8 ± 81.9	278.0 ± 96.2	251.2 ± 80.7
	Relative carbohydrate intake (g/kg/day)	3.6 ± 1.1	4.3 ± 1.3	3.7 ± 1.1
	Protein intake (g/day)	110.6 ± 32.8	102.0 ± 16.5	109.4 ± 230.7
	Relative protein intake (g/kg/day)	1.6 ± 0.4	1.6 ± 0.4	1.6 ± 0.4
	Fat intake (g/day)	66.5 ± 16.6	70.7 ± 24.9	67.1 ± 16.8
	Relative fate intake (g/kg/day)	0.98 ± 0.2	1.1 ± 0.4	0.9 ± 0.3
Expenditure	Activity energy expenditure (kcal)	692.9 ± 177.8	654.5 ± 159.1	685.8 ± 170.4
status	Energy availability (kcals/kg FFM/day)	20.98 ± 5.2	30.58 ± 0.2	22.36 ± 5.9

Values represented as mean ± standard deviation; LEA, low energy availability; FFM, fat-free mass.

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TABLE 4 Relationships between energy availability, body composition, and upper- and lower-body plyometric variables.

	Body mass (kg)	BF% (%)	FFM (kg)	FFMI ($kg \cdot m^2$)	FM (kg)	EA (kcal/kg FFM)	REI (kcal)	CMJ height (m)	CMJ RSI_{mod} (AU)	CMJ PWR_{peak} (w)	PP Height (m)	PP RSI_{mod} (AU)	PP PWR_{peak} (w)
Body mass (kg)	1	−0.234	0.921[b]	0.732[b]	−0.063	−0.101	−0.227	−0.103	−0.013	−0.264	0.0,264	0.324	0.096
BF% (%)		1	−0.595[a]	−0.504	0.984[b]	0.136	−0.082	−0.647[a]	−0.547[a]	−0.445	−0.219	−0.051	−0.102
FFM (kg)			1	0.806[b]	−0.447	−0.140	−0.156	0.172	0.207	0.442	−0.138	0.285	0.116
FFMI ($kg \cdot m^2$)				1	−0.385	−0.301	−0.261	0.082	0.110	0.210	−0.108	0.493	−0.009
FM (kg)					1	0.125	−0.122	−0.675[b]	−0.561[a]	−0.391	−0.252	0.014	−0.076
EA (kcal/kg FFM)						1	0.845[b]	0.212	0.206	0.289	0.339	0.187	0.408
REI (kcal)							1	0.273	0.136	0.254	0.474	0.146	0.520
CMJ-Height (m)								1	0.768[b]	0.553[a]	0.324	0.103	0.144
CMJ-RSI_{mod} (AU)									1	0.593[a]	0.088	−0.015	−0.059
CMJ-PWR_{peak} (w)										1	0.458	0.051	0.483
PP-Height (m)											1	0.039	0.831[b]
PP-RSI_{mod} (AU)												1	0.0–82
PP-PWR_{peak} (w)													1

[a]Correlation is significant at the 0.05 level; [b]Correlation is significant at the 0.01 level; BF%, percent body fat; FFM, fat-free mass; FM, fat mass; REI, relative energy intake; EA, energy availability; CMJ, countermovement jump; PP, plyometric push-up; RSI_{mod}, modified reactive strength index; PWR_{peak}, peak power.

Energy Availability Questionnaire (DEAQ), underscoring the challenge of making direct comparisons between studies due to differences in measurement techniques (9). LEA prevalence rates of 25% (33), 30% (34), 66% (35), and 70% (10), have been reported in male endurance athetes from non-aesthetic sports like cycling, distance running, and race walking. While LEA rates are notably lower than those observed in the current study, these studies had larger sample sizes ($n = 21–53$) and longer assessment durations (≥7 days). The absence of a standardized reference time frame for dietary or exercise assessments complicates absolute comparisons of LEA prevalence (36). Further, these studies involved well-trained, elite adult, or masters participants (age range: 18–50 years), potentially lacking the unique constraints experienced by the collegiate athletes in the current study, such as limited fueling opportunites due to demanding academic schedules, which could hinder access to optimal nutritional resources (37). It is important to note that the cross-sectional nature of these studies makes it unclear whether the causes of LEA are intentional, such as disordered eating behaviors, or inadvertent, stemming from factors like high-volume training or lack of education (9, 21, 38). With the exception of high-level endurance sports, which require high-volumes of training, low energy intake likely underpins the high prevalence of LEA for most aesthetic sport athletes. For example, the activity energy expenditure observed in the current study (692.9 ± 177.8 kcal) would not be considered an excessively high activity level. As such, it is apparent that nutrient deficiencies, primarily insufficient macronutrient energy consumption, plays a major role in influencing LEA prevalence (10).

The mean EA value observed in the current study was 22.36 ± 5.9 kcals/kg FFM/day, falling below the threshold of <30 kcals/kg FFM/day used to classify individuals with LEA (17). Surprisingly, it is lower than previously reported values in pre-teenage (60.04 kcal/kg FFM/day), teenage (29.7 kcal/kg FFM/day), and adult (31.5 kcals/kg FFM/day) female gymnasts (5, 19). Differences in sample size, measurement techniques, and assessment durations may partially explain the disparities in EA. However, the notably lower EA observed in the current study suggests potential issues with energy intake among men collegiate gymnasts that warrant further investigation. In part, lower EA values may be explained by the greater amounts of FFM in male gymnasts compared to females. It is plausible that men and women gymnasts may have comparable levels of energy expenditure; thus if the men neglected to compensate by increasing energy intake, lower EA values will result. The mean EA for athletes in the current study is comparable to previous season average values reported for male cyclists (20 kcals/kg FFM/day) (10), but appears lower than previously reported preseason values for male distance runners and racewalkers (27–36 kcals/kg FFM/day) (39, 40). Further, the mean FFM (61.3 ± 5.7 kg) for the gymnasts is comparable to that previously reported for male cyclists (63.8 ± 3.8 kg). These findings contribute to the growing body of evidence suggesting that male athletes are vulnerable to the adverse effects of LEA, particularly those engaged in high-volume training (e.g., distance runners), weight-category sports (e.g., wrestlers), and aesthetic-based

competitions (e.g., gymnasts, dancers) (9) due to their higher energy requirements and greater amounts of FFM.

The primary contributor of LEA appears to stem from insufficient energy intake, namely from a low carbohydrate intake. Scientific societies have provided recommendations regarding the optimal carbohydrate intake for athletes based on their specific sports disciplines (41, 42). For men's and women's gymnastics, a relative carbohydrate intake of 5–7 g/kg/day is suggested (42). However, while previous reports indicated an average carbohydrate intake of 5.1 ± 2.3 g/kg/day for adult female gymnasts without LEA (19), gymnasts in the current study consumed 3.7 ± 1.1 g/kg/day, a level similar to that reported for teenage female gymnasts with LEA (3.7 ± 1.3 g/kg/day) (5). Given the limited evidence indicating substantial differences in carbohydrate utilization between adolescents and adults (43), it seems that inadequate carbohydrate intake is the most probable contributor to energy deficiency in male and female gymnastics athletes, irrespective of age (5, 43). In support, protein (1.6 ± 0.4 kcal/kg/day) and fat (0.9 ± 0.3 kcal/kg/day) intake were within appropriate ranges (41, 42) considering the type and amount of physical activity performed by the men gymnasts. However, the focus on leanness in gymnastics often results in weight-control measures, including carbohydrate restriction, a trend also noted in competitive male cyclists (10). Research highlights the prevalence of fad diets advocating carbohydrate restriction in sports emphasizing leanness (10), which stresses the importance of sport nutrition education, practical dietary skills development, and behavior change interventions, which may be necessary to effectively address nutritional deficiencies among athletes (16).

The current investigation is the first to report PWR_{peak} and RSI_{mod} values for a sample of collegiate men gymnasts. Notably, the CMJ-PWR_{peak} (3,663.9 ± 563.6 w) exceeds previous reports for NCAA DI women gymnasts (32,100 ± 350 w) (27) and substantially surpasses findings for male youth gymnasts (1,400 ± 543 w) (28). While average RSI_{mod} has not been documented in other gymnastics populations (e.g., women, youth), the observed average RSI_{mod} in current study (0.48 ± 0.10 w) surpasses values reported for men's baseball (0.41 ± 0.08 w), men's tennis (0.30 ± 0.07 w), and men's soccer (0.44 ± 0.05 w) athletes (44). The notably higher RSI_{mod} observed in men gymnasts compared to athletes in field or court sports underscores the unique demands of gymnastics, where athletes heavily rely on the SSC during plyometric activities, as they maneuver their bodies acrobatically on various apparatuses. Over time, this likely leads to sport-specific adaptations, which help develop lower-body power and explosiveness. Currently, PP-PWR_{peak} (1,678.9 ± 295.3 w) or PP-RSI_{mod} (0.19 ± 0.06 w) values have not been reported for gymnastics or other aesthetic sport athletes.

A secondary aim of the current study was to investigate the relationships between EA, body composition metrics, and upper- and lower-body plyometric performance metrics, namely RSI_{mod} and PWR_{peak}, in a sample of collegiate men's gymnastics athletes. While no correlations were found, this finding contrasts with a recent study conducted by Jurov et al., which observed decreases in CMJ height following 14 days of induced LEA with a 50% reduction in EA in trained male endurance athletes (45). In a subsequent study, the same research group observed decreases in PWR_{peak}, relative power, and CMJ-height following 14 days of induced LEA with a 25% reduction in EA (46). In a final study, where EA was reduced in progressive stages of 25%, 50%, and 75%, Jurov et al. discovered CMJ height decreased proportionately with increased EA reductions, and most of the negative effects occurred in the range of 9–25 kcals/kg FFM/day (47). Differences between the aforementioned studies and the current one in subject populations, sport demands, and study design, may account for the disparate results between EA and plyometric performance. However, it is noteworthy that the EA observed in the current study (22.36 ± 5.9 kcals/kg FFM/day) falls within the upper range associated with the most pronounced negative effects on performance as reported by Jurov et al. (47). Further, reports suggesting that suboptimal EA detrimentally affects explosive power even prior to hormonal changes indicate the potential utility of monitoring changes in plyometric performance as early indications of the adverse effects of LEA (46). However, more research is needed to elucidate the specific impact of LEA on plyometric performance in men's gymnastics. Additionally, the current study reported significant negative associations ($r = -0.547$) between CMJ-RSI_{mod} and BF%, emphasizing the importance of maintaining low BF%. This is critical for not only enhancing movement efficiency and promoting aesthetic presentation but also for optimizing strength-to-mass ratio and gaining various biomechanical advantages during set routines (3). Lastly, our findings revealed no significant correlation between EA and body composition variables, contrasting with reports from studies on athletes in other sports (48). This may be due to the lack of standardized protocols for calculating EA, potential errors in self-reported dietary intake and activity expenditure measurements, and individual variability in physiological responses (49). The complex nature of body composition changes in response to EA further complicates the detection of clear correlations (49). Future research with larger sample sizes, accurate measurement techniques, and standardized protocols is necessary to elucidate this relationship.

While the current study has provided valuable insights into dietary behaviors and plyometric performance for a sample of collegiate male gymnasts, a few limitations should be considered. First, the study's small sample size ($n = 14$) may limit the generalizability of the findings. Additionally, the reliance on a single time point, heart rate-derived activity energy expenditure measures, and self-reported dietary intake might not fully capture the dynamic nature of athletes' energy balance. It should also be noted that the Polar H9 heart rate monitors, used in this study to estimate activity energy expenditure, have not been validated for measuring exercise energy expenditure in activities like gymnastics that often involve substantial anaerobic components. Further, under-reporting is a common issue in dietary self-reporting, and there is the potential for subjects to alter their dietary intake during the recording period, which might not reflect their long-term intake patterns (49). Lastly, the lack of information on whether certain athletes were undergoing intentional weight-cutting during data collection is a notable limitation, as such phases could influence energy intake and

expenditure, potentially skewing the results. Future research with larger cohorts and longitudinal designs is warranted to clarify these relationships in greater detail.

5 Conclusion

The current research is novel in that it provides the first report of LEA prevalence and upper- and lower-body plyometric performance metrics in a sample of men collegiate gymnasts. Results indicate there may be a high prevalence of LEA, underscoring the need for attention to nutritional practices within this population. Findings suggest that athletes are underfueling, particularly in terms of carbohydrates, which is critical for meeting the energy demands of intense gymnastics training and competition. Failure to address LEA may have implications for performance outcomes among athletes participating in weight-control sports. While the relationship between EA and plyometric performance has been documented in sports such as distance running, it has yet to be determined in men's gymnastics, underscoring the need for further research. Additionally, prolonged LEA can compromise various aspects of athlete health, including bone metabolism, hormonal function, and immune response, thereby elevating the risk of injuries and illness. Interventions aimed at optimizing EA and carbohydrate intake are important for safeguarding the well-being, and potentially physical performance, of men collegiate gymnasts.

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 Springfield College Institutional Review Board. 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

NK: Writing – original draft, Writing – review & editing, Data curation, Formal Analysis. MJ: Writing – original draft, Writing – review & editing, Conceptualization. AJ: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. MM: Data curation, Formal Analysis, Writing – review & editing. LW: Data curation, Project administration, Visualization, Writing – original draft, Writing – review & editing. JF: Methodology, Project administration, Supervision, 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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A low-carbohydrate, high-fat diet leads to unfavorable changes in blood lipid profiles compared to carbohydrate-rich diets with different glycemic indices in recreationally active men

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Objective: In addition to recent discussions of low-carbohydrate, high-fat diets (LCHF) from a performance perspective, there is a paucity of knowledge regarding influence of the combined effect of an exercise and nutritional intervention, which varies in carbohydrate (CHO) intake and glycemic indices, on blood lipid levels in recreationally active men.

Methods: A total of 65 male runners (VO_2 peak = 55 ± 8 mL·min^{-1}·kg^{-1}) completed a 10-week *ad libitum* nutritional regimen (LOW-GI: ≥ 65% low GI CHO per day, n = 24; HIGH-GI: ≥ 65% high GI CHO per day, n = 20; LCHF: ≤ 50 g CHO daily, n = 21) with a concurrent prescribed endurance training intervention. Fasting total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined before and after the intervention. Additionally, 24-h dietary recalls were completed twice weekly.

Results: Following the intervention, TC was significantly higher in LCHF (196 ± 37 mg·dL^{-1}) compared to both LOW-GI (171 ± 41 mg·dL^{-1}) and HIGH-GI (152 ± 28 mg·dL^{-1}, $p < 0.001$). Additionally, LDL-C levels increased in LCHF (+17 ± 21 mg·dL^{-1}, $p = 0.001$), while they decreased in both CHO groups ($p < 0.05$, respectively). Only the HIGH-GI group demonstrated a significant reduction in HDL-C (−3 ± 9 mg·dL^{-1}, $p = 0.006$), while a decrease in TG was only significant in LOW-GI (−18 ± 36 mg·dL^{-1}, $p = 0.008$).

Conclusion: Although mean blood lipid levels remained within the normal range, the data indicate that a low-carbohydrate, high-fat (LCHF) diet leads to unfavorable changes in individual blood lipid profiles compared to carbohydrate-rich diets. Therefore, it is recommended that the impact of a low-carbohydrate diet on blood lipids be considered when counseling active and healthy individuals.

KEYWORDS

blood lipids, cholesterol, lipoproteins, triglycerides, carbohydrates, low-carb, glycemic index

1 Introduction

In recent times, low-carbohydrate-high-fat (LCHF) diets have become a popular choice amongst endurance athletes seeking to enhance their capacity to utilize fat as a fuel source (1). A reduction in carbohydrate (CHO) intake results in a shift in substrate utilization toward a reliance on fat in circumstances where carbohydrate stores in the form of glycogen would typically be used (2–4). However, since carbohydrate stores are finite and a LCHF diet might promote increased fat oxidation while sparing CHO stores, this theory still appeals to some endurance athletes, despite official guidelines recommending a high-carbohydrate diet (5–8). In addition to the potential enhancements in their substrate metabolism, some athletes may also observe the favorable effects on weight and cardiometabolic health of a LCHF diet that have been evidenced in the general population. In this cohort, it has been demonstrated that an LCHF diet can have a beneficial impact on conditions such as obesity (9), metabolic syndrome (10) and type 2 diabetes (11). In light of these arguments, it appears reasonable to conclude that some endurance athletes view an LCHF diet as a healthy and effective approach.

In addition to the debate as to whether a LCHF should be recommended from a performance perspective (12–14), the cardiometabolic health benefits of a LCHF diet are frequently misinterpreted by endurance athletes in practice. Given that the majority of athletes already have a favorable health status, the interpretation of studies reporting health benefits of a LCHF diet must be approached with caution, as the reported improvements were often observed in individuals with overweight or existing metabolic disturbances. The assumption that an athlete's physical condition and lifestyle are sufficient to protect them from cardiometabolic diseases, such as dyslipidemia, leads most athletes to be less concerned about the impact of their daily diet on their cardiometabolic health status. Instead, they focus on optimizing their daily intake of nutrients in order to enhance their training adaptations and competition performance.

Indeed, there is limited evidence, that a LCHF might lead to unfavorable alterations in blood lipid concentrations in endurance athletes (15). Athletes who follow a LCHF diet may experience an increase in total cholesterol (TC) levels. However, only three trials were included in the meta-analyses. Nevertheless, the findings should be regarded with respect by athletes who adhere to or plan to employ a LCHF diet, as they may already be at an elevated risk for arterial wall stiffening and myocardial fibrosis due to the high training volumes they engage in (16). In light of the ambiguous evidence regarding the performance benefits of a LCHF diet, endurance athletes who adhere to this dietary regimen may unintentionally elevate their risk of cardiovascular dysfunction. This could potentially negate the favorable cardiometabolic health outcomes achieved through training.

In addition, it still remains uncertain whether the findings on the impact of the glycemic index (GI) on blood lipids can be extrapolated to physically active individuals. Previous research has shown that reducing the GI can lead to beneficial changes in blood lipid levels in people with type 1 and 2 diabetes (17, 18) or obesity (19), as well as in young adults (20, 21). In fact, a meta-analysis of 28 randomized controlled trials in overweight and obese subjects has consistently shown that a diet containing low GI CHO can reduce TC and LDL-C. However, no effects on HDL-C or triglycerides (TG) were found (22). Compared to a LCHF diet, a low GI diet was associated with longer-lasting positive changes in cardiometabolic parameters, such as TC and HDL-C (19).

Despite the existing research on the impact of carbohydrate restriction on blood lipid levels in athletes (23–32), the current literature on this topic is still evolving and the acquisition of further data can contribute to a more nuanced understanding. Furthermore, the additional investigation of the GI should provide new insights into the effects of a high or low GI on blood lipids in athletic individuals. Thus, the main aim of this study was to investigate the impact of different nutritional regimens, which vary in carbohydrate content and GI, on blood lipid levels in recreationally active runners enrolled in a 10-week prescribed endurance training program. It was hypothesized that a LCHF diet would lead to higher levels of TC, LDL-C, and TG when compared to a carbohydrate-rich diet. Moreover, we considered that a low GI would have a beneficial impact on blood lipid profiles, in comparison to a high GI.

2 Materials and methods

This study is a secondary analysis from an investigation conducted at the University of Vienna (33). The original study followed an open, randomized, non-blinded design. The primary outcome variables in the present observation are distinct, with only the interventional data and subjects' characteristics being shared. Accordingly, a comprehensive overview of the methodological approaches employed in the current study has been published elsewhere (33), but are summarized here for clarity. Specific methods only applied in this study will be provided in further detail. The registration of this study is located at ClinicalTrials.gov with the Identifier NCT05241730. This study adhered to all CONSORT guidelines (34). The original study protocol for this clinical trial, including the CONSORT diagram demonstrating participant flow, can be found in Moitzi, Kršňák (33).

2.1 Participants

A summary of the subject's characteristics can be found in Table 1. The initial cohort comprised 87 participants, who were randomly assigned to one of the three interventional groups. For various reasons, including non-compliance, infection with the SARS-CoV-2 virus, and personal withdrawal, 65 of the initial 87 recruited runners completed the study in its entirety.

TABLE 1 Subject characteristics.

	LOW-GI	HIGH-GI	LCHF	*p*-value
N	**24**	**20**	**21**	–
Age [years]	30 ± 4	29 ± 4	27 ± 4	0.112
Height [cm]	182 ± 7	180 ± 6	182 ± 7	0.740
Weight [kg]	79.5 ± 8.1	77.1 ± 11.3	81.5 ± 10.8	0.354
BMI [kg·m^{-2}]	24.1 ± 2.8	23.7 ± 3.2	24.6 ± 3.3	0.661
Active days per week	3 ± 1	3 ± 1	3 ± 1	0.886
VO_2 peak [mL·min^{-1}·kg^{-1}]	54 ± 7	55 ± 7	55 ± 9	0.967

The inclusion criteria required recreationally active (2–3 training sessions per week) male endurance athletes without any medical conditions. Exclusion criteria included experience within the last 6 months with one of the interventional diets, contraindication to physical activity according to the American College of Sports Medicine Guideline (35), use of medications or dietary supplements that could affect measurements or are prohibited by the WADA code, chronic diseases, and arterial hypertension.

The study protocol underwent review by the Ethical Committee of the Medical University of Vienna (EK Nr: 2105/2021), the Ethical Committee of the University of Vienna (Reference number: 00871), and was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participant prior to beginning of the intervention.

2.2 Intervention

The intervention lasted for a period of 10 weeks. Before the initial visit, all participants underwent a screening process that included a medical examination and an evaluation of their readiness for physical activity using the PAR-Q. Additionally, anthropometric and performance data, such as body composition and a graded exercise test, were collected; all of which are described in detail elsewhere (33). Enrolled participants were assigned to one of three groups (LOW-GI, HIGH-GI, and LCHF) based on their VO_2 peak to minimize performance-related outcomes, as proposed by Hopkins (36).

Diet prescription is outlined in detail by Moitzi, Kršśák (33). In brief, participants were instructed to adhere to their respective dietary patterns. All dietary regimens were designed as *ad libitum*, with subjects preparing their own meals in accordance with the respective group guidelines:

- LOW-GI: 50–60% carbohydrates with ≥65% of energy from low glycemic index (GI < 50) carbohydrates per day
- HIGH-GI: 50–60% carbohydrates with ≥65% of energy from high glycemic index (GI > 70) carbohydrates per day
- LCHF: ≥ 65% fat, maximum of 50 g carbohydrates per day.

The endurance exercise intervention was prescribed for all groups and consisted of five running sessions per week (three session constant moderate load, two sessions heavy strenuous load), with an average of 230 training minutes per week. The training zones were adjusted individually based on the results of the graded exercise test. The training was conducted individually by the test subjects, allowing for personal preferences regarding the time of day or the running route to be taken into account. The training sessions were uploaded onto the sports watch (Polar Vantage M, Polar Electro Oy, Kempele, Finnland) in advance, and during the session, the subjects received feedback from the watch via vibration indicating whether they were in the desired zone. Sessions were therefore recorded with a watch and a heart rate belt (Polar H10, Polar Electro Oy, Kempele, Finland) and controlled weekly by the study management. It was stated prior to the study that participants must complete a minimum of 75% of the prescribed training minutes to be included in the final analysis. Of the 87 participants, 11 were unable to achieve the requisite 75% of the prescribed training minutes.

2.3 Compliance evaluation

To monitor nutritional compliance, participants were instructed to record their food intake for one weekday and one weekend day per week. Trained dieticians reviewed the records using nut.s software (Dato Denkwerkzeuge, Wien, Austria). The food consumed was entered and analyzed in the software. In addition to the energy intake and macronutrient values, the fatty acids and fiber consumed were also analyzed in this study. Compliance during the study was assessed by calculating the mean of 20 24-h recalls per subject, which was then used for further calculations.

To assess nutrition prior to the intervention a 24-h recall and a food frequency questionnaire were used. The validated DEGS1-FFQ collects the frequency and quantity of 53 food items eaten in the last 4 weeks (37). The questionnaire was completed online and converted to nutritional intake according to previous proposed methods (38). For the baseline value the mean of the 24-h recall and the FFQ was used.

The determination of the GI of the diets was based on Atkinson, Brand-Miller (39) and Atkinson, Foster-Powell (40). To calculate the average GI of each recall, the percentage contribution of each individual CHO-containing food was multiplied by its glycemic index. The sum of these products was then divided by the number of meals and was taken as the GI of this recall. Finally, the mean GI of all protocols was determined for each subject in the carbohydrate groups. Given the markedly low carbohydrate intake observed in the LCHF cohort, the GI was not calculated for any of the participants in LCHF group.

2.4 Blood lipid biomarker analysis

Participants were instructed to arrive at the laboratory in the morning after an 8-h fasting period for the blood draw, both before and after the 10-week intervention. To minimize circadian influences blood samples were taken ±1 h before and after the intervention. A trained phlebotomist obtained an 8.5-ml blood sample through venipuncture. The serum sample (BD Vacutainer SST II Advance, Belliver Industrial Estate, Plymouth, United Kingdom) was sent to at a certified laboratory (Synlab, Institut für medizinische und chemische Labordiagnostik GmbH, Vienna, Austria) for subsequent analysis of total cholesterol (TC), HDL-C, LDL-C, and triglycerides (TG). There analyses for TC, HDL-C and TG were performed using an enzymatic color test in a clinical chemistry analyzer (AU5822, Beckman Coulter, Brea, USA). The respective Beckman Coulter kit numbers were OSR6216 for TC, OSR6287 for HDL-C, and ORS61118 for TG. LDL-C was calculated using the Friedewald equation, where:

$$LDL - C = TC - HDL - C - \frac{TG}{5}.$$

2.5 Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences Software (SPSS for Windows, Version 28, SPSS Inc., Chicago, IL). Figures were created using GraphPad Prism (GraphPad Prism Version 8.0.2 for Windows, GraphPad Software,

San Diego, California, United States). The level of significance was set at $\alpha = 0.05$. Results are presented as mean ± standard deviation (SD).

The normality of the distribution was evaluated using the Shapiro–Wilk test. The differences between groups at baseline and different changes during the intervention were evaluated using a one-way ANOVA and in case of no given normal distribution a Kruskal-Wallis test was used. If significant differences were identified, a Tukey post-hoc test was conducted to ascertain which groups exhibited significant differences. To assess differences in time (within-subject factor), group (between-subject factor), and time x group interaction effects, a two-way mixed ANOVA with Tukey-corrected *post hoc* analyses was conducted. In the event of a significant interaction, simple main effects for group and time were analyzed. For significant results, we displayed effect sizes for one-way ANOVA (ηp^2) and simple time effects (Cohen's *d*). Due to observed baseline differences in LDL-C levels among the groups, a one-way ANCOVA was employed to adjust for these initial disparities and assess the effect of the interventions on follow-up LDL-C levels. Baseline LDL-C level was included as a covariate to control for initial differences among participants. *Post hoc* comparisons were performed using the Bonferroni correction to adjust for multiple testing.

In order to ascertain the relationship between alterations in blood lipid levels, body weight and dietary intake, a Pearson's correlation was conducted. If normal distribution was not given, Spearman was used. Therefore, the change was calculated as value post minus value pre (Δ). Variables used for the correlation analysis included TC, HDL-C, LDL-C, TG, body weight, energy intake, relative macronutrient intake, glycemic index, fatty acids intake and fiber intake.

Lastly, a multiple linear regression was conducted to refine predictive models for changes in blood lipid concentrations (ΔTC, ΔHDL-C, ΔLDL-C and ΔTG), initially including changes in energy intake, relative macronutrient intake (CHO, proteins, fat), glycemic index, fiber intake, body weight and composition (fat mass, fat-free mass) as potential predictors. The analysis utilized a backward elimination method, with a criterion set at a probability of F-to-remove greater than or equal to 0.100. The predictors with the highest adjusted R^2 from the backward elimination method were included in the final model. A multiple linear regression was calculated with those predictors using the enter method. Linearity was assessed by visual interpretation of partial regression plots and a plot of studentized residuals against the predicted values. Homoscedasticity was assessed via visual inspection of a plot of studentized residuals versus unstandardized predicted values. When independent variables had a correlation coefficient $R > 0.8$ and the multicollinearity was harmed, one of the parameters was removed from the model.

Eight participants lacked baseline data on dietary fiber and fatty acid intake due to issues with data collection. Nevertheless, the energy intake and macronutrient intake of these participants were evaluated using the FFQ.

3 Results

3.1 Study population

As already stated detailed information about subjects, body weight and composition and performance-measurements are published in Moitzi, Kršśák (33). For clarity, subjects baseline characteristics are shown in Table 1.

3.2 Nutritional intervention

Detailed nutritional data are already published in Moitzi, Kršśák (33). Data on energy intake and relative macronutrient intake will be presented in brief in the following. Energy intake was significantly reduced in LOW-GI (T-0: 2178 ± 556 kcal vs. T-10: 1784 ± 502 kcal, $p < 0.001$, $d = 0.802$). During the intervention, intake was significantly higher in HIGH-GI (2124 ± 462 kcal) compared to the LCHF (1755 ± 468 kcal, $p = 0.043$, $\eta p^2 = 0.109$). Analysis revealed significant interaction effects for relative carbohydrate, protein, and fat intake. During the intervention, LOW-GI (50.5 ± 5.4%) and HIGH-GI (53.5 ± 5.6%) had a significantly higher relative carbohydrate intake compared to LCHF (10.6 ± 3.7%, $p < 0.001$, $\eta p^2 = 0.941$). All groups experienced a significant change in relative protein intake. LOW-GI (+3.4 ± 2.4%, $p < 0.001$, $d = 1.452$) and LCHF (+9.3 ± 4.7%, $p < 0.001$, $d = 1.975$) had an increase in relative protein intake, while HIGH-GI (−1.1 ± 2.3%, $p = 0.042$, $d = 0.487$) had a decrease. There were significant differences in protein intake between all groups (for all pairwise comparisons $p < 0.050$, $\eta p^2 = 0.773$). The LOW-GI group experienced a decrease in relative fat intake (−3.1 ± 6.9%, $p = 0.034$, $d = 0.461$), while the LCHF group experienced an increase (+27.9 ± 8.9%, $p < 0.001$, $d = 3.152$) and intake in HIGH-GI remained unchanged (−2.0 ± 5.2%, $p = 0.104$). Fat intake also differed significantly between the LCHF group and the two carbohydrate groups ($p < 0.001$, $\eta p^2 = 0.906$). The study evaluated the glycemic index for LOW-GI and HIGH-GI. The study found that the glycemic index decreased in the LOW-GI group (−22 ± 9, $p < 0.001$, $d = 1.469$) and increased in the HIGH-GI group (+7 ± 7, $p < 0.001$, $d = 0.993$). There was a significant difference in the glycemic index between the two groups during the intervention (LOW-GI: 41 ± 3, HIGH-GI: 64 ± 3, $p < 0.001$, $d = 7.555$).

In addition to macronutrient intake, the intake of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 (n-3-FA) and omega-6 fatty acids (n-6-FA), total fiber, soluble and insoluble fiber were determined from the food protocols and are shown in Table 2. SFA intake was significantly reduced in LOW-GI (−7.7 ± 15.4 g·day^{-1}, $p = 0.025$, $d = 0.529$), while in LCHF, SFA intake increased (+34.3 ± 16.6 g·day^{-1}, $p < 0.001$, $d = 2.065$). Intake during the study was significantly higher in the LCHF group compared to the LOW-GI or HIGH-GI group ($p < 0.001$, $\eta p^2 = 0.458$). Intake of MUFA showed no difference between the LOW-GI and HIGH-GI group, whereas intake in the LCHF group increased significantly (+31.6 ± 30.5 g·day^{-1}, $p < 0.001$, $d = 1.036$) and was significantly higher during the study compared to both other groups ($p < 0.001$, $\eta p^2 = 0.513$). Additionally, n-3-FA intake increased in LCHF (+3.4 ± 4.0 g·day^{-1}, $p = 0.004$, $d = 0.860$) with a significantly higher intake during the intervention compared to LOW-GI or HIGH-GI ($p < 0.001$, $\eta p^2 = 0.454$). Intake of PUFA or n-6-FA showed no significant interaction effect. However, the main effect for the group showed a significantly higher mean PUFA-intake in LCHF (24.2 ± 13.7 g·day^{-1}) compared to LOW-GI (16.0 ± 6.0 g·day^{-1}, $p = 0.008$, $\eta p^2 = 0.163$) independent of time.

The fiber intake during the study was significantly higher in LOW-GI compared to LCHF or HIGH-GI ($p < 0.001$, $\eta p^2 = 0.369$).

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TABLE 2 Fatty acids and fiber intake before (T-0) and during the intervention.

	Group	T-0	During the intervention (T-10)	Time x Group	Simple group effect at T-10
SFA [g·day^{-1}]	LOW-GI	35.9 ± 16.6	26.9 ± 13.2 ^{c,*}	**<0.001**	**<0.001**
	HIGH-GI	31.9 ± 20.3	36.0 ± 14.2 ^{c}		
	LCHF	30.4 ± 16.3	60.4 ± 19.7 ^{a,b,*}		
MUFA [g·day^{-1}]	LOW-GI	30.0 ± 12.8	27.9 ± 10.6 ^{c}	**<0.001**	**<0.001**
	HIGH-GI	34.0 ± 21.1	31.6 ± 11.5 ^{c}		
	LCHF	36.2 ± 24.6	63.2 ± 22.7 ^{a,b,*}		
PUFA [g·day^{-1}]	LOW-GI	16.0 ± 7.2	16.0 ± 5.1	0.135	**0.008 # (LCHF vs. LOW-GI)**
	HIGH-GI	19.8 ± 11.8	18.5 ± 6.0		
	LCHF	20.5 ± 15.6	27.0 ± 12.2		
N-3-FA [g·day^{-1}]	LOW-GI	2.1 ± 1.5	2.3 ± 0.7 ^{c}	**<0.001**	**<0.001**
	HIGH-GI	4.7 ± 6.0	3.3 ± 2.1 ^{c}		
	LCHF	3.2 ± 2.0	6.5 ± 2.8 ^{a,b,*}		
N-6-FA [g·day^{-1}]	LOW-GI	13.9 ± 7.1	13.7 ± 4.6	0.473	0.073 #
	HIGH-GI	15.9 ± 11.1	15.2 ± 5.5		
	LCHF	17.4 ± 14.6	20.5 ± 10.0		
Fiber [g·day^{-1}]	LOW-GI	35.2 ± 12.9	40.8 ± 8.9 ^{b,c}	**0.038**	**<0.001**
	HIGH-GI	32.9 ± 8.6	28.0 ± 8.8 ^{a}		
	LCHF	28.2 ± 13.9	25.9 ± 9.4 ^{a}		
Soluble fiber [g·day^{-1}]	LOW-GI	10.7 ± 4.0	12.1 ± 2.8 ^{c}	**0.014**	**<0.001**
	HIGH-GI	11.6 ± 3.2	10.4 ± 4.4 ^{c}		
	LCHF	8.4 ± 4.5	5.9 ± 2.4 ^{a,b,*}		
Insoluble fiber [g·day^{-1}]	LOW-GI	24.1 ± 9.4	28.7 ± 6.3	0.068	**<0.001 # (LCHF vs. LOW-GI)**
	HIGH-GI	21.3 ± 7.7	19.0 ± 5.2		
	LCHF	19.1 ± 10.4	18.1 ± 7.6		

^{a,b,c} Significantly different intake between groups during the intervention: ^{a}compared to LOW-GI, ^{b}compared to HIGH-GI, ^{c}compared to LCHF. T-0: baseline values, T-10: values during the intervention. * significant different from T-0. # reflects *p*-value of main effects. Bold numbers represent a significant *p*-value.

Intake of soluble fiber decreased in LCHF (−2.9 ± 4.7 g·day^{-1}, $p = 0.026$, $d = 0.619$) and differed significantly from LOW-GI and HIGH-GI during the study ($p < 0.001$, $\eta p^2 = 0.407$). The LOW-GI group showed a significantly higher mean intake in insoluble fiber compared to LCHF group ($p < 0.001$, $\eta p^2 = 0.307$) independent of time.

3.3 Exercise intervention

The training minutes were divided into basic and interval sessions, and no significant difference was found between the groups ($p > 0.05$). Additionally, there were no differences in total training minutes between the LOW-GI (2,125 ± 294 min), HIGH-GI (2072 ± 285 min), and LCHF (2,103 ± 256 min) groups ($p = 0.824$).

3.4 Blood lipid levels

At baseline measurement, blood lipid levels did not differ except for LDL-C. LDL-C was significantly higher in LOW-GI compared to HIGH-GI ($p = 0.035$, $\eta p^2 = 0.095$). Two-way mixed ANOVA showed significant time group interactions for TC, HDL-C and LDL-C ($p < 0.05$, respectively, see Table 3). The study found that TC levels significantly decreased in both LOW-GI (−21 ± 24 mg·dL^{-1}, $p < 0.001$, $d = 0.845$) and HIGH-GI (−15 ± 23 mg·dL^{-1}, $p = 0.007$, $d = 0.669$), while it increased in LCHF (+21 ± 29 mg·dL^{-1}, $p = 0.004$, $d = 0.706$). After the intervention, TC was significantly higher in LCHF compared to LOW-GI or HIGH-GI ($p < 0.001$, $\eta p^2 = 0.201$) and increase was significantly greater in LCHF ($p < 0.001$, $\eta p^2 = 0.589$). There was a significant decrease in HDL-C in HIGH-GI (−3 ± 9 mg·dL^{-1}, $p = 0.006$, $d = 0.374$), while no changes were observed in LOW-GI or LCHF. Changes in HDL-C were significantly different between HIGH-GI and LCHF ($p = 0.043$, $\eta p^2 = 0.098$). At T-10, there were no differences in HDL-C. LDL-C decreased significantly in LOW-GI (−14 ± 20 mg·dL^{-1}, $p = 0.002$, $d = 0.723$) and HIGH-GI (−13 ± 18 mg·dL^{-1}, $p = 0.005$, $d = 0.702$) and increased in LCHF (+17 ± 21 mg·dL^{-1}, $p = 0.001$, $d = 0.818$). The change in LDL-C was significantly higher in LOW-GI and HIGH-GI compared to LCHF ($p < 0.001$, $\eta p^2 = 0.359$). Due to baseline differences in LDL-C concentration a one-way ANCOVA was employed to compare groups at T-10. Prior to conducting the ANCOVA, assumptions were assessed. Linearity between the baseline LDL-C and follow-up LDL-C was confirmed using scatterplots within each group. The homogeneity of regression slopes assumption was tested by examining the

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interaction between the group and baseline LDL-C levels, which was not statistically significant [F (2, 29) = 1.798, $p = 0.175$], indicating that the assumption was met. Normality of residuals was evaluated using Q-Q plots and the Shapiro–Wilk test, confirming normal distribution ($p > 0.05$). Homogeneity of variances was assessed using Levene's Test of Equality of Error Variances, which was not significant [F(2, 62) = 2.054, $p = 0.137$]. After adjustment for pre-intervention LDL-C concentration, there was a statistically significant difference in LDL-C concentration at T-10 [F(2, 61) = 20.391, $p < 0.001$, $\eta p^2 = 0.401$]. Adjusted LDL-C concentration at T-10 was statistically significantly greater in LCHF ($M = 115$, SE = 4 mg·dL^{-1}) compared to LOW-GI ($M = 87$, SE = 4 mg·dL^{-1}, $p < 0.001$) and HIGH-GI ($M = 82$, SE = 4 mg·dL^{-1}, $p < 0.001$). TG showed no significant interaction effect. However, one way ANOVA of Δ-values revealed a significant higher decrease in LOW-GI compared to HIGH-GI or LCHF ($p = 0.009$, $\eta p^2 = 0.149$, Figure 1).

TABLE 3 Blood lipid levels at baseline (T-0) and after the intervention (T-10).

	Group	T-0	T-10	Time x Group	Simple group effect at T-10
TC [mg·dL^{-1}]	LOW-GI	191 ± 43	171 ± 41 *,c	**<0.001**	**<0.001**
	HIGH-GI	167 ± 37	152 ± 28 *,c		
	LCHF	175 ± 33	196 ± 37 *,a,b		
HDL-C [mg·dL^{-1}]	LOW-GI	61 ± 13	59 ± 11	**0.048**	0.197
	HIGH-GI	61 ± 12	57 ± 11 *		
	LCHF	60 ± 14	64 ± 13		
LDL-C [mg·dL^{-1}]	LOW-GI	109 ± 32 [b]	95 ± 34 *,b,c *87 ± 4* [c]	**<0.001**	**<0.001**
	HIGH-GI	86 ± 32 [a]	73 ± 21 *,a,c *82 ± 4* [c]		
	LCHF	98 ± 28	115 ± 30 *,a,b *115 ± 4* [a,b]		
TG [mg·dL^{-1}]	LOW-GI	102 ± 49	85 ± 36	0.074	0.574[#]
	HIGH-GI	100 ± 74	106 ± 75		
	LCHF	85 ± 38	89 ± 36		

[a,b,c] significantly different concentration between groups after the intervention: [a]compared to LOW-GI, [b]compared to HIGH-GI, [c]compared to LCHF. *significant different from T-0. [#]reflects p-value of main effects. Bold numbers represent a significant p-value. Italic numbers represent estimates ± standard error and results of ANCOVA analysis.

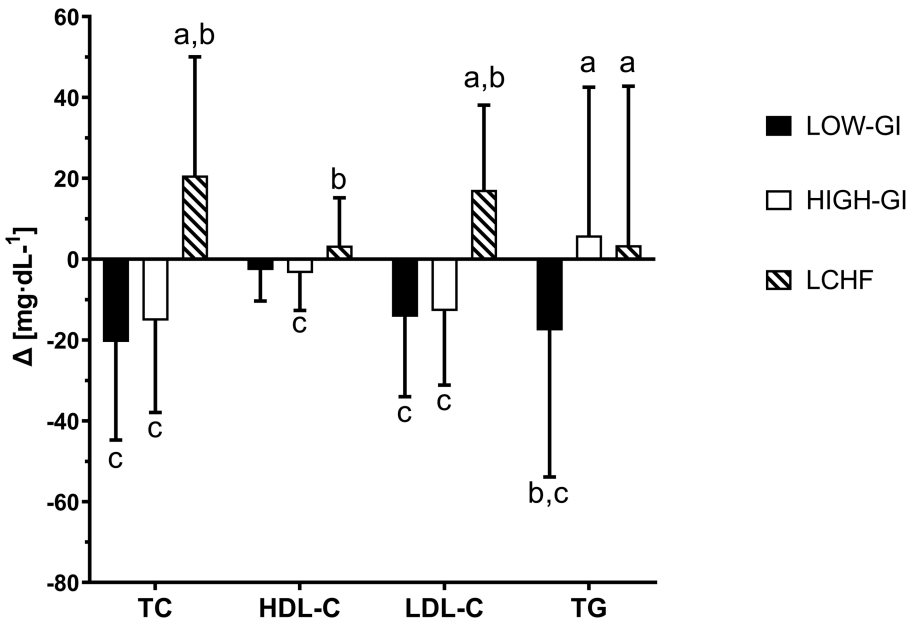

FIGURE 1
Different changes in blood lipid levels during 10-week intervention: a compared to LOW-GI, b compared to HIGH-GI, c compared to LCHF. TC, total cholesterol; TG, triglyzerides.

3.5 Correlation between changes in blood lipid levels, body weight, and nutrition

Changes in TC were significantly correlated with changes in relative protein intake ($p = 0.017$, $r = 0.295$), relative CHO intake ($p < 0.001$, $r = -0.580$), relative fat intake ($p < 0.001$, $r = 0.639$), SFA intake ($p < 0.001$, $r = 0.461$), MUFA intake ($p < 0.001$, $r = 0.494$) and n-3-FA intake ($p = 0.048$, $r = 0.264$). Changes in relative protein intake ($p = 0.043$, $r = 0.252$), relative CHO intake ($p = 0.009$, $r = -0.323$), relative fat intake ($p = 0.008$, $r = 0.328$) and MUFA intake ($p = 0.012$, $r = 0.332$) were significantly correlated with changes in HDL-C. Changes in LDL-C were significantly correlated with changes in relative protein intake ($p = 0.014$, $r = 0.305$), relative CHO intake ($p < 0.001$, $r = -0.588$), relative fat intake ($p < 0.001$, $r = 0.655$), SFA intake ($p < 0.001$, $r = 0.502$), MUFA intake ($p < 0.001$, $r = 0.487$) and n-3-FA intake ($p = 0.006$, $r = 0.360$). Only changes in body weight ($p = 0.011$, $r = 0.314$) were correlated with changes in TG. All other comparisons yielded insignificant correlations. Highest correlation of TC, HDL-C, LDL-C and TG with respective parameters are shown in Figure 2.

3.6 Multiple linear regression

The backward elimination method was employed to ascertain the most appropriate predictor variables for Δ total cholesterol. The results indicated that the predictor variables ΔBMI, Δ relative fat intake and Δ energy intake exhibited the highest adjusted R^2 of 0.210. The multiple regression model statistically significantly predicted ΔTC [$F(3, 61) = 20.158$, $p < 0.001$]. R^2 for the model was 49.8% with an adjusted R^2 of 47.3%. Δ BMI and Δ relative fat intake added statistically significantly to the prediction ($p < 0.05$). Regression coefficients and standard errors can be found in Table 4.

For the ΔHDL-C concentration most appropriate variables according to backward elimination method are Δ fiber intake, Δ BMI, Δ relative fat intake, Δ relative CHO intake and Δ energy intake (adjusted $R^2 = 0.097$). Δ relative CHO intake was removed from the final model due to a R greater 0.8. R^2 for the final model was 20.0% with an adjusted R^2 of 13.9%. The parameters statistically significantly predicted ΔHDL-C [$F(4, 52) = 3.253$, $p = 0.019$]. Δ BMI and Δ relative fat intake added statistically significantly to the prediction ($p < 0.05$). Results are presented in Table 5.

Backward elimination method revealed Δ BMI, Δ fat-free mass, Δ fat mass, Δ fiber intake, Δ energy intake and Δ relative fat and CHO intake to be the most appropriate predictors for ΔLDL-C concentration (adjusted $R^2 = 0.123$). The final model was constructed without Δ relative CHO intake, Δ fat-free mass and Δ fat mass, as $R > 0.8$ or redundant information was present, which resulted in a decrease in adjusted R^2. The multiple regression model statistically significantly predicted ΔLDL-C concentration [$F(4, 52) = 10.756$, $p < 0.001$, $R^2 = 0.453$, adjusted $R^2 = 0.411$]. Δ BMI and Δ relative fat intake added statistically significantly to the prediction ($p < 0.05$, Table 6).

The backward method revealed that the following variables were suited predictors of ΔTG: Δ BMI, Δ fat-free mass, Δ fat mass, Δ energy intake, Δ relative CHO and fat intake and Δ fiber

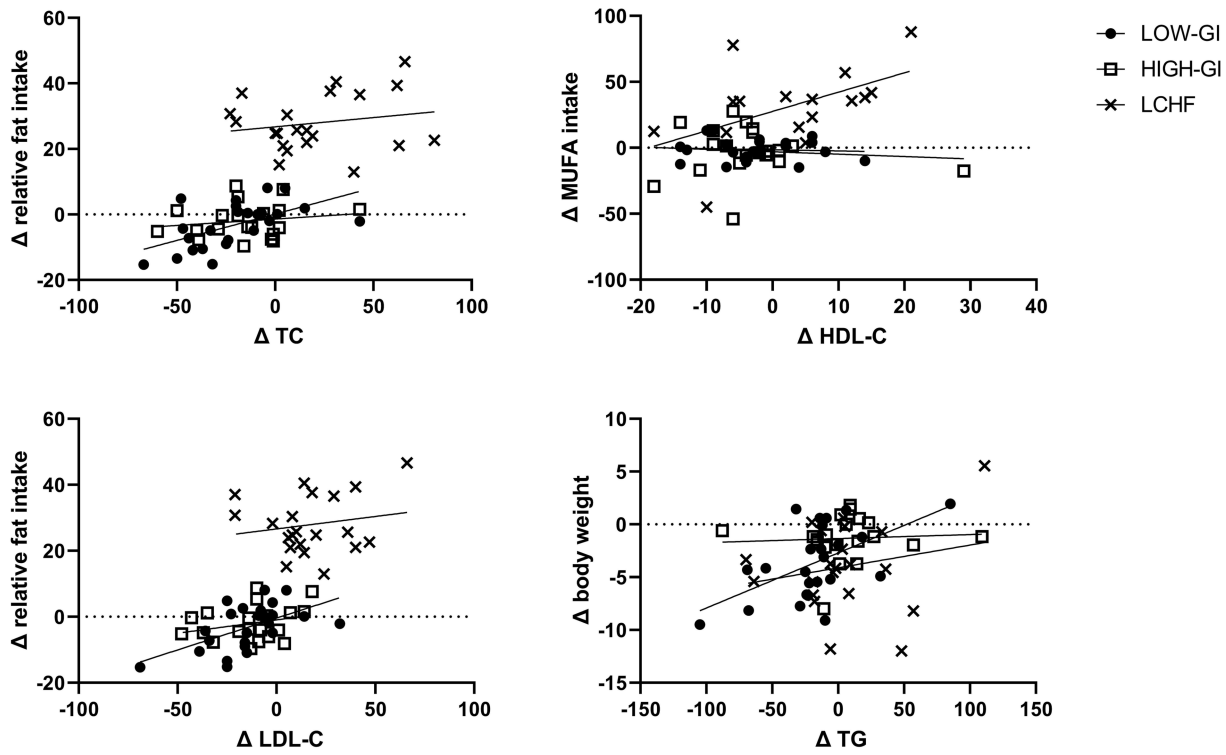

FIGURE 2
Significant correlations between changes in blood lipids and dietary intake or body weight. TC, total cholesterol; MUFA, monounsaturated fatty acids; TG, triglyzerides.

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TABLE 4 Multiple regression results for Δ total cholesterol concentration.

Total cholesterol	*B*	95% CI for B		SE B	Beta	R^2	ΔR^2
		LL	UL				
Model						0.50	0.47
Constant	−6.35	−14.39	1.68	4.02			
ΔBMI [kg·m−2]	8.98**	3.38	14.58	2.80	0.30		
ΔEnergy intake [kcal]	0.00	−0.01	0.01	0.01	0.01		
ΔRelative fat Intake [% of total EI]	1.30***	0.94	1.65	0.18	0.67		

Model, "enter" method in SPSS statistics; EI, energy intake; *B*, unstandardized regression coefficient; CI, confidence interval; LL, lower limit; UL, upper limit; SEB, standard error of the coefficient; beta, standardized coefficient; R^2, coefficient of determination; ΔR^2, adjusted R^2; $^{**}p < 0.01$; $^{***}p < 0.001$.

TABLE 5 Multiple regression results for ΔHDL-C concentration.

HDL-C	*B*	95% CI for B		SE B	Beta	R^2	ΔR^2
		LL	UL				
Model						0.20	0.14
Constant	−1.43	−4.57	1.72	1.57			
ΔBMI [kg·m−2]	2.54*	0.21	4.87	1.16	0.29		
ΔEnergy intake [kcal]	0.00	−0.01	0.00	0.00	−0.20		
ΔRelative Fat Intake [% of total EI]	0.24**	0.09	0.39	0.08	0.40		
ΔFiber intake [g·day−1]	0.06	−0.13	0.25	0.09	0.09		

Model, "enter" method in SPSS statistics; EI, energy intake; *B*, unstandardized regression coefficient; CI, confidence interval; LL, lower limit; UL, upper limit; SEB, standard error of the coefficient; beta, standardized coefficient; R^2, coefficient of determination; ΔR^2, adjusted R^2; $^{*}p < 0.05$; $^{**}\ p < 0.01$.

intake (adjusted $R^2 = 0.357$). However, the final model was constructed without the inclusion of Δ CHO intake and Δ fat mass ($R > 0.8$). The remaining predictors were found to significantly predict ΔTG [$F(5, 51) = 3.772$, $p = 0.006$, $R^2 = 0.270$, adjusted $R^2 = 0.198$]. Δ energy intake and Δ fiber intake were identified as significant contributors to the prediction ($p < 0.05$, Table 7).

4 Discussion

The data presented indicate that a 10-week LCHF diet may result in unfavorable changes in blood lipid panel when compared to a carbohydrate-rich diet in recreationally active runners. In particular, the data show increased levels of TC and LDL-C after the LCHF diet, whereas TC and LDL-C decreased during the carbohydrate rich diets. Additionally, a significant reduction in HDL-C was observed in the HIGH-GI group.

Previous studies have similarly demonstrated the onset of chronic hypercholesterolemia following the adoption of a low-carbohydrate, high-fat (LCHF) diet (25, 26, 28, 31, 32). The notable elevation in TC in the present observation may be attributed to the considerable rise in LDL-C, while no alterations were discerned in HDL-C following the LCHF diet. This differs from other studies, in which an increase in TC was accompanied by an increase in both HDL-C and LDL-C (26, 31) or HDL-C alone (25, 28). Additionally, in contrast to previous observations (24, 25, 30, 31), a LCHF diet was not found to have a "triglyceride-lowering" effect. It is possible that discrepancies may arise in the outcomes due to variations in the methodological approaches (*ad libitum*, isoenergetic diets or free-living), the different types of training (aerobic or anaerobic activities) and the populations (endurance or power athletes) studied. To elucidate the presented outcomes, we propose several potential explanations.

Compared with normative thresholds for dyslipidemia and cardiovascular risk (41), the lipid panel of the CHO-rich diets was in range. For the LCHF group, only the LDL-C concentration was above optimal (LDL-C: 115 mg·dL^{-1}) with the TC concentration still being in range. The increases in TC and LDL-C following an LCHF diet were anticipated for several reasons. Diets were designed *ad libitum* without any restrictions regarding the fatty acid intake. In general, it is recommended that a LCHF diet should have a higher intake of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, while limiting the intake of saturated fatty acids (SFA) (42, 43). During the *ad libitum*, non-restrictive LCHF diet, our group of recreationally active men experienced a significant change in dietary fat intake to meet energy needs. While the intake of SFA decreased in LOW-GI and remained unchanged in HIGH-GI, it increased by around 50% in LCHF compared to baseline intake. Furthermore, a moderate positive correlation was observed between changes in SFA intake and TC and LDL-C. This suggests that higher SFA intake may stimulate cholesterol biosynthesis, leading to increased circulating cholesterol (44). It is important to note that an optimal intake of SFA, MUFA, and PUFA can significantly influence serum cholesterol and lipoprotein levels (45). However, it was observed that in the LCHF group, the higher intake of MUFA and n-3-FA did not lead to favorable changes in TC, lipoprotein levels, or TG. This may be partly explained by the concurrent higher intake of SFA, as the observed correlation between SFA intake and TC or LDL-C was stronger compared to the correlation between n-3-FA and TC or LDL-C. On the other hand, low GI nutrition led to a decrease in SFA intake, accompanied by a decrease in TC and

TABLE 6 Multiple regression results for ΔLDL-C concentration.

LDL-C	*B*	95% CI for B		SE B	Beta	R^2	ΔR^2
		LL	UL				
Model						0.45	0.41
Constant	−6.29	−12.62	0.03	3.15			
ΔBMI [kg·m^{-2}]	5.55*	0.87	10.23	2.33	0.26		
ΔEnergy intake [kcal]	0.00	−0.02	0.01	0.01	−0.10		
ΔRelative fat intake [% of total EI]	0.97***	0.67	1.28	0.15	0.67		
ΔFiber intake [g·day^{-1}]	0.25	−0.13	0.63	0.19	0.15		

Model, "enter" method in SPSS statistics; EI, energy intake; *B*, unstandardized regression coefficient; CI, confidence interval; LL, lower limit; UL, upper limit; SEB, standard error of the coefficient; R^2 = coefficient of determination; ΔR^2 = adjusted R^2; *$p < 0.05$, ***$p < 0.001$.

TABLE 7 Multiple regression results for ΔTG concentration.

Triglycerides	*B*	95% CI for B		SE B	Beta	R^2	ΔR^2
		LL	UL				
Model						0.27	0.20
Constant	7.85	−5.77	21.47	6.78			
ΔBMI [kg·m^{-2}]	11.47	−0.19	23.13	5.81	0.30		
ΔFat-free mass [kg]	−1.05	−8.71	6.62	3.82	−0.04		
ΔEnergy intake [kcal]	0.02*	0.00	0.05	0.01	0.28		
ΔRelative Fat Intake [% of total EI]	0.47	−0.17	1.12	0.32	0.19		
ΔFiber intake [g·day-1]	−1.00*	−1.78	−0.22	0.39	−0.34		

Model, "enter" method in SPSS statistics; EI, energy intake; *B*, unstandardized regression coefficient; CI, confidence interval; LL, lower limit; UL, upper limit; SEB, standard error of the coefficient; beta, standardized coefficient; R^2, coefficient of determination; ΔR^2, adjusted R^2; *$p < 0.05$.

LDL-C. No significant changes in fatty acid intake were observed in the HIGH-GI group. However, it could be speculated that, endurance exercise may have resulted in a decrease in TC and LDL-C levels.

It is clear that regular moderate endurance exercise of around 150 min or 75 min of vigorous exercise per week provides reliable protection against cardiovascular disease (46). Our subjects increased their endurance exercise from an average of three sessions per week to five sessions per week during the intervention, and there was no difference between the groups. Aerobic exercise seems to have a greater impact on HDL-C levels compared to LDL-C or TG. This is because aerobic exercise increases the concentration and activity of lipoprotein lipase in skeletal muscles, which in turn increases HDL-C levels (47). However, the improved function of HDL-C, specifically in terms of increased reverse cholesterol transport and lipid peroxide transport clearing, requires further investigation (48). The effects of aerobic exercise on LDL-C are still unclear and require more data. Aerobic exercise might reduce smaller and less dense LDL-subfractions, which are directly linked to cardiovascular events (49). Additionally, exercise can lead to lower TG concentrations, because it appears that there is an inverse relationship between HDL-C and TG (50). It is unclear to what extend the endurance exercise program had an impact on our results, because subjects were already moderately trained before enrolment. However, it seems that the higher dietary fat intake in the LCHF group contributed greater to alterations in blood lipids compared to the regular endurance exercise.

Weight loss and energy intake has a large beneficial effect on circulating blood lipids, making it difficult to separate dietary effects from other factors (51, 52). Particularly in this case, subjects in all groups experienced significant reductions in body weight, BMI and absolute fat mass [data presented in Graybeal et al. (33)], with significantly greater losses in the LOW-GI and LCHF groups. However, as both groups (LCHF and LOW-GI) experienced similar weight loss, the increase in TC and LDL-C may be partly due to the significantly higher fat intake in LCHF compared to LOW-GI (53). Furthermore, energy intake was markedly diminished in the LOW-GI and LCHF groups, exhibiting lower energy intake than the HIGH-GI group. It can thus be concluded that energy intake alone is not responsible for changes in the blood lipid panel.

Furthermore, we found some additional effects of glycemic index on TC or subfractions. The low GI diet resulted in significantly higher reductions in TG compared to the high GI and the LCHF diet. Epidemiological evidence suggests an inverse correlation between GI and HDL-C (54, 55) and a positive effect on total and LDL cholesterol (22, 56). The higher GI in the HIGH-GI group might therefore be responsible for the significant decrease in HDL-C after 10 weeks. A similar decline has been documented in previous studies (21, 57–59). Nevertheless, the underlying mechanism remains to be elucidated. Chronic hyperinsulinemia might stimulate a series of interconnected metabolic processes (60), including for example an increased production of very-low-density lipoproteins via the upregulation of cholesteryl ester transport protein (61, 62), altered HDL-C composition (63), or reduced cholesterol efflux (64) thereby reducing reverse cholesterol transport (65, 66). Moreover, it appears that a combination of vascular dysfunction and endothelial damage, which has also been observed during chronic

hyperinsulinemia, may be responsible for the reduction in HDL-C levels following a HIGH-GI diet (58). Additionally, dietary fiber and GI work together to affect lipid absorption and synthesis. Sources rich in insoluble fiber appear to have a smaller effect on serum lipids compared to sources rich in soluble fiber, which have been shown to effectively lower lipids (53, 67). In this investigation, the intake of soluble fiber was significantly higher in the LOW-GI group compared to the HIGH-GI or LCHF groups, resulting in the greatest decrease in TC (not significant compared to HIGH-GI), LDL-C (not significant compared to HIGH-GI) and TG in the LOW-GI group. However, no correlation was found between the change in fiber intake and the changes in blood lipids. The responsible mechanisms still need to be addressed, but two main factors have been proposed to influence the decrease in blood lipids after fiber intake. Firstly, increased dietary fiber intake leads to reductions in bile acid and cholesterol absorption from the ileum, which inhibits hepatic cholesterol synthesis. Secondly, a low GI diet can lead to reduced insulin secretion, which in turn reduces the activity of hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme of cholesterol synthesis (44). Nevertheless, according to this explanation, the LCHF group with reduced insulin production should also experience favorable changes in the lipid profile. Therefore, more data are needed to understand the underlying mechanism in active individuals.

Finally, additional data are required to ensure the accurate prediction of changes blood lipid levels based on dietary intake, body weight, and composition. The adjusted R^2 for the calculated models ranged from 14 to 47%. Examining the relationship between change in TC reveals that relative fat intake and BMI serve as reliable predictors. Positive correlations were observed between changes in BMI and relative fat intake. Specifically, an increase in BMI of 1 $kg \cdot m^{-2}$ is associated with a 9 $mg \cdot dL^{-1}$ rise in TC, which is consistent with the findings of previous studies in patients with diabetes (68) and obese individuals (69). Additionally, a 10% increase in relative fat intake is associated with a 13 $mg \cdot dL^{-1}$ rise in TC. These findings contrast with previous studies on overweight individuals (70, 71), which recommend an increased fat intake for weight loss and improved lipid profiles. However, research on athletic populations indicates that the results may not be directly applicable to recreationally active subjects (25, 26, 28, 31, 32). This data suggests that an increase in relative fat intake may contribute to the development of hypercholesterolemia. The predictors for changes in HDL-C exhibited low reliability, with an adjusted R^2 of only 14%. The anticipated relationship between BMI and relative fat intake was not observed. The model suggests that an increase in BMI of 1 $kg \cdot m^{-2}$ and 10% increase in relative fat intake are associated with increases in HDL-C of 3 $mg \cdot dL^{-1}$ and 2 $mg \cdot dL^{-1}$, respectively. Further data are required to substantiate these findings (72). A positive correlation was identified between changes in BMI and relative fat intake and changes in LDL-C. An increase in BMI by 1 $kg \cdot m^{-2}$ was associated with a 6 $mg \cdot dL^{-1}$ increase in LDL-C, and a 10% increase in relative fat intake resulted in an increase of 10 $mg \cdot dL^{-1}$ in LDL-C. These findings differ from results in obese subjects (70, 71, 73), but align with previous reports in athletes (26, 31) or normal-weight adults (72). Dietary fiber and energy intake were reliable predictors of changes in TG levels. A 1000 kcal increase in energy intake resulted in a 23 $mg \cdot dL^{-1}$ increase in TG, a finding consistent with previous studies (51). Additionally, changes in fiber intake and TG were inversely correlated, with a 10-gram increase in daily fiber intake leading to a 10 $mg \cdot dL^{-1}$ reduction in TG. This observation warrants further analysis, as it has yet to be empirically validated (74, 75). Therefore, further investigation is required to ascertain the reliability of regression models in predicting changes in blood lipid concentrations based on change in nutrient intake and body weight in healthy, active individuals. The lack of a sufficiently large sample size precludes any definitive conclusions. Nevertheless, the results suggest that, in addition to BMI, fat intake significantly influences blood lipids. Therefore, individuals who are regularly physically active and considering a LCHF diet should carefully evaluate its potential effects on their blood lipid profile. Existing literature on obese patients cannot be extrapolated to this population.

4.1 Limitations

In addition to the study's strengths, such as the intervention's duration and free-living conditions, it is important to mention some limitations. The diet's compliance was evaluated through 24-h recalls for 2 days per week, which may have caused distortions. Additionally, it is important to note that this trial only included male athletes. It remains unclear whether sex has an impact on the response of blood lipid levels to diet and exercise. Moreover, it is currently not possible to ascertain from the available data how long the effects of a LCHF diet on blood lipids will persist. It is unclear how long it will take for blood lipid levels to return to their original baseline levels following a transition to the habitual diet.

5 Conclusion

A LCHF diet is often recommended for weight loss and fat oxidation in active individuals. However, caution should be exercised when proposing this diet based on current data. The data suggests that despite a regular exercise program, subjects on a LCHF diet showed a significant increase in TC and LDL-C during the 10-week intervention, possibly due to the higher intake of SFA and reduced intake in fiber. When combined with endurance exercise, the carbohydrate rich diets led to reduced levels of TC and LDL-C. However, the reduction in GI had a positive effect on the change in TG, while the high GI resulted in a decrease in HDL-C.

In summary, the data suggests that in active individuals, a diet low in carbohydrates and high in fat may lead to unfavorable alterations in blood lipid levels, while a diet rich in carbohydrates does not have such a detrimental effect on blood lipids. Additionally, reducing the glycemic index of consumed carbohydrates may result in a favorable change in TG concentration. Based on the findings of this study, it is recommended that active individuals who engage in regular exercise should be mindful of the potential impact of their diet on blood lipid levels. It would be beneficial for future research to consider the glycemic index when comparing the effects of a low- and high-carbohydrate diet on blood lipid levels, in order to gain further insights.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethical Committee of the Medical University of Vienna (EK Nr: 2105/2021) and Ethical Committee of the University of Vienna (Reference number: 00871). 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

AK: Writing – original draft, Writing – review & editing, Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Data curation, Project administration, Validation. AF: Investigation, Writing – review & editing, Data curation. DK: Conceptualization, Methodology, Supervision, Writing – review & editing, Resources.

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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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Habitual water intake impacted the body composition of young male athletes in free-living conditions: a cross-sectional study

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The study aimed to explore the associations between water intake and body composition and differences of body composition in different water itake and hydration statuses among young male athletes. A cross-sectional study was conducted among 111 young male athletes in Beijing, China. Total drinking fluids (TDF) and water from food were assessed using a 7-day, 24-h fluid intake record questionnaire and the duplicate portion method, respectively. The osmolality of 24-hour urine and blood samples was tested. Body composition was measured using a bioelectrical impedance analyzer twice at 5-min intervals. Participants were divided into two groups based on the recommendations of total water intake (TWI) and TDF in China, as well as into three groups based on 24-h urine osmolality. Pearson's correlation coefficients were calculated to determine the relationship between water intake and body composition. Chi-square tests and Student's *t*-tests were used to compare differences. A total of 109 participants completed the study. TDF ($r = 0.230$, $p = 0.016$; $r = 0.234$, $p = 0.014$; $r = 0.242$, $p = 0.011$) and TWI ($r = 0.275$, $p = 0.004$; $r = 0.243$, $p = 0.011$; $r = 0.243$, $p = 0.011$) were positively correlated with total body water (TBW), intracellular water (ICW), and extracellular water (ECW). TBW/body weight (BW) was positively associated with TDF percentage of BW (TDF/BW) ($r = 0.267$, $p = 0.005$), water from food percentage of BW ($r = 0.217$, $p = 0.024$), and TWI percentage of BW (TWI/BW) ($r = 0.316$, $p = 0.001$). Participants who met the TDF recommendation of China had 1.3 kg higher skeletal muscle mass (SMM), 0.9 kg higher ICW, and 0.5% higher TBW/BW than those who did not (all $p < 0.05$), with fat-free mass (FFM) and TBW being higher ($p = 0.051$; $p = 0.050$). Those who met the TWI recommendation of China had 1.3 kg higher SMM, 2.4 kg higher FFM, 1.1 kg higher ICW, 0.6 kg higher ECW, and 1.7 kg higher TBW than their counterparts (all $p < 0.05$). Moderate associations were found between water intake and body composition. No significant differences were observed among participants in three hydration statuses (all $p > 0.05$). Participants who met the TWI or TDF recommendations had better body composition distribution than

their counterparts. Thus, habitual water intake, not hydration status, affects body composition among athletes in free-living conditions.

KEYWORDS

body composition, total water intake, total drinking fluids, association, hydration status, young athletes

1 Introduction

Water is essential for life. Within the human body, water is distributed in intracellular and extracellular compartments. Intracellular water (ICW), which represents 60% of total body water (TBW), is the main determinant of cell volume. Extracellular water (ECW), which represents the other 40% of TBW, includes plasma, interstitial fluid, and other transcellular fluids such as cerebrospinal fluid, synovial fluid, and vitreous body fluid (1). Without water, humans can survive only for a few days. The human body obtains water from total drinking fluids (TDF), water from food, and metabolic water. TDF and food each contribute about 50% of total water intake (TWI), whereas metabolic water represents about 250–350 ml/day (2, 3). Therefore, water intake is important for the distribution of body water.

There is a dynamic balance between water intake and water output. When water intake is roughly equal to the output, people are in an optimal hydration status. Otherwise, individuals may experience hypohydration when the water intake does not compensate for water losses. A large body of research has reported that dehydration induces deficits in cognitive performance among adults (4–6). Even mild hypohydration is associated with an increased prevalence of obesity, insulin resistance, diabetes, and metabolic syndrome (7–9). Nevertheless, the evidence was regarded the non-athletic population, for athletes, the situations of hypohydration on health maybe more serious. It is accepted that the core body temperature increases during exercise, and blood flow to the skin increases concurrently to remove heat through sweat. Therefore, sweat loss during exercise that exceeds water intake may lead to dehydration among athletes (10). Additionally, a study conducted among mice showed that muscle is the first organ to lose water; thus, fluctuations in hydration status may directly affect muscle function (11). In this sence, evidence of effects of hydration status in neuromuscular function has been collected among athletes (12, 13). Indeed, dehydration over 2% of body weight impairs endurance exercise performance (14), including endurance cycling performance (15), and maximal aerobic capacity (16), by reducing blood volume, muscle blood flow, and thermoregulation (17). Moreover, that a deficit in body water may also impacted the performance during exercise through an increase in mood disturbance and subjective discomfort (18, 19), and may contribute to the decreased in endurance performance with hypohydration (20). Although strategies to maintain proper hydration status for athletes have been proposed (21–26), a substantial number of studies (27–31) indicate that a significant percentage of athletes are undehydrated before, during, and after exercise. Future research should help raise awareness of hydration status among athletes.

Studies have revealed a positive association between water intake and body water content (32–35). Individuals with different levels of water intake show different hydration biomarkers, including urine osmolality and urine specific gravity (USG), among young adults and athletes (36–39). Furthermore, young adults who meet the total fluids intake recommendation of China have higher ICW, ECW, and TBW than those who do not, in free-living conditions (32). Moreover, relative water turnover in participants with active physical activities is significantly greater than that in the sedentary group (40). Body composition may differ between athletes and non-athletes among adults. However, in China, few studies have evaluated the body composition of young adults (32, 41–43) and even fewer have focused on athletes or explored the associations between water intake and body composition, which needs more attention. Additionally, a large proportion of athletes in China are not in euhydration status (44, 45), which may cause deleterious effects on performance, especially in hot weather. To date, differences in body composition among athletes with different hydration statuses have not been investigated.

Monitoring body composition, including TBW, ICW, and ECW, may represent an additional and valuable approach to control for potential body composition changes linked to physical performance in athletes. TBW and ICW are closely correlated with muscle mass, both in male and female elderly people, indicating that those with higher ICW have better functional performance and lower frailty risk (46). Furthermore, a reduction of 3%–4% in TBW caused by dehydration would attenuate muscular strength and power. ICW determines cell volume and is believed to affect metabolism, as water impacts protein structure and enzymatic activity (47, 48). Moreover, ICW is a good predictor of strength and power in athletes (11, 49), with decreases in ICW correlated with impaired muscle strength. Additionally, the ECW/ICW ratio can predict knee extension force and gait speed, unaffected by age and sex (50). Therefore, considering the importance of body composition for athletes, frequent investigations should take place. Although scientific studies investigating the body composition of athletes are growing, they are still limited. Therefore, more studies need to be initiated.

This study aimed to explore the associations between water intake and body composition and investigate differences in body composition among young athletes who meet the recommendation of TWI or TDF or not. Furthermore, we also aimed to compare the values of body water compartments

Abbreviations

ICW, intracellular water; ECW, extracellular water; TBW, total body water; FFM, free fat mass; SMM, skeletal muscle mass; BW, body weight; TWI, total water intake; TDF, total drinking fluids.

among participants with different hydration statuses. With this research, we aimed to contribute to the provision of a science-based education on fluid intake for young adults.

2 Methods

2.1 Participants

A cross-sectional study was designed, and 111 young adult males were recruited in Beijing, China. The inclusion criteria were as follows: healthy adult male college students aged 18–25 years with a regular exercise training plan (more than five moderate-intensity exercises per week). The exclusion criteria were as follows: those with chronic diseases such as oral cavity (including the mouth ulcer or chronic cheilitis), endocrine, kidney, gastrointestinal tract, and metabolic diseases; sports injuries; cognitive impairment; and those who have taken drugs, vitamins, or other health products within the past month. The study protocol was approved by the Peking University Institutional Review Committee. The ethical approval project identification code is IRB00001052-19051. The study protocol has been registered on the Chinese clinical trial registry website, and the identification code is Chi CTR 1900025710. This study was conducted according to the guidelines of the Declaration of Helsinki. All subjects signed an informed consent form before participating in the study.

2.2 Sample size calculation

In a similar study (51), more than 75% of the random urine samples of 4.4% of the participants were of optimal hydration status, with the set $t = 1.96$ ($\alpha = 0.05$), $e = 4\%$. Thus, according to the formula for calculating the sample size of simple random sampling, $n = t^2p\ (1\text{-}p)/e^2$, the maximum sample size is 101. Considering the dropout rate, set at 10%, 111 participants were needed for this research.

2.3 Study procedure

A total of 111 participants were recruited, and 109 of them completed the study, totalizing a completion rate of 98.2%. The athletes were all classified at least as tear 3 according to Mckay and colleagues (52). The study spanned 7 consecutive days, including 5 weekdays and 2 weekends. On the first study day, anthropometric measurements, including height, weight, and waist circumference, were taken. For recording fluid intake from water and other beverages, all participants were instructed several times on how to record the related information on the self-designed 7-day, 24-h fluid intake questionnaire. In addition, over the 7 consecutive days, all the foods that the participants ate were weighed and recorded for 3 consecutive days (2 weekdays and 1 weekend day, from day 3 to day 5). Furthermore, during these 3 days, 24-h urine samples, including the first morning urine, were collected by the participants. On day 4, the fasting venous blood samples of all participants were collected. Moreover, the indoor and outdoor temperature and humidity were recorded each day for 7 days. The study procedure is shown in Figure 1.

2.4 Measurement of TWI

A 7-day, 24-h fluid intake record questionnaire was used to assess the TDF (53–55), which was designed by the investigator, as described previously (36). Participants were asked to complete the questionnaire for 7 consecutive days (5 weekdays and 2 weekends). The type and amount of fluid intake for each time were measured by a standard cup provided by the investigator. Furthermore, to ensure completeness and accuracy, the investigators checked the questionnaire every day. Before filling the records, a certified researcher provided instructions about how to recorded fluids intake each time. Furthermore, in order to avoid forgetting to record information about drinking water, notices were sent through WeChat, an instant messaging apps widely used by college students, to remind them to record (Tencent Holdings Ltd., Shenzhen, China).

All foods that the participants ate for the 3 days were weighed before and after eating, to calculate the amount of water from food (YP20001, SPC, Shanghai, China). Water from food was assessed using the duplicate portion method. Moreover, all food samples were collected by the investigators and sent to the laboratory for immediate storage. All food samples were measured according to the national standard GB 5009.3-2016, and the water from fruits or other snacks was assessed according to the China Food Composition Table (2016). The water from food was separated into five categories, as described in our previous study (36). Before three days of foods intake survey, participants were showed how to eat the foods, such as how to place chicken bones or fish bones, etc.

2.5 Temperature and humidity of the environment

The indoor and outdoor temperature and humidity were recorded each day for 7 days (WSB-1-H2, Exasace, Zhengzhou, China). The average temperature was 24.2°C ± 5.8°C at 10 a.m. each day, and the average humidity was 29.5% ± 15.8% RH during the study.

2.6 Anthropometric measurements

Height, weight, and waist circumference were measured twice by trained investigators using standard procedures in the morning of the first day of the study, with height was measured twice to the nearest 0.1 cm in bare feet and light clothes and fasting body weight (participants were asked to voided) was measured twice to the nearest 0.1 kg (HD2M-300; Huaju, Zhejiang, China; Accu Measure, Greenwood Village, CO, USA).

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	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Anthropometric measurements	√						
Physical activity	√						
Environment	√	√	√	√	√	√	√
7-day 24-hour fluid- intake questionnaire	√	√	√	√	√	√	√
Water from food			√	√	√		
Blood biomarkers				√			
Urine biomarkers			√	√	√		

FIGURE 1
Study procedure.

Averages for height and weight were calculated. BMI was calculated as weight (kg)/height squared (m^2).

The body composition was tested by a trained investigator using a bioelectrical impedance analyzer in the fifth day and seventh day of the study days (BIA; Inbody 230, Inbody, Seoul, Korea). Fasting body composition was evaluated with participants were asked to voided and with light clothes and bare feet. Each participant was tested twice. Before being tested, participants were asked not to eat or drink anything and to remain quiet for at least 15 min. The researcher entered the participant's number, age, and sex into the BIA, and the participants stood on the foot electrodes while holding the hand electrodes tightly. The test lasted about 3 min. During the test, participants were asked to stand still with an angle of about 15° between their trunk and upper limbs. After the data were displayed on the BIA, the participant stepped off the instrument and then stood on the foot electrodes again for the second measurement. The value used was the average of the two examinations.

2.7 Urine and plasma biomarkers

During the 3 consecutive days (2 weekdays and 1 weekend, Thursday, Friday, and Saturday), which was from the fifth day to the seventh day of the study days, participants were asked to use customized urine collection bags to collect all random urine and filled out the urination behavior questionnaire, recording the time and volume of each urination. Furthermore, all the urine samples were collected, except the first urine samples on the morning of the fifth day. The collected urine was immediately sent to the laboratory. Trained investigators weighed the collected urine (YP20001, SPC, Shanghai, China), recorded and sampled it, and then stored the urine in a refrigerator at +4°C. In order to avoid the mistakes in recording urine, there were three actions. Firstly, participants should record the related information of each urine on the questionnaire and the urine collection bags; secondly, investigators were asked to recorded each urine the participants sent to the lab; thirdly, the amounts of the TWI and the volume of the urine would be checked. If there were some mistakes in the collection of the urine, the investigators would report to the researchers to find solutions.

Urine osmolality, USG, pH, urea, creatinine, and urine electrolyte concentrations (including sodium, potassium, chloride, calcium, magnesium, and phosphate) were tested using standard procedures (45). Fasting venous blood samples were collected by trained investigators to measure the concentrations of Na, K, Cl, testosterone, cortisol, creatinine, and copeptin, as described in our previous study (45). Osmolality of urine samples and plasma were assessed with freezing point method by osmotic pressure molar concentration meter (SMC 30C; Tianhe, Tianjin, China). USG, pH, urea and creatinine were tested by automatic urinary sediment analyzer with uric dry-chemistry method (H-800; Dirui, Changchun, China). The electrolyte concentrations of urine and plasma samples (including sodium, potassium, chloride, calcium, magnesium and phosphate) were evaluated by automatic biochemical analyzer with the ion-selective electrode potentiometer method (AU 5800; Beckman, Brea, CA, USA). The testosterone, cortisol, creatinine were determined by a trained investigator using Imark microplate reader (Bio-Rad 680, Bio-Rad, Hercules, CA, USA). The creatinine level was assessed using the sarcosine oxidase method (C011-2-1, Jiancheng, Nanjing, China).

Participants were divided into three groups: optimal hydration status (defined as ≤500 mOsm/kg), medium hydration status (defined as 500 mOsm/kg <urine osmolality ≤800 mOsm/kg), and dehydration [defined as urine osmolality >800 mOsm/kg

(1, 52, 56, 57)]. Moreover, participants were also assigned to two groups: those met the recommendation of TWI of China (≥AI), and those failed to meet the recommendation of TWI of China (<AI). Finally, participants were split into two groups: those met the recommendation of TDF of China (≥AI), and those failed to meet the recommendation of TDF of China (<AI).

2.8 Statistics

SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Data are presented as mean ± standard deviation or median and interquartile ranges if the data were not normally distributed. Chi-square tests and Student's *t*-tests were used to compare differences among groups. Pearson's correlation coefficients were calculated to determine the relationship between water intake and body composition. The significance level was set at 0.05.

3 Results

Table 1 shows the characteristics of the participants. Age, height, weight, BMI, and skeletal muscle of the participants were 20.8 years, 178.7 cm, 70.7 kg, 22.1 kg, and 34.9 kg, respectively (Supplementary Table S1). The amounts of TWI, TDF and water from food are displayed in Supplementary Table S2. The amounts of the TWI, TDF and water from food were 2,701 ml, 1,789 ml and 955 ml, respectively; with TDF contributing about 65.0% to TWI. Furthermore, the main contributor of TDF was water, which was 1,181 ml; while, the intake of sugar sweetened beverage was 469 ml.

The urinary and plasma biomarkers of the participants are showed in Supplementary Tables S3, S4. The volume, osmolality, specific gravity, pH and the concentrations Na, K and Cl of 24 h urine were 850 ml, 764 mOsm/kg, 1.020, 6.3, 202 mmol/L, 45.21 mmol/L and 221 mmol/L, respectively. Almost about of the participants were hypohydrated only 15.6% of them were in optimal hydration status.

3.1 Intracellular and extracellular fluid and fluid intake of the participants

Table 1 presents the differences in the mean body composition of participants who met the recommended adequate intake of water or not. Participants who met the recommendation for TDF in China had higher skeletal muscle mass (SMM), fat-free mass (FFM), ICW, and TBW/body weight (BW) (all $p < 0.05$), with TBW and ECW tending to be significantly different ($p = 0.050$; $p = 0.059$), when comparing with those falied meeting the recommendation. Similarly, those who met the recommendation for TWI in China had higher SMM, FFM, ICW, ECW, and TBW than their counterparts (all $p < 0.05$). It should be noted that the values of SMM, FFM, ICW, ECW, and TBW were all assessed by BIA in the current study.

3.2 Correlation between intracellular and extracellular fluid and fluid intake

Table 2 shows that TDF and TWI were positively correlated with TBW, respectively ($r = 0.232$, $p = 0.015$; $r = 0.275$, $p = 0.004$); simultaneously, significant associations were found between TDF and TWI with ICW, respectively ($r = 0.234$, $p = 0.014$; $r = 0.243$, $p = 0.011$); TDF and TWI were significantly associated with ECW, respectively ($r = 0.243$, $p = 0.011$; $r = 0.242$, $p = 0.011$). A significant correlation was also found only between ICW and water from food ($r = 0.199$, $p = 0.038$), but no significant associations were found between ECW and water from food ($r = 0.161$, $p = 0.094$), TBW and water from food, respectively ($r = 0.185$, $p = 0.054$).

3.3 Body composition of participants with different hydration statuses

No statistically significant differences were noted in SMM, FFM, ICW, ECW, TBW, BW, ICW/FFM, ECW/FFM, ICW/

TABLE 1 Body composition of participants who met or did not meet the adequate intake of TWI and TDF of China.

Variable	TWI/China				TDF/China				Total
	≥AI	<AI	*F*	*p*	≥AI	<AI	*F*	*p*	
SMM	35.7 ± 4.0#	34.4 ± 2.6	−2.186	0.031	35.4 ± 3.8*	34.2 ± 2.4	−1.990	0.049	34.9 ± 3.3
FFM	62.7 ± 6.7#	60.3 ± 4.2	−2.258	0.026	62.2 ± 6.2	60.1 ± 4.0	−1.974	0.051	61.3 ± 5.4
ICW	29.0 ± 3.1#	27.9 ± 2.0	−2.241	0.027	28.7 ± 2.9*	27.8 ± 1.8	−2.016	0.046	28.3 ± 2.5
ECW	16.9 ± 1.8#	16.3 ± 1.1	−2.247	0.027	16.8 ± 1.7*	16.3 ± 1.1	−1.913	0.058	16.5 ± 1.5
TBW	45.9 ± 4.9#	44.2 ± 3.1	−2.251	0.026	45.5 ± 4.5*	44.0 ± 2.9	−1.986	0.050	44.8 ± 3.9
BW	72.3 ± 8.8	70.2 ± 6.1	−1.473	0.144	71.9 ± 8.4	70.0 ± 5.7	−1.326	0.188	71.0 ± 7.3
ICW/FFM (%)	46.2 ± 0.2	46.2 ± 0.2	0.351	0.726	46.2 ± 0.3	46.2 ± 0.2	−0.514	0.609	46.2 ± 0.3
ECW/FFM (%)	27.0 ± 0.3	27.0 ± 0.3	0.062	0.951	27.0 ± 0.3	27.0 ± 0.3	0.392	0.696	27.0 ± 0.3
ICW/TBW (%)	63.1 ± 0.4	63.1 ± 0.3	0.073	0.942	63.1 ± 0.4	63.1 ± 0.3	−0.447	0.656	63.1 ± 0.4
ECW/TBW (%)	36.9 ± 0.4	36.9 ± 0.3	−0.082	0.934	36.9 ± 0.4	36.9 ± 0.3	0.457	0.649	36.9 ± 0.4
ECW/ICW (%)	58.5 ± 1.0	58.5 ± 0.8	−0.092	0.927	58.5 ± 0.9	58.4 ± 0.9	0.447	0.656	58.5 ± 0.9
TBW/BW (%)	63.6 ± 2.8	63.1 ± 3.2	−0.890	0.375	63.5 ± 3.1	63.0 ± 3.1	−1.990	0.049	63.3 ± 3.1
TBW/FFM (%)	73.2 ± 0.2	73.2 ± 0.1	0.692	0.490	73.2 ± 0.2	73.2 ± 0.1	−0.131	0.896	73.2 ± 0.2

Note: Values are shown as mean ± standard deviation (SD).
*Significant difference was found between the group ≥AI of TDF/China and <AI of TDF/China ($p < 0.05$).
#Significant difference was found between the group ≥AI of TWI/China and <AI of TWI/China ($p < 0.05$).

TABLE 2 Correlation between water intake normalized by body weight from all the sources analyzed with anthropometric and body composition variables of males.

Variable	TDF/weight (ml/kg)		Water from food/ weight (ml/ kg)		TWI/weight (ml/kg)	
	r	*p*	*r*	*p*	*r*	*p*
BW	−0.177	0.065	−0.194	0.043	−0.231	0.016
FFM	−0.061	0.526	−0.102	0.291	−0.095	0.326
FM	−0.264	0.006	−0.237	0.013	−0.321	0.001
TBW	−0.058	0.549	−0.103	0.288	−0.092	0.339
TBW/BW	0.267	0.005	0.217	0.024	0.316	0.001
ICW	−0.009	0.928	−0.078	0.423	−0.076	0.430
ECW	−0.007	0.945	−0.105	0.279	−0.080	0.407

Note: Positive correlations were found for TDF and TWI with TBW, ICW and ECW, respectively ($r = 0.232$, $p = 0.015$; $r = 0.275$, $p = 0.004$; $r = 0.230$, $p = 0.016$; $r = 0.279$, $p = 0.003$; $r = 0.233$, $p = 0.015$; $r = 0.265$, $p = 0.005$); moreover, significant correlation was found between ICW and water from food ($r = 0.199$, $p = 0.038$), but no significant differences were found for ECW and TBW with water from food, respectively ($r = 0.161$, $p = 0.094$; $r = 0.185$, $p = 0.054$).

TBW, ECW/TBW, ECW/ICW, TBW/BW, and TBW/FFM among participants in the three hydration groups ($p > 0.05$) (Table 3).

4 Discussion

The present study is the first to investigate differences among athletes with different water intakes and hydration status and to evaluate the relationship between water intake and body composition in China. The main findings of the current study were threefold: (1) moderate associations between water intake and body composition; (2) young male athletes with adequate water intake had a significant differences in body water; (3) participants in different hydration statuses had similar body water.

It is worth noting that the body composition of the participants were assessed by BIA. BIA is a technology that quantitatively measures body composition through impedance that occurs when an electric current flows through the human body. The instrument measuring the body composition in this study utilizes the molecular method to quantify four body components of body water, protein, minerals, and body fat. The instrument uses 8-point contact electrodes (two thumb electrodes, two palm electrodes, two sole electrodes and two heel electrodes) to measure 30 impedance values at 5 segments (left and right upper limbs, trunk and lower limbs) at 6 different frequencies (1 kHz, 5 KHz, 50 KHz, 250 kHz, 500 KHz and 1,000 kHz), so as to accurately analyze the total water content. BIA has been widely used to quantify body composition, including ICW, ECW, and TBW of athletes (58–63), and has been compared with densitometry and dilution techniques as references (60, 64, 65).

In this study, ICW, ECW, and TBW were found to be 28.3 kg, 16.5 kg, and 44.8 kg, respectively, with TBW accounted for 63.3% of BW. In a study conducted among the young general population (32), TBW, ICW, and ECW were reported as 32.8 kg, 20.5 kg, and 12.4 kg, respectively, which were 12.0 kg, 7.8 kg, and 4.1 kg lower than the values reported in the current study, indicating that the body composition of athletes differ from that of the general population; these findings are consistent with those of a study conducted in Japan, in which the TBW of athletes was 3.2 kg higher than that of non-athletes (66). Furthermore, previous work has reported differences in ECW/TBW between athletic and non-athletic children (67). The results of the study confirmed differences between athletes and the general population. Additionally, due to differences in sweat output during increased energy expenditure from physical activity, the contribution of dietary composition to water turnover rates differs between active and sedentary men (40). In another study, TBW, ICW, and ECW among athletes were 47.46 kg, 28.86 kg, and 18.60 kg, respectively, which were all higher than the values reported in the present study, even though participants were of the same age (63). This may be attributed to racial and ethnic differences (68). Furthermore, FFM hydration (73.3%) reported in the present study was similar to that reported in a previous study (66).

Optimal body composition plays a significant role in athletic performance (69). ICW determines cell volume and impacts cell metabolism, and its depletion impairs the availability of nutrients and may produce intracellular catabolic effects (47, 49). ICW is an indicator of muscle quality and cell hydration and is related

TABLE 3 Body composition of participants with different hydration statuses.

Variable	Optimal hydration	Medium hydration	Dehydration	*F*	*p*
SMM	34.0 ± 2.8	35.1 ± 3.4	35.0 ± 3.3	0.733	0.483
FFM	60.0 ± 4.8	61.6 ± 5.6	61.4 ± 5.5	0.605	0.548
ICW	27.6 ± 2.2	28.5 ± 2.6	28.4 ± 2.5	0.736	0.481
ECW	16.3 ± 1.3	16.6 ± 1.5	16.6 ± 1.5	0.376	0.688
TBW	43.9 ± 3.5	45.1 ± 4.1	45.0 ± 4.0	0.590	0.556
BW	69.9 ± 6.9	71.5 ± 8.1	71.0 ± 6.8	0.286	0.752
ICW/FFM (%)	46.1 ± 0.3	46.2 ± 0.2	46.2 ± 0.2	1.534	0.220
ECW/FFM (%)	58.8 ± 1.1	58.3 ± 0.9	58.5 ± 0.8	2.307	0.105
ICW/TBW (%)	63.0 ± 0.4	63.2 ± 0.4	63.1 ± 0.3	2.169	0.119
ECW/TBW (%)	37.0 ± 0.4	36.8 ± 0.4	36.9 ± 0.3	2.191	0.116
ECW/ICW (%)	58.8 ± 1.1	58.3 ± 0.9	58.5 ± 0.8	2.194	0.116
TBW/BW (%)	63.0 ± 2.4	63.3 ± 3.6	63.5 ± 2.8	0.143	0.867
TBW/FFM (%)	73.2 ± 0.2	73.2 ± 0.2	73.2 ± 0.1	0.616	0.542

Note: Values are shown as mean ± standard deviation (SD).

to muscle strength and functionality (70, 71). In the present study, participants who met or exceeded the recommendations for TWI had higher SMM, FFM, ICW, ECW, and TBW than their counterparts. Furthermore, those who met the recommendation for TDF in China had higher SMM and ICW than those who did not. A similar trend was found, including the ECW/TWI and TBW/BW, among general young adults aged 18–23 years in a previous study (32). In addition, a finding in agreement with other studies in the literature was that our present results confirmed moderate associations between water intake and body composition among athletes in China. Hence, those who achieved the adequate intake of TWI had better distribution of body composition and may also have better physical performance than those who did not. These results also indicated that water intake could impact the distribution of body composition among athletes. Nevertheless, it has been reported that LOW athletes exhibit higher USG when comparing with those with higher TWI; while TBW, ICW, ECW, and FFM hydration were not statistically different between groups (39). The population composition and methodologies in the two studies explained the differences of results. The study included males and females; the body water as measured by dilution techniques; the USG was the diagnosis of hydration status, and the participants were split into two groups with their habitual TWI obtained by seven-day food records, according to the standard of EFSA. While, in the present study, only young male athletes were included; BIA was used to assess the body water; the hydration status was evaluated by the osmolality of 24 h urine; the TWI was measured by questionnaire for 7 consecutive days of TDF survey (measured by a cup to the nearest 5 ml and recorded fluids intake each time) and 3 days of water from food survey (duplicate portion method) and participants were divided according to the recommendation of TWI or TDF China. Further studies were needed to explore this issue.

Regarding to the TDF and hydration status, in a previous study, a higher proportion of participants with higher habitual TDF had optimal hydration status than their counterparts (36). Thus, those with higher habitual TDF may have a lower risk of being dehydrated. Especially, the current study presents the need for athletes to maintain adequate water intake and the importance of educating athletes on proper hydration.

As previously mentioned, hydration is important for athletes, and hypohydration, mainly as a result of sweat loss among athletes, impairs physical performance, particularly reducing endurance performance (12, 13, 72, 73). Furthermore, hydration status also affects body composition. In our study, ICW, ECW, and TBW among athletes with different hydration statuses did not differ, similar to the results of another study (74). The study exploring the water compartments of athletes with differing hydration statuses demonstrated that ICW, ECW, TBW, and other variables of body composition did not differ between different hydration groups (74). Notwithstanding, in some acute heat-induced or water restriction-induced hypohydration cases, the body composition changed when comparing the hypohydration with baseline or rehydration. One study conducted among young adults showed that individuals who were hypohydrated had higher ICW/TBW, and lower ECW and ECW/TBW than baseline values (42). Another study conducted among athletes revealed that participants seemed to be dehydrated after an Ironman triathlon compared with baseline values (75). Thus, hydration status may not impact body composition among athletes in free-living conditions. Differences in the methods used in the studies and the indices evaluating hydration status and body composition could explain the controversial results of the research mentioned above, with the current study being a cross-sectional study, whereas the others were self-controlled or randomized controlled studies.

The body water compartments of the athletes in our study were evaluated by BIA. Some studies have demonstrated the validity and reliability of the BIA in assessing the body water (60). In contrast, some studies came to the conclusions that overestimation of body fat mass and lower estimation of TBW should be considered when evaluating the body composition in health-clinical practice. The measurements of the BIA maybe impacted by factors including the nutritional status, hydration statuses, machine specifications and the technical skill (76–78). Maybe such a lack of validity could attributed to the differences of the present study and other studies, therefore, more studies should be conducted to explore the issue further.

Our findings highlight the need for tracking body composition, specifically ICW, ECW, TBW, and FFM hydration, in athletes to avoid reductions. The study had strenghs and limitations. In regard to the strengths, it was the first study investigated the correlations between water intake and body water among athletes with different total drinking fluids. Moreover, 7-day 24 h fluid intake questionnaire and the duplicate portion method were used to assess the total drinking fluids for seven consecutive days and amounts of water from all the food the participants ate for three consecutive days which would reduce the recall bias. There are limitations to this investigation to consider. The study was conducted only among male young athletes, not including females. Additionally, future studies including a larger population of different ages of adults are needed to verify our findings.

5 Conclusions

The present results confirmed moderate associations between water intake and body composition among athletes with habitual water intake in free-living conditions in China. Furthermore, water intake, but not hydration statuses, was found to affect body composition among athletes in free-living conditions.

Data availability statement

The datasets of this study is available from the corresponding author on reasonable request.

Ethics statement

The studies involving humans were approved by Peking University Institutional Review Committee (the ethical approval project identification code is IRB00001052-19051). 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

JZ: Writing – original draft, Methodology, Investigation, Formal Analysis. NZ: Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation. YL: Writing – original draft, Investigation, Formal Analysis, Data curation. HH: Writing – original draft, Methodology, Investigation. GS: Writing – original draft, Investigation, Formal Analysis, Data curation. JC: Writing – original draft, Investigation. YY: Writing – review & editing, Validation, Supervision, Resources, Project administration. GM: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fspor.2024.1458242/full#supplementary-material

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The effect of protein intake on athletic performance: a systematic review and meta-analysis

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Background: The impact of a protein-rich diet and protein supplements on athletic performance remains a topic of debate. Does protein intake offer benefits for athletes? If so, which specific aspects of athletic performance are most influenced by protein?

Methods: This study aimed to explore the relationship between protein intake and athletic performance. A systematic database search was conducted to identify randomized controlled trials (RCTs) examining the effects of protein intake on athletes' performance. The databases searched included PubMed, Scopus, Web of Science, EBSCO, and Ovid. The meta-analysis included a total of 28 studies involving 373 athletes. The meta-analysis employed both the fixed-effects model and the random-effects model to investigate the impact of protein intake on sports performance. Subgroup analyses were conducted to provide solid evidence to explain the results of the meta-analysis. Sensitive analysis and funnel plots were used to assess the risk of bias and data robustness.

Results: Overall, protein intake did not show a statistically significant improvement in athletic performance (standardized mean difference [SMD] = 0.12, 95% confidence interval [CI]: −0.01 to 0.25). However, in subgroup analysis, the protein group demonstrated a statistically significant improvement in endurance performance, as indicated by the forest plot of final values (SMD = 0.17, 95% CI: 0.02 to 0.32). Additionally, the change value in the forest plot for endurance performance showed even greater statistical significance than the final value (SMD = 0.31, 95% CI: 0.15 to 0.46). In the subgroup analysis based on physiological indices, muscle glycogen showed a statistically significant improvement in the protein group (standardized mean difference [SMD] = 0.74, 95% confidence interval [CI]: 0.02 to 0.32). Furthermore, subgroup analyses based on protein supplementation strategies revealed that co-ingestion of protein and carbohydrates (CHO) demonstrated statistically significant improvements in endurance performance (SMD = 0.36, 95% CI: 0.11 to 0.61), whereas high protein intake alone did not.

Conclusion: Protein intake appears to provide modest benefits to athletes in improving their performance, particularly by enhancing endurance. Subgroup analysis suggests that protein intake improves muscle glycogen levels and that the co-ingestion of protein with CHO is more effective for endurance athletes than high protein intake alone.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, Identifier CRD42024508021.

KEYWORDS

protein intake, athletic performance, macronutrients, physiological index, endurance ability, muscle strength

1 Introduction

With the rapid development of technology and society, diet and supplements have garnered significant attention from scientists and scholars, particularly in the field of sports. Professional athletes often manage their daily dietary intake under the guidance of dietitians to prepare for upcoming competitions. Protein, as a crucial macronutrient, plays an essential role in human nutrition and warrants further investigation. According to the *NSCA's Guide to Sport and Exercise Nutrition*, protein can be metabolized for energy, and adequate protein ingestion is especially important for athletes participating in energy-demanding aerobic endurance sports (1).

However, protein is not the body's preferred fuel source and is metabolized more slowly for energy than carbohydrates CHO. In contrast, CHO is the preferred energy source due to its rapid metabolism, and there is a well-established consensus in sports nutrition that CHO, rather than protein, should constitute the majority of energy intake during prolonged physical activities. Some prestigious authors have supported this viewpoint. Jager et al. concluded that dietary protein is not an ideal energy source and does not enhance endurance performance when adequate CHOs are consumed (2).

According to *ACSM's Nutrition for Exercise Science*, CHOs have a protein-sparing effect, meaning that protein's unique roles, such as stimulating muscle protein synthesis (MPS) or reducing muscle protein breakdown, are only activated when the body's energy needs are met through sufficient CHO intake. Therefore, protein consumption adequacy can only be viewed in the context of whether sufficient total energy has been consumed (3). Phillips and Van Loon concluded that post-exercise protein consumption may enhance adaptation by aiding in glycogen restoration, but this effect seems to occur primarily when carbohydrate intake is insufficient (4).

Proteins are organic compounds composed of a genetically determined sequence of amino acids that serve as protein building blocks. Protein is the diet's primary source of these essential amino acids (EAAs). Without dietary sources of EAAs, the body must metabolize its protein stores (e.g., muscle) to provide EAAs to meet essential protein needs (1), and intense exercise will increase protein needs (1, 5). People in a general fitness program can generally meet protein needs by ingesting 0.8 to 1.0 g/kg daily. However, competitive athletes, or those who engage in intense training, require more protein than this to adequately respond to the stimulus that training provides (1). The World Health Organization (WHO) suggests an intake of 0.83 g protein/kg weight/day of good-quality protein for all healthy adults of both genders and ages. Typical recommendations for endurance athletes are 1.2 to 1.4 g protein/kg weight/day and for strength-trained athletes from 1.6 to 1.7 g protein/kg weight/day (2, 3, 6).

Although athletes may need more protein to supplement energy, the effect of protein intake on athletic performance is still debatable. The measurement method for athletic performance in cycling and running after a protein intervention is diverse and comprehensive. The completed time, the time to exhaustion (TTE), and the peak power acquired after the time trial (TT) can well reflect running or cycling athletes' endurance ability and observe their body condition. The consensus is that TTE and TT are well-established endurance performance tests commonly used to examine the influence of experimental interventions on endurance (7, 8). Many studies used these testing methods to explore the relationship between protein ingestion and endurance performance. However, some authors agree that a high-protein diet or supplement may hinder endurance performance in time trials, particularly in activities such as cycling or running (2, 9–12). Additionally, some studies suggest that protein consumption during exercise may not provide immediate ergogenic benefits, especially when carbohydrate intake is limited (13, 14).

Increased protein intake does not necessarily lead to greater power or muscle gain; more data is needed to demonstrate its effectiveness. Athletes who consume excessive protein may experience the opposite results. On the one hand, Rosenbloom et al. determined that increasing protein intake may decrease carbohydrate intake, causing athletes to feel exhausted and perform poorly during training (15). On the other hand, several researchers reported that increased protein intake (higher than or equal to 1.5 g/kg daily) had no additional benefits or drawbacks for athletes' athletic performance, such as endurance or maximum strength (16–20). Knuiman et al. also mentioned that the evidence of the role of protein on endurance training adaptations and performance is scarce, and there is still no direct evidence that individuals performing endurance training benefit from additional protein (21).

The efficacy of protein plus carbohydrate co-ingestion is also unclear. Some authors concluded that protein plus carbohydrate could not improve athletic performance in athletes compared with carbohydrate ingestion alone (19, 22–25), and protein plus carbohydrate co-ingestion could not enhance muscle glycogen synthesis (26). McCartney et al. discovered that the impact of protein, CHOs, and water on real-world endurance performance in cycling and running was likely negligible, indicating no practical benefits or harms. They recommended prioritizing the consumption of CHOs over protein. The participants included in their study were healthy people (27). Jager et al. stated that protein co-ingestion with additional dietary ingredients may have a favorable impact on muscle strength (13). As a result, it is necessary to collect more data from published studies to investigate the relationship between protein and endurance performance.

Abbreviations: PRO, Protein; CHO, Carbohydrates; BMI, Body mass index; SD, Standardized deviation; CMJ, Counter-movement jump; SMD, Standardized mean deviation; RCTs, Randomized controlled trials; 1RM, One-repetition maximum; ROB, Risk of bias; ΔSD, Mean change difference with corresponding standard deviation; MVC, Maximum voluntary contraction; M, Mean; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; NSCA, National Strength and Conditioning Association; MeSH, Medical Subject Headings; PICOS, Population, intervention, comparison, outcome and study type; PRISMA, Preferred reporting items for systematic review and meta-analysis; 95% CI, 95% Confidence interval; TTE, Time to Exhaustion; TT, Time Trials.

On the contrary, some studies have proved the efficacy of protein plus carbohydrate co-ingestion on athletic performance in athletes (28–30), but the source of this benefit is unknown. CHOs are still the primary supplement choice for athletes because they are the main and preferred fuel. Therefore, the performance enhancement resulting from co-ingesting protein with CHOs may be due to the additional energy from either protein or CHOs rather than an isolated effect of the protein itself. It is recommended that a ratio of 3–4:1 of CHO: Protein is optimal for both health and performance in athletic populations (27).

Factors such as blood glucose, heart rate, blood lactate, and muscle glycogen influence athletes' performance. The extracted data have shown the relationship between protein intake and these factors. A large number of studies have proven the benefits of protein for endurance performance. After the experiment, some scholars mentioned that high protein intake could improve athletic performance and decrease the feeling of fatigue during and/or after exercise, especially in endurance performance, and suggested athletes increase the daily ingestion of protein to 1.5 g/kg a day compared with common people (31–37). Co-ingestion of protein and CHOs could change the perception of exertion by reducing central fatigue, increasing protein oxidation, potentially sparing endogenous CHOs, and enhancing both aerobic and anaerobic endurance under varying Vo_{2max} loads (28–30, 38, 39). A Bayesian meta-analysis (40) found that plant-based protein could improve athletic ability, including muscle strength, endurance performance, and muscle protein synthesis rate in healthy people, but plant-based protein appears to be less effective than other types of proteins, such as beef, whey, or milk protein. Finally, a review (41) summarized the efficacy of dietary protein on endurance and found that periodized protein ingestion has been shown to augment the remodeling of muscle and whole-body proteins with endurance training. Changes in muscle protein synthesis primarily determine protein remodeling, which plays a crucial role in the acute recovery process after exercise and ultimately contributes to the adaptations associated with endurance training, such as increased muscle power and aerobic capacity.

Protein ingestion appears to link with muscle glycogen, an indirect factor that affects athletic performance. In their study, Williams et al. found that ingesting recovery beverages provided following exercises that greatly deplete muscle glycogen stores resulted in an increase in glycogen storage, and the rate of glycogen storage during the CHO co-ingested with PRO treatment was 128% greater than that of the sports beverage treatment. The CHO-PRO beverage contained 53 g of CHOs and 14 g of protein in a serving (355 mL). However, this rise may be attributed to an increase in carbohydrate intake (0.8 g/kg), and further evidence is needed to support the efficacy of protein intake (39).

Rustad et al. found that consuming a protein-plus-carbohydrate beverage immediately after intense exercise accelerated recovery, leading to improved performance 18 h later. The ingestion of CHOs post-exercise was also more beneficial than fasting, possibly due to increased muscle glycogen stores, reduced protein degradation, or a combination of both. In this study, the participants consumed 0.8 g of $CHO{\cdot}kg^{-1}{\cdot}h^{-1}$ and 0.4 g of whey $protein{\cdot}kg^{-1}{\cdot}h^{-1}$ (42). Muscle glycogen storage proved to be a key factor in maintaining athletic performance after high-intensity exercise.

Several studies have demonstrated the effectiveness of protein intake in improving muscle strength. A meta-analysis (43) investigated the effect of protein supplements on resistance training-induced gains in muscle mass and strength in healthy adults. They found protein supplements enhanced change in muscle strength and fat-free mass in trained people, but this increase in 1RM and fat-free mass was largely induced by resistance exercise training, and protein intake augmented its efficacy. A network meta-analysis by Lam et al. concluded that whey protein supplements would assist athletes in strength at a longer period of consumption with physical activities (44). In the same way, a review exploring the effect of protein on athletic performance concluded that beef protein and whey protein combined with exercise training both could improve lower limb muscle strength, and this increase seems to be attributed to the better stimulation of MPS by protein (45). Bagheri et al. concluded that an intake of 1.6 g/kg a day of protein combined with resistance training appeared sufficient to maximize gains in lean mass, muscle strength, performance, and aerobic capacity, indicating this daily protein amount is effective and safely tolerated in young, healthy adults. Participants consumed 40 g of an isolated whey protein beverage containing 110 calories upon cessation of every training session (46). High-protein dairy milk combined with resistance training could effectively increase total energy intake and augment lean mass and muscle performance in young resistance-trained males already ingesting 1.5 g/kg of protein. Participants ingested 250 mL of 156 kcal high-protein dairy milk containing 30 g of a mixture of whey (6 g) and casein (24 g) with 10 g carbohydrate (47), and Fritz et al. concluded that vegan protein supplements with probiotics improved body weight and skeletal muscle mass in 19 players. The improved body weight and skeletal muscle mass were probably attributed to the change in the gut microbiota composition and better protein absorption. The composition of Biotech vegan protein contains pea protein, rice protein, and soy lecithin (48). Therefore, it is meaningful to combine studies with different results and data through meta-analysis to provide robust conclusions in the field of protein supplements.

When athletes, especially runners or cyclists, eat protein after a TTE or TT simulated test, their endurance may improve. This could provide benefits such as allowing them to perform longer in a race. This improvement may be linked to protein oxidation, extra energy, or less central fatigue. For instance, Highton et al. concluded that the ability to sustain high-intensity running is particularly relevant for sprint athletes, and CHOs with protein beverages could provide a meaningful (small to moderate) advantage over CHOs at the phase of a multiple-sprint sport in which fatigue-related performance deterioration is most likely to have an impact. Therefore, they likely attributed the enhanced performance to either altered central fatigue or increased protein oxidation (28). Another study has provided the same conclusion. Saunders et al. summarized that cyclists who ate protein-carbohydrate gels could decrease the incidence rate of muscle injury, prolong the time to exhaustion, and increase protein oxidation. Therefore, protein's ergogenic effects may come from raising the upper limit of exogenous caloric uptake or oxidation above what can be done with treatments that only use CHOs (38).

Currently, there is a lack of comprehensive evidence regarding the impact of protein on athletes' athletic performance. Few studies have investigated the efficacy of protein ingestion on athlete populations, particularly in endurance athletes, and have reached a clear conclusion. Jager et al. described that very few studies have investigated the effects of prolonged periods (1 week or more) of dietary protein manipulation on endurance performance (13). Therefore, it is necessary to synthesize all available evidence and arrive at a robust conclusion.

This meta-analysis aims to examine athletes' physiological responses to protein intake from various sources, including food bars, whey protein, plant protein, and other supplements. By synthesizing current evidence, this study seeks to provide clearer insights into the effectiveness of protein on athletic performance. We hypothesize that protein intake can improve athletic performance through muscle strength and endurance performance in athletes. The results of this study may have practical implications for athletes and their advisors in planning protein supplements to enhance competitive ability.

2 Method

This study was registered in PROSPERO (CRD42024508021) and reported in accordance with PRISMA guidelines. We performed a meta-analysis using Review Manager 5.3, Stata 12, Get Data Graph Digitizer 2.26, and SPSS.

2.1 Search strategy

In January 2024, 1,246 studies were extracted by two authors (S.Z. & Y.X.) from 5 databases, including Web of Science, Ovid, Scopus, Pubmed, and Ebsco, into the software Endnote X9 to be screened, and no more studies were from other resources. The keywords and subject heading were determined through two reviewers' discussions, and these words were collected from the Pubmed Mesh database. The confirmed words were as follows: "High protein, diet OR Plant protein, dietary OR Vegetable protein OR Whey protein OR Egg protein OR White protein OR Amino acid OR athletic performance OR Sports performance OR Endurance performance OR Muscle strength." Each previously mentioned database utilized these terms.

2.2 Inclusion and exclusion criteria

We excluded non-human studies, including those on animals, plants, and so on. The studies without a control group or protein group were not considered. Studies that lacked available data to be extracted and non-original studies, including letters, reviews, or editorials, were excluded during the selection process. The studies that used other languages instead of English were excluded.

We included the randomized controlled trial, which included male and female athletes. The included studies must include both a control group and a protein group, with the control group demonstrating a significant difference in protein intake compared to the protein group. Studies must provide quantitative measurements of athletic performance or physiological indices, such as aerobic performance, anaerobic performance, heart rate, blood glucose, and so on.

2.3 Selection process

Figure 1 shows the selection process and information sources. The automatic tool recommended by PRISMA was used to make the flow chart (49). The literature selection started in February 2024. Two authors, S.Z. and Y.X., independently searched and evaluated the literature using the previously confirmed inclusion and exclusion criteria to exclude duplicates. The Endnote auto tool initially eliminated duplicate literature, followed by screening by two authors. In the first step, 1,046 articles were screened based on their title and abstract, marking 993 articles as irrelevant. We advanced 53 articles to the next step for further full-text assessment, while 25 articles were excluded due to data ambiguity and participant irrelevance. During the full-text screening process, two articles on plant-based protein with more than 50 participants were discovered, but they were not chosen for the final data extraction and meta-analysis due to the absence of a comparable group. The meta-analysis ultimately included 28 articles.

2.4 Study risk of bias assessment

The risks of bias for all included studies were independently assessed using the guidelines and criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions. Two authors (S.Z. & Y.X.) assessed the included literature through the Cochrane risk of bias (ROB) assessment tool. Seven areas of bias risk were assessed: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; and (7) other bias. The risk of bias was classified into low, high, and unclear. Two reviewers performed the Kappa consistency test using the software SPSS after the quality assessment. If the Kappa results were poor, we asked the third or fourth reviewers (S.D. & J.L.) to reassess the quality of all included studies and discuss how to correctly adjust the quality assessment results until we got a good Kappa value.

After the data extraction process and meta-analysis, the risk of bias was assessed using the funnel plot and the p value from Egger's and Begg's tests. Funnel plots were generated in Stata 12.0, displaying the symmetry of the included data, with circle dots distributed evenly on both sides of the plot. A p-value less than 0.05 in both Egger's and Begg's tests indicated no significant risk of bias. Any discrepancies between reviewers at any stage of the process were resolved through discussion and consensus. The final data were presented in the results section.

2.5 Certainty in evidence

GRADEprofiler software was used to assess each main result. The quality of protein evidence was assessed using the GRADE approach, which provided a clear method to rate the quality across studies by evaluating the risk of bias, inconsistency of results, indirectness, and imprecision of effect estimates. The GRADE approach classifies the quality of evidence as high, moderate, low, or very low.

2.6 Data extraction and analysis

Two authors collected data using Endnote X9. The authors appropriately extracted the eligible data into the Excel template. The original data types were mean plus standardized deviation, mean plus standardized error, or mean plus confidential interval. Data were presented as mean plus standard deviation (M ± SD). Review Manager was used to convert data not initially in M ± SD format.

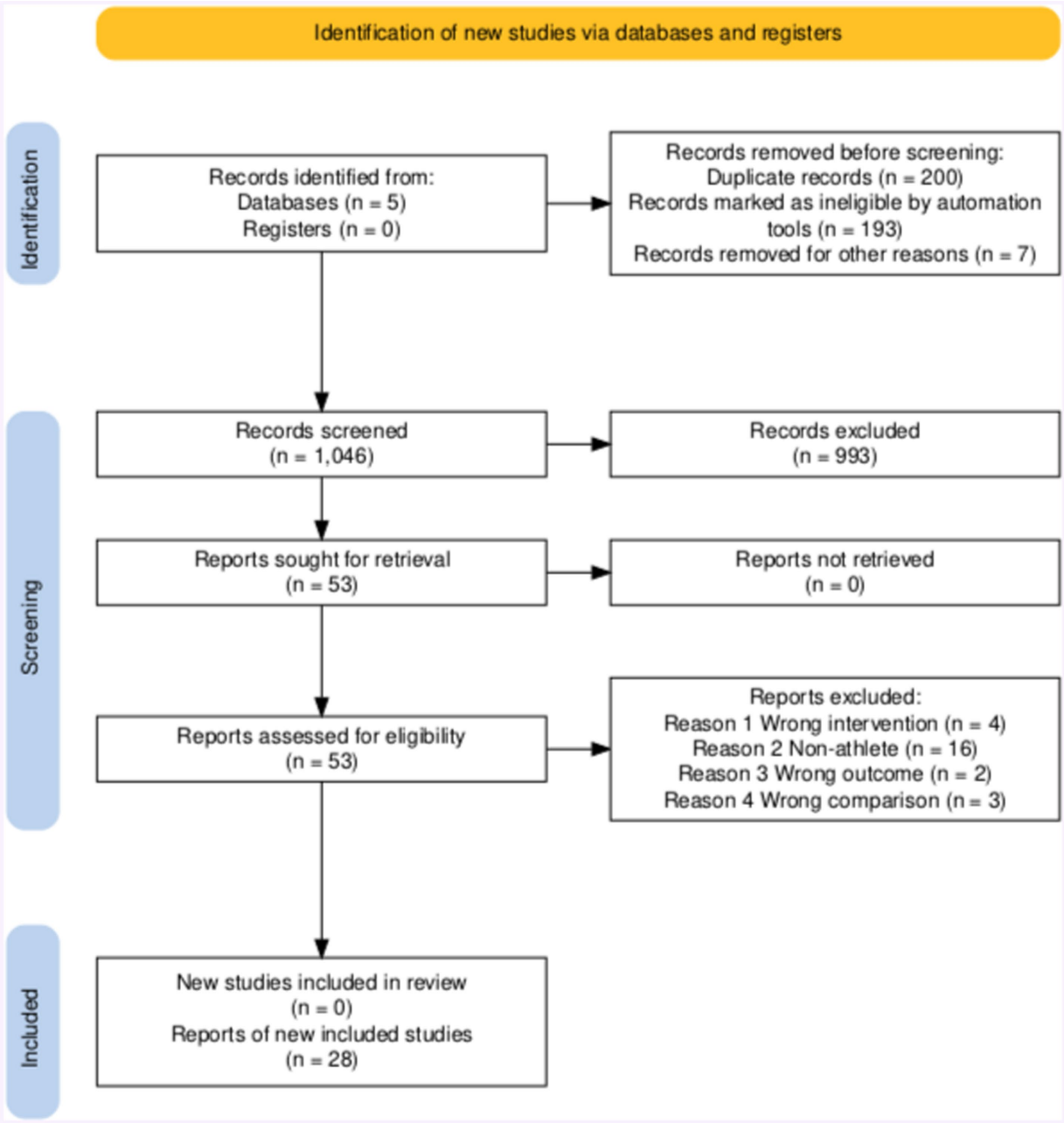

FIGURE 1
Prisma flow chart for the identification of the included studies.

The information we extracted was as follows: country, author, publication years, participants' age, total number, gender, classification of athletes, and types of dietary interventions (control and experimental group), and the available outcome data included the time to exhaustion, peak power, deadlift, squat, blood glucose, Vo_{2max}, heart rate, blood lactate, bench press, maximum voluntary contraction (MVC), Wingate test, counter-movement jump (CMJ), running average speed, and maximum speed. Physiological indices include blood glucose, muscle glycogen, heart rate, and blood lactate.

All of the data collected were continuous variables. The effect sizes calculated in the meta-analysis were standardized mean difference (SMD) or mean difference (MD). When the article did not provide accurate data, the Get Data Graph Digitizer software extracted data from the graph, including the desired outcomes. We divided all original data from the 28 articles into two parts. One was the mean change difference and corresponding standard deviation (ΔSD) of the outcomes of interest from text, tables, and graphs to compare the changes through interventions between the protein and control group. The other was the final value after the intervention to compare the difference between the two groups. The data were mean plus standardized deviation (M ± SD). When the ΔSD was not reported in the study, we estimated the ΔSD by calculating the correlation coefficient (corr) according to the formula provided by the Cochrane Handbook for meta-analysis of intervention:

$$\text{Corr} = \left(\text{SDpre}^2 + \text{SDpost}^2 - \text{SDchange}^2\right) / \left(2 \times \text{SDpre} \times \text{SDpost}\right)$$

The ΔSD was then calculated using the formula:

$$\Delta\text{SD} = \sqrt{\left(\text{SDpre}^2 + \text{SDpost}^2 - 2 \times \text{corr} \times \text{SDpre} \times \text{SDpost}\right)}$$

All of the data were exported into an Excel template to make the literature characteristic chart, and we also used it to make a forest plot and did the test for heterogeneity to calculate the I^2value to represent. All forest plots chose the fixed-effect model because the calculated heterogeneity $I^2 < 50\%$. In the meta-analysis, some articles used three groups, one control group and two experimental groups, to carry out their research. To address this, we compared the control and two experimental groups in the forest plot, respectively, which means we used the control group's data twice.

2.7 Subgroup analysis

Despite the relatively low heterogeneity, subgroup analysis was conducted to fully understand the impact of protein intake on athletic performance. Subgroup analysis was divided into several parts: (1) subgroup analysis based on the types of athletic performance; (2) subgroup analysis based on protein supplementation strategy; (3) subgroup analysis based on each athletic performance test and blood parameters; and (4) subgroup analysis based on energy matching and the amount of protein ingestion (1.5 g/kg daily or 1.5 g/kg daily).

2.8 Sensitive analysis

Stata 12.0 conducted a sensitive analysis to evaluate the credibility and robustness of all included data. The sensitive analysis used the leave-one-out method to assess whether the study's included data was robust.

3 Results

3.1 Study characteristics

All studies included in the meta-analysis were randomized controlled trials (RCTs). This meta-analysis included the randomized crossover design (RCD), a type of RCT. 10 studies were RCD. Tables 1, 2 provide the details and characteristics of the 28 studies. This meta-analysis analyzed data from 373 participants. A total of 14 studies included cyclists as participants, while one study (18) did not specify the type of athletes involved. Participants in 2 studies were soccer players (28, 31), and 2 studies' participants were triathletes (23, 33). Other participants were rugby players, sailing players, and climbing players (28, 32, 36). The majority of participants in the included studies were male, and three studies did not collect information on participants' gender. The publication years spanned from 2001 to 2023, and the participants were from 13 countries on the following continents: Europe, North America, and South America.

Twelve studies' intervention methods were carbohydrate (CHO) plus protein (PRO) co-ingestion. Six studies had three groups: one control group and two experimental groups. Five studies used a placebo (PLA) group as the control group. The difference in protein content was the main distinction between the control and experimental groups.

3.2 Quality assessment

The details of the quality assessment can be seen in Figures 2, 3. The risk of bias in the included studies was assessed using the ROB scale, and the results were presented using the software Reviewer Manager 5.3. Some studies' randomized methods were insufficient. The use of incomplete random allocation methods, such as block randomization, resulted in these studies being classified as high risk (4 articles, accounting for 14%) (24, 33, 34, 50). Some studies did not provide enough information on allocation concealment (57%), and one study did not conceal the allocation process to participants (3.5%) (34). Some studies failed to provide comprehensive details about participant and personnel blinding (32%), while 82% of the included studies lacked sufficient information on outcome data blinding, resulting in their classification as unclear risk. One study (35) was marked as high risk (3.5%) due to its failure to conceal the outcome data from participants and researchers during the test. Only one study (16) incurred an unclear risk of incomplete data due to participant withdrawal during the experiment (3.5%).

After two reviewers (S.Z. & Y.X.) assessed all of the included studies, we did the Kappa consistency test. The result was 0.286 at first, so we invited the third scholar (S.D.) to be the reviewer to reassess all studies. After three reviewers' discussions, we obtained the final result. The final Kappa value was 0.761, indicating excellent agreement in quality assessment between reviewers. Table 3 illustrates this.

3.3 Quality grade in each main outcome

Data from nine outcomes were assessed (Figure 4). The outcome of endurance performance was rated as high quality. The outcome of athletic performance was rated as moderate quality due to statistical insignificance. The outcomes of muscle strength, physiological index, and muscle strength presented by change value were rated as low quality due to the small sample size and statistical insignificance. The outcomes of muscle strength and endurance performance intervened by high protein ingestion were rated as low quality due to the small sample size and statistical insignificance. The outcome of endurance performance intervened by protein plus carbohydrate ingestion was rated moderate due to the small sample size. The outcome of endurance performance presented by the change value was rated moderate due to the small sample size.

3.4 Meta-analysis

All included studies compared the effect of the protein group vs. the non-protein group on athletic performance. A total of 27 studies provided 361 participants' athletic performance data in the forest plot presented in Figure 5 (final value), including the running maximum speed and average speed, peak power in the Wingate test, the time to exhaustion of cycling with different percentages of Vo_{2max}, Vo_{2max}, cycling completed time, and mean power in time trial, 1RM, MVC,

TABLE 1 The characteristics of the included studies.

Code	Region	Author	Year	Study design	Sample	Sport Subjects	Gender (Male/ Female)	Age (Mean ± SD)	Amount of protein
1	UK	Highton	2012	RCD	9	Soccer & rugby players	9/0	23.4 ± 1.2	0.5 g/kg daily
2	Australia	Hall	2012	RCD	10	Cyclists	10/0	29.7 ± 5.01	0.54 g/kg daily
3	Malaysia	Ghosh	2010	RCD	8	Cyclists	8/0	21.5 ± 1.1	0.3 g/kg daily
4	Switzerland	Furber	2021	RCT	16	Endurance runners	16/0	26 + 4.5	4.6 g/kg daily
5	USA	Grubic	2019	RCD	12	Resistance-trained athletes	12/0	22 ± 1.8	0.73 g/kg daily
6	USA	Saunders	2007	RCT	13	Cyclists	8/5	24.2 ± 6.8	0.038 g/kg
7	UK	Röhling	2021	RCT	23	Endurance athletes	16/7	Not clear	0.75 g/kg daily
8	USA	Schroer	2014	RCT	8	Cyclists	4/4	22.3 ± 5.6	1.93 g/kg daily
9	New Zealand	Rowlands	2011	RCD	12	Cyclists	0/12	30 ± 7	2.8 g/kg daily
10	UK	Witard	2011	RCD	8	Cyclists	8/0	27 ± 8	3 g/kg daily
11	Greece	Kritikos	2021	RCD	10	Soccer players	10/0	21 ± 1.5	1.5 g/kg daily
12	France	Portier, H.	2008	RCT	12	Sailing players	12/0	36.15 + 8.85	3.2 g/kg daily
13	USA	Campbell	2018	RCT	17	NA	0/17	21.2 ± 2.1	2.5 g/kg daily
14	Canada	Naclerio	2017	RCT	24	Triathletes	24/0	46.17 + 7.98	1.6 g/kg daily
15	UK	Furber	2022	RCT	16	Endurance runners	Not clear	26.6 + 4.33	4.6 g/kg daily
16	Not clear	Laskowski	2003	RCT	12	Judo players	Not clear	16.25 + 2.33	0.50 g/kg daily
17	UK	Mettler	2010	RCT	20	Not clear	20/0	25.25 + 5.11	2.31 g/kg daily
18	France	Bourrilhon	2010	RCD	10	Climbing Players	10/0	30 ± 2.85	4.1 g/kg daily
19	Brazil	Finger	2018	RCT	13	Triathletes	13/0	29.7 ± 7.7	0.3 g/kg daily
20	UK	Naclerio	2019	RCT	25	Endurance athletes	25/0	32.28 + 8.35	0.3 g/kg daily
21	UK	Toone	2010	RCT	12	Cyclists	12/0	23.4 ± 3.2	0.3 g/kg daily
22	New Zealand	Thomson	2011	RCT	10	Cyclists	10/0	33 ± 9	0.75 g/kg daily
23	Spain	Valenzuela	2023	RCT	24	Cyclists	Not clear	19.33 + 1.73	3.5 g/kg daily
24	Denmark	Hansen	2016	RCT	18	Cyclists	18/0	19.5 + 2	0.95 g/kg daily
25	Norway	Sollie	2018	RCD	8	Cyclists	8/0	22.9 ± 3.39	0.76 g/kg daily
26	New Zealand	Macdermid	2006	RCT	7	Cyclists	7/0	33.6 ± 5.0	3.3 g/kg daily
27	USA	Williams	2003	RCD	8	Cyclists	8/0	28.4 ± 4.8	0.5 g/kg daily
28	UK	Jentjens	2001	RCT	8	Cyclists	8/0	27.1 ± 7.35	0.85 g/kg daily

jump test, and CMJ. The mean athletic performance effect size was 0.12 with a 95% confidence interval of −0.01 to 0.25, $p = 0.06$, $Z = 1.86$, and $I^2 = 16\%$. The effect of the control and protein groups on athletic performance has not been observed with any statistical significance.

3.5 Subgroup analysis

The subgroup analysis was divided into five parts: (1) subgroup analysis based on athletic performance types; (2) subgroup analysis based on protein supplementation strategies; (3) subgroup analysis based on each athletic performance test and blood parameters; (4) subgroup analysis based on energy matching or not between protein group and non-protein group; and (5) subgroup analysis based on the amount of daily protein ingestion in the protein group (1.5 g/kg daily or 1.5 g/kg daily). Parts 1 to 3 aimed to explore the effect of protein intake on different types of athletic performance. Parts 4 and 5 aimed to avoid imprecision in the meta-analysis because of the difference in energy matching or the amount of protein ingestion.

3.5.1 Subgroup analysis based on the amount of daily protein ingestion in protein group (<1.5 g/kg daily or ≥ 1.5 g/kg daily)

Table 4 presents the subgroup analysis based on protein intake. The protein ingestion that was lower than 1.5 g/kg daily showed statistical significance, and the protein ingestion that was higher than or equal to 1.5 g/kg daily showed insignificance. Thirteen of the fifteen studies implemented a carbohydrate plus protein intervention, while all twelve implemented a high protein intervention. This subgroup analysis result aligns with the subgroup analysis based on the different types of protein supplementation strategies. Thus, the difference in the amount of protein ingestion did not affect the result of this meta-analysis or cause deviation.

TABLE 2 The characteristics of diet intervention and athletic performance measure method.

Code	Author	Diet intervention			Athletic performance measure method
		Control group	Experiment group		
1	Highton	CHO	CHO + PRO		Modified Loughborough intermittent shuttle test
2	Hall	CHO	CHO + PRO		VO2max test; cycling time trial; Blood sampling
3	Ghosh	PLA	CHO	CHO + PRO	VO2max test; Time to exhaustion test; Blood sampling
4	Furber	CHO	PRO		Running distance and speed; Cycling time trial; and Blood sampling
5	Grubic	CHO	CHO + PRO		Maximum voluntary contraction (MVC); Resistance exercise; Sprint performance and Blood sampling
6	Saunders	CHO	CHO + PRO		Physical fitness assessment Experimental ride with blinded treatment
7	Röhling	PLA	PRO		Running completed time and speed; VO2max test; and Blood sampling
8	Schroer	PLA	ALA	PRO	Cycling time trial; VO2max test; and Blood sampling
9	Rowlands	CHO	PRO		Sprint performance, strength performance, and Blood sampling
10	Witard	Normal diet	PRO		VO2max test; Cycling time trial; and Blood sampling
11	Kritikos	PLA	Whey Protein	Soy protein	Speed-endurance training, MVC, and Blood sampling
12	Portier	Normal diet	PRO		Physical performance test (Jump test and Handgrip Strength)
13	Campbell	Low protein diet	PRO		Maximal strength Resistance and high-intensity interval training and blood sampling
14	Naclerio	CHO	Beef protein	Whey protein	VO2max test
15	Furber	CHO	PRO		Time trial to exhaustion performance
16	Laskowski	Normal diet	PRO		VO2max test; Wingate test (peak power)
17	Mettler	Normal diet	PRO		MVC; jump test; Wingate test (peak power); Blood sampling
18	Bourrilhon	High carbohydrate diet	PRO		MVC
19	Finger	PLA	CHO	CHO + PRO	Cycling test; Running test
20	Naclerio	CHO	CHO + PRO		VO2max test; maximal aerobic speed
21	Toone	CHO	CHO + PRO		Cycling time trial; blood sampling
22	Thomson	Normal diet	PRO		Maximum aerobic power; repeat-sprint performance test
23	Valenzuela	CHO + PLA	Pre-sleep protein	Afternoon protein	Counter-movement jump (CMJ); cycling time trial
24	Hansen	CHO	CHO + PRO		10 s peak power test; 5 min all-out performance test
25	Sollie	CHO	CHO + PRO		Time to exhaustion test; Sprint power; Blood sampling
26	Macdermid	CHO	PRO		Time Trial Performance; Blood sampling
27	Williams	CHO	CHO + PRO		Time to exhaustion test; Blood sampling
28	Jentjens	CHO	CHO + PRO		Graded exercise test to exhaustion; Blood sampling

3.5.2 Subgroup analysis based on the types of athletic performance

Five forest plots (final value & change value) present three mean effect sizes of endurance performance, muscle strength, and physiological indices. Three forest plots included the final value data, while two included the change value data. The summary of the overall effect size can be seen in Table 5 (Final value) and Table 6 (Change value).

The mean endurance performance effect size was 0.19 with a 95% confidence interval of 0.04 to 0.33, $p = 0.02$, $Z = 2.42$, and $I^2 = 31\%$. Compared with the control group, the protein group had a greater gain in endurance performance, which includes aerobic and anaerobic capacity (Figure 6).

Seventeen studies provided 272 participants' data about endurance performance in the forest plot presented in Figure 7 (change value). The mean endurance performance effect size was 0.31 with a 95% confidence interval of 0.15 to 0.46, $p = 0.0001$, $Z = 3.81$, and $I^2 = 0\%$. Compared with the control group, the protein group had a greater gain in endurance performance, which includes aerobic and anaerobic capacity.

Figure 8 summarized the mean effect size of muscle strength, which included MVC, jump height, 1RM chest press, 1RM bench press, and 1RM squat. This forest plot included seven studies and 105 participants. The mean muscle strength effect size was −0.05 with a 95% confidence interval of −0.3 to 0.19, $p = 0.68$, $Z = 0.42$, $I^2 = 0\%$. No difference between the protein and control groups was observed.

In the forest plot shown in Figure 9 (change value), nine studies provided 105 participants' muscle strength data. The mean muscle strength effect size was −0.05 with a 95% confidence interval of −0.19

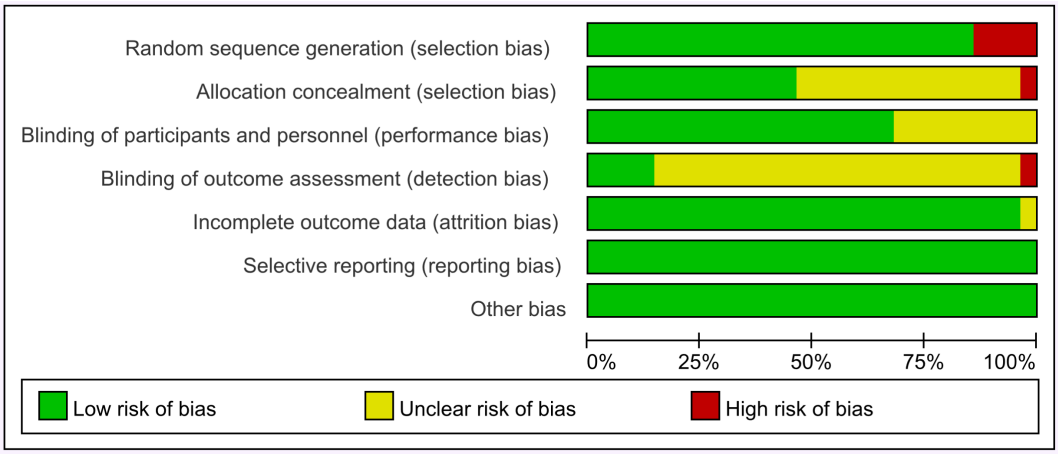

FIGURE 2
Risk of bias summary.

to 0.3, $p = 0.67$, Z = 0.42, $I^2 = 0\%$. No difference between the protein and control groups was observed.

Figure 10 summarizes the mean effect size of the physiological indices, which include blood glucose, blood lactate, heart rate, and muscle glycogen. This forest plot included 18 studies and 206 participants. The mean physiological index effect size was 0.06 with a 95% confidence interval of −0.10 to 0.22, $p = 0.48$, Z = 0.71, $I^2 = 0\%$. No difference between the protein and control groups was observed.

3.5.3 Subgroup analysis based on protein supplementation strategy

Table 7 summarizes the subgroup analysis based on the different protein supplementation plans. To conduct the subgroup analysis, the protein supplementation program was divided into two sections: (1) Protein plus Carbohydrate (PRO+CHO) and (2) High Protein intake.

The forest plot of endurance performance after protein plus carbohydrate intervention included twelve studies and 142 athletes. The mean effect size was 0.29 with a 95% confidence interval of 0.09 to 0.49, $p = 0.005$, Z = 2.84, $I^2 = 0\%$. The protein group showed a greater improvement in endurance performance. Figure 11 provides the details.

The forest plot of endurance performance after the high-protein intervention included 12 studies and 164 athletes. The mean effect size was 0.09 with a 95% confidence interval of −0.10 to 0.27, $p = 0.36$, Z = 0.92, and $I^2 = 31\%$. No difference between the protein and control groups was observed. Figure 12 provides the details.

Following the high-protein intervention, the forest plot for muscle strength included seven studies and 117 athletes. The mean effect size was −0.08 with a 95% confidence interval of −0.37 to 0.26, $p = 0.56$, Z = 0.58, and $I^2 = 0\%$. No difference between the protein and control groups was observed. Figure 13 provides the details.

3.5.4 Subgroup analysis based on each athletic performance test and blood parameters

Table 8 displays the subgroup analysis of each athletic performance test and blood parameters. The protein group showed statistical significance in the subgroup analysis of average speed, Wingate test, time to exhaustion ($Vo_{2max} \leq 90\%$), and muscle glycogen. The results showed a greater gain for the protein group. The subgroup analysis of time to exhaustion (95% Vo_{2max}) revealed that the non-protein group had a higher gain. The remaining data did not show statistical significance in both protein and non-protein groups.

3.5.5 Subgroup analysis based on the energy matching or not between protein group and non-protein group

Table 9 displays the subgroup analysis based on energy matching. The meta-analysis in both the energy-matching and non-energy-matching groups did not show statistical significance. The result of the subgroup analysis is the same as the meta-analysis, which means the energy mismatch between the control and experimental groups did not affect the result or cause deviation.

3.6 Sensitivity analysis

The results of the sensitive analysis can be seen in Figures 14, 15. In the sensitive athletic performance analysis, this estimated result was 0.126, with a 95% confidential interval of −0.001 to 0.254. The estimated outcome closely matched the results of the meta-analysis on athletic performance. The estimated result in the sensitive analysis of the physiological indices was 0.063, with a 95% confidential interval of −0.099 to 0.226. The estimated result was highly close to the meta-analysis result in the physiological index. After the leave-one-out test, the results remained the same as the meta-analysis result, indicating the excellent robustness of all included data.

3.7 The risk of bias

Stata 12 was used to assess the risk of bias in the included studies on athletic performance and physiological indices. Figures 16, 17 summarize the risk of bias in athletic performance studies using Begg's and Egger's assessment methods. The Egger assessment result ($p = 0.826$) indicates no significant risk of bias in these studies. Similarly, Figures 18, 19 summarize the risk of bias in studies related

FIGURE 3
Risk of bias graph.

to physiological indices, with the Egger assessment result ($p = 0.301$) also indicating no risk of bias in this category. The funnel plot shows symmetrically distributed circle dots, suggesting a low risk of bias but also indicating a study population of potentially low quality.

4 Discussion

This is the first meta-analysis to investigate the effect of protein intake, including protein diets and protein supplements, on athletes' endurance performance, muscle strength, and physiological indices. High protein ingestion could not improve athletic performance, including endurance performance and muscle strength in athletes, but protein co-ingested with carbohydrate supplements was found to have statistical significance through subgroup analysis in improving endurance performance in athletes, especially in anaerobic endurance.

The present systematic review and meta-analysis summarize evidence for the effect of (1) protein intake on muscle strength; (2) protein intake during and/or before the initial bout of exercise on subsequent athletic performance, including aerobic and anaerobic performance and muscle strength; and (3) high protein ingestion versus protein plus carbohydrate co-ingestion on athletic performance in subgroup analysis.

4.1 The effect of protein intake on muscle strength

The meta-analysis failed to detect a relationship between protein intake and muscle strength. Many studies have demonstrated that protein intake cannot improve muscle strength in athletes. Protein sources are not likely to have an impact on muscle strength (51, 52), and some researchers found statistical significance between protein intake and lean mass and concluded that lean mass gain may not necessarily translate to strength improvements (51, 53, 54). Schoenfeld et al. concluded that protein timing had a small to moderate effect on muscle hypertrophy, with no significant effect on muscle strength (55). Researchers did not detect a statistically significant effect of protein intake on different age groups except for athletes. A meta-analysis of protein supplements (56) also did not find a statistical significance between the control and protein groups in muscle strength in common people, even including those who suffered from disease and were older. Seven studies' data were collected to calculate SMD of muscle strength. No correlations between single or combined protein intake and leg muscle power, leg muscle strength, or handgrip strength were observed in older people (57). Regardless of age, it appears that protein intake has no significant impact on muscle strength.

4.2 The effect of protein intake on endurance performance

The meta-analysis revealed that protein intake significantly improved endurance performance, including anaerobic and aerobic capacity, as demonstrated by the subgroup analysis of running speed, Wingate test, and time to exhaustion. The effect size in the forest plot of endurance performance presented by the change value was found to have greater statistical significance than the final value. The forest

TABLE 3 The Kappa consistency test of quality assessment.

Kappa				
	Value	Asymptotic standard error	Approximate *T*	Approximate significance
Measurement of agreement	0.761	0.063	12.85	0.0001
N of valid cases	196			

Protein intake for Athletic performane

Bibliography: . [Intervention] for [health problem]. Cochrane Database of Systematic Reviews [Year], Issue [Issue].

Outcomes	No of Participants (studies) Follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects: Risk with Control	Risk difference with Protein intake (95% CI)
Endurance performance	726 (23 studies)	⊕⊕⊕⊕ **HIGH**			The mean endurance performance in the intervention groups was **0.19 standard deviations higher** (0.04 to 0.33 higher)
Muscle strength	253 (7 studies)	⊕⊕⊖⊖ **LOW**[1,2] due to imprecision			The mean muscle strength in the intervention groups was **0.05 standard deviations lower** (0.3 lower to 0.19 higher)
Physiological index	597 (18 studies)	⊕⊕⊖⊖ **LOW**[1,2] due to imprecision			The mean physiological index in the intervention groups was **0.06 standard deviations higher** (0.1 lower to 0.22 higher)
CHO plus PRO: Endurance performance	404 (12 studies)	⊕⊕⊕⊖ **MODERATE**[1] due to imprecision			The mean cho plus pro: endurance performance in the intervention groups was **0.29 standard deviations higher** (0.09 to 0.49 higher)
High PRO: Muscle Strength	190 (7 studies)	⊕⊕⊖⊖ **LOW**[1,2] due to imprecision			The mean high pro: muscle strength in the intervention groups was **0.08 standard deviations lower** (0.37 lower to 0.2 higher)
H PRO: Endurance Performance	372 (10 studies)	⊕⊕⊖⊖ **LOW**[1,2] due to imprecision			The mean h pro: endurance performance in the intervention groups was **0.06 standard deviations higher** (0.15 lower to 0.27 higher)
Overall: Athletic Performance	782 (21 studies)	⊕⊕⊕⊖ **MODERATE**[2] due to imprecision			The mean overall: athletic performance in the intervention groups was **0.11 standard deviations higher** (0.03 lower to 0.26 higher)
Endurance Performance(change value)	648 (34 studies)	⊕⊕⊕⊖ **MODERATE**[1] due to imprecision			The mean endurance performance(change value) in the intervention groups was **0.31 standard deviations higher** (0.15 to 0.46 higher)
Muscle strength (change value)	258 (14 studies)	⊕⊕⊖⊖ **LOW**[1,2] due to imprecision			The mean muscle strength (change value) in the intervention groups was **0.05 standard deviations higher** (0.19 lower to 0.3 higher)

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Small sample size
[2] P > 0.05

FIGURE 4
The quality grade of athletic performance.

plot of endurance performance presented by the change value was considered the baseline difference among participants, and the results were more convincing than the final value. Many studies had similar results. A review (58) concluded that using protein supplements during recovery can enhance subsequent exercise capacity, particularly when sub-optimal carbohydrate delivery. Lin et al. concluded that protein supplements increased aerobic capacity, stimulated lean mass gain, and improved time trial performance during chronic endurance training in healthy and clinical populations (56). It was also found by Stearn et al. that eating protein and carbs together increased endurance performance when assessed by time to exhaustion and where supplements were matched for CHOs. However, the ergogenic effect of protein observed in studies on isocarbohydrates might be due to the general effect of adding calories (fuel) rather than a specific benefit of protein. Before drawing a clear conclusion, further research is necessary (59).

In the subgroup analysis, protein intake did not show statistical significance in increasing the Vo_{2max} compared to the control group. Protein intake did not provide additional benefits for participants who had already achieved substantial improvement in Vo_{2max} through high-volume training, suggesting that the Vo_{2max} improvement from protein intervention had reached a ceiling effect in these athletes.

However, Lin et al. found statistical significance in Vo_{2max} improvements in the protein group among individuals who were weak or ill, concluding that protein supplements probably provided more benefits to those with lower aerobic capacity but offered few additional

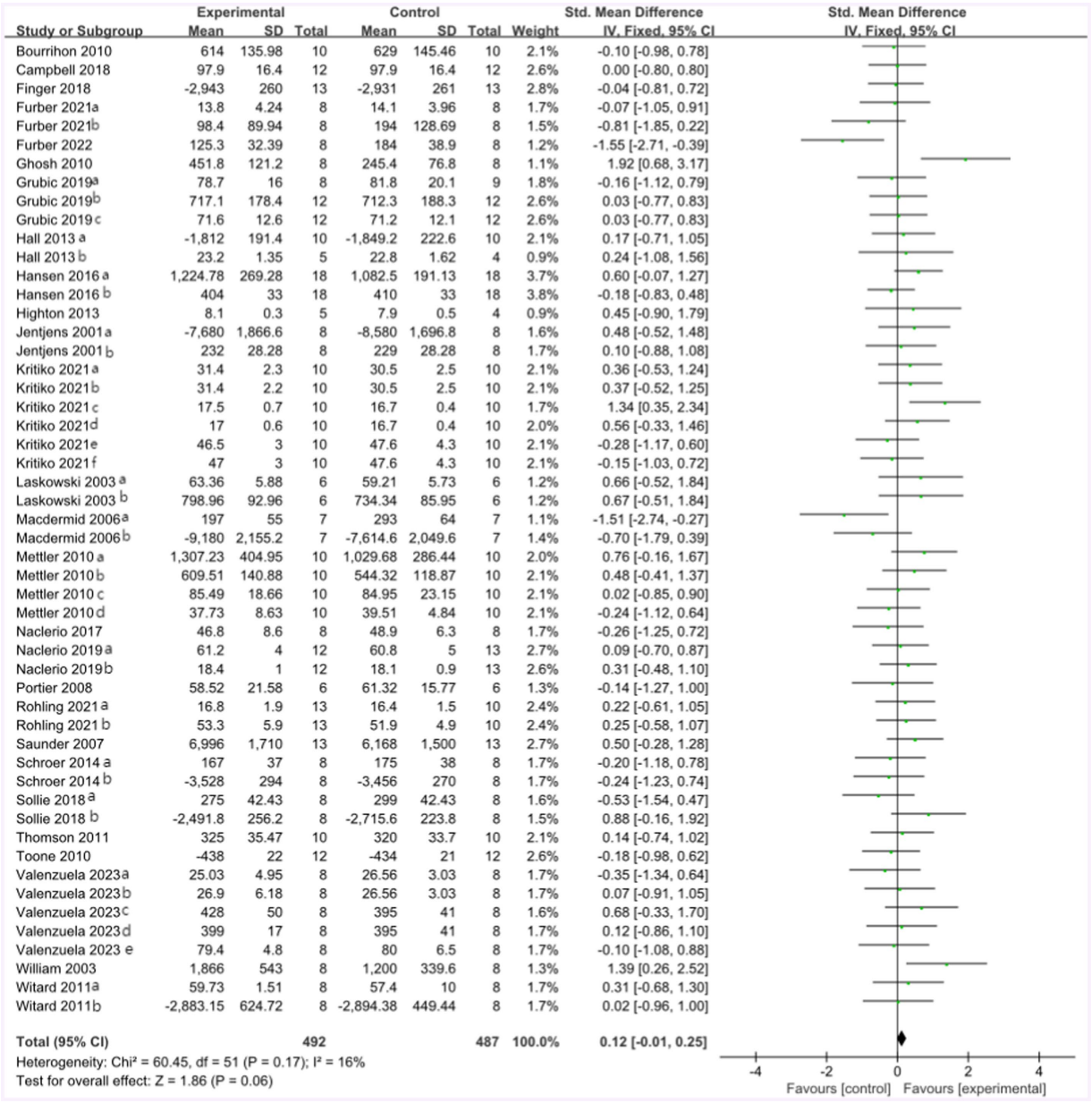

FIGURE 5
The forest plot of athletic performance (no protein group vs. protein group, and final value).

TABLE 4 Subgroup analysis based on the amount of protein ingestion (<1.5 g/kg daily or ≥ 1.5 g/kg daily).

Subgroup name	Studies number	SMD (95% CI)	*p*	*Z*	I^2
Protein ingestion (lower than 1.5 g/kg daily)	15	0.24 (0.06 to 0.42)	0.008	2.63	0%
Protein ingestion (higher than or equal to 1.5 g/kg daily)	12	0.00 (−0.17 to 0.18)	0.98	0.02	19%

TABLE 5 The summary of different athletic performance (final value).

Name	No. of studies	No. of participants	Effect size (SMD)	*p*	*Z*	I^2
Endurance performance	23	310	0.19 [0.04, 0.33]	0.03	2.42	31%
Physiological index	18	206	0.06 [−0.10, 0.22]	0.48	0.71	0%
Muscle strength	7	105	−0.05 [−0.30, 0.19]	0.68	0.42	0%

10.3389/fnut.2024.1455728

TABLE 6 The summary of different athletic performance (change value).

Name	No. of studies	No. of participants	Effect size (SMD)	*p*	*Z*	I^2
Endurance performance	17	272	0.31 [0.15, 0.46]	0.0001	3.81	0%
Muscle strength	9	105	−0.05 [−0.19, 0.30]	0.67	0.42	0%

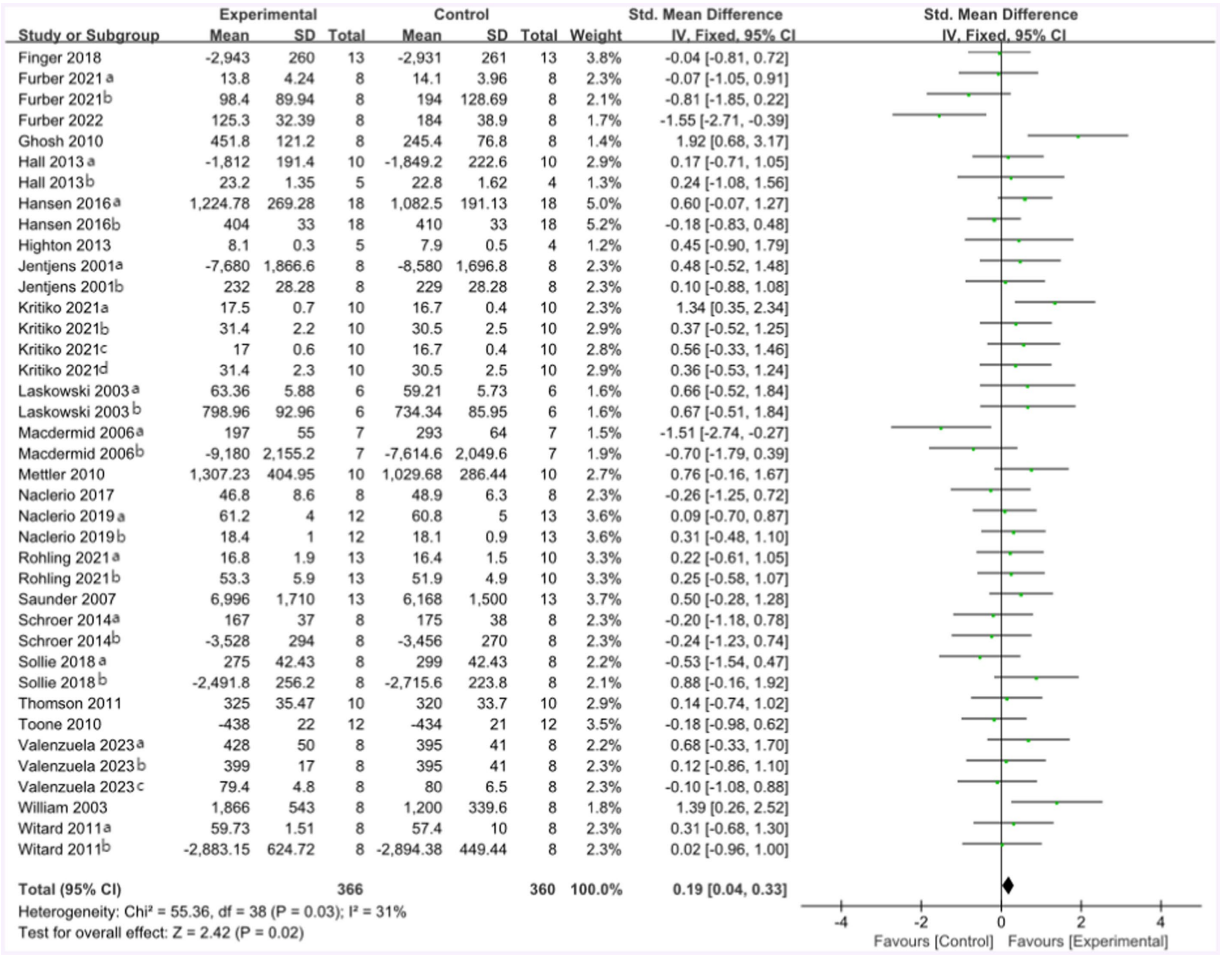

FIGURE 6
The forest plot of endurance performance (no protein group vs. protein group and final value).

benefits to participants who had already achieved substantial improvements in VO_{2max} through high-intensity endurance training (56). Additionally, some researchers discovered that protein supplements increased the production of myofibrillar protein but not mitochondrial protein, indicating that protein intake did not improve whole-body aerobic capacity (i.e., VO_{2max}) (60, 61). This finding provides supportive evidence from a molecular perspective.

The time to exhaustion in cycling and running exerted by different loads of Vo_{2max} (95%, 85% & 75%) presented opposing results. The control group experienced a greater increase in the time to exhaustion when running at the load of 95% Vo_{2max}, indicating that protein intake was not beneficial in improving anaerobic performance. However, it is important to note that the same author wrote all studies in the forest plot of time to exhaustion in the running and published them in different years. The number of athletes was limited. It still needs further investigation. The time to exhaustion during cycling at 90, 85, and 75% loads of Vo_{2max} exhibited moderate heterogeneity of 52%, which can be attributed to the variation in Vo_{2max} exerted during the test.

Overall, protein ingestion seems to have a beneficial effect on endurance performance, and several studies have given supportive evidence. Ingesting protein during exercise may have an ergonomic effect, potentially delaying the time to exhaustion in tests requiring significant physical strength (62). The provision of protein/amino acids supports increased rates of protein synthesis and positive protein balance following endurance exercise (63). Overall, protein intake has greater benefits for endurance athletes, such as cyclists or runners, than for other types of athletes. Although protein intake may improve endurance performance, more studies are still needed to enhance its credibility.

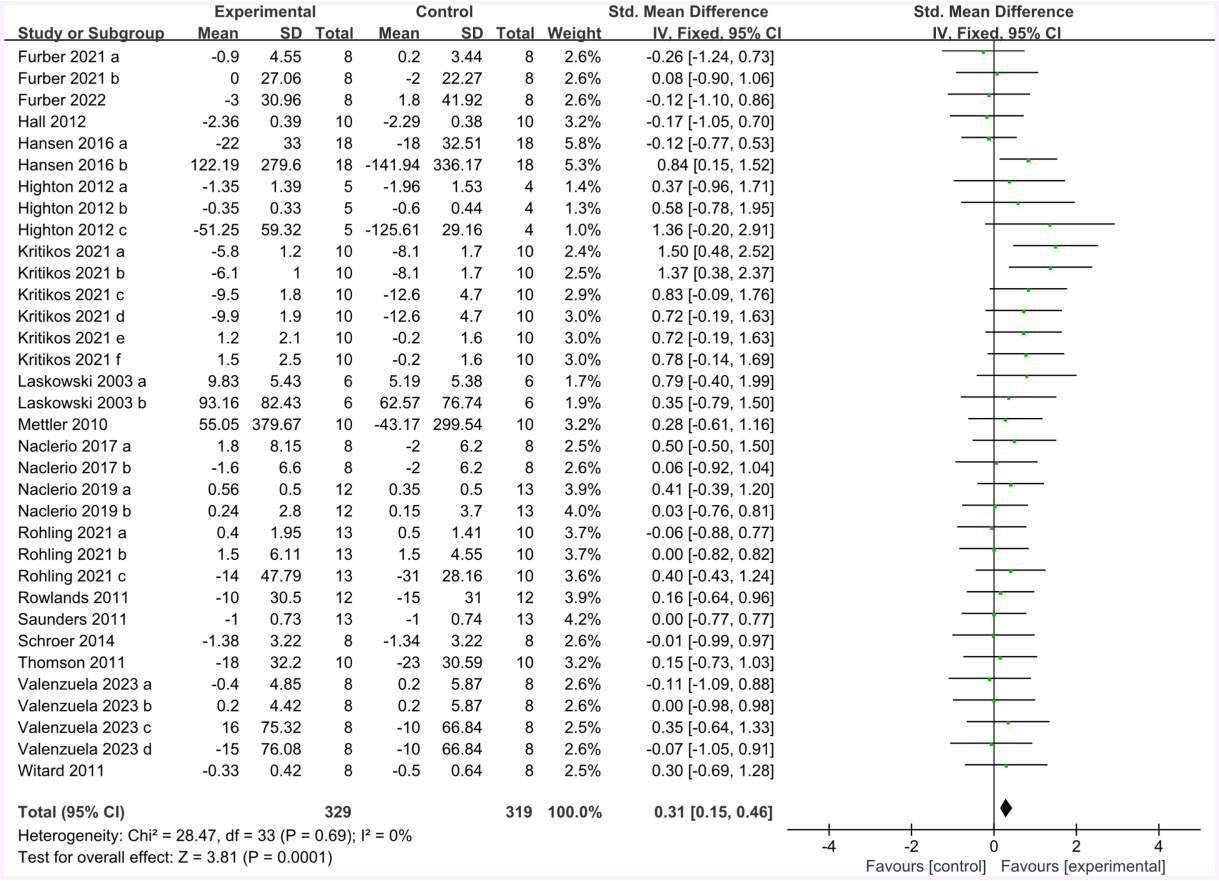

FIGURE 7
The forest plot of endurance performance (no protein group vs. protein group and change value).

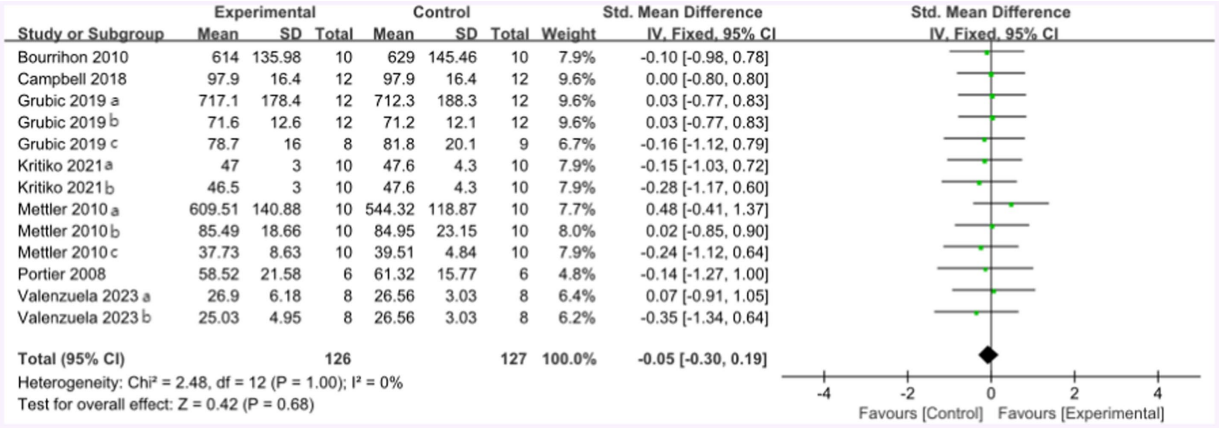

FIGURE 8
The forest plot of muscle strength (no protein group vs. protein group, final value).

4.3 The effect of protein intake on physiological indices

In the meta-analysis of the physiological indices, we did not find a statistical significance between the protein group and control group, but in the subgroup analysis, the muscle glycogen was found to be statistically significant in the protein group. A sufficient amount of muscle glycogen, increased by protein ingestion, could indirectly improve performance in athletes. A narrative review written by Larsen et al. tried to find the relationship between muscle glycogen and

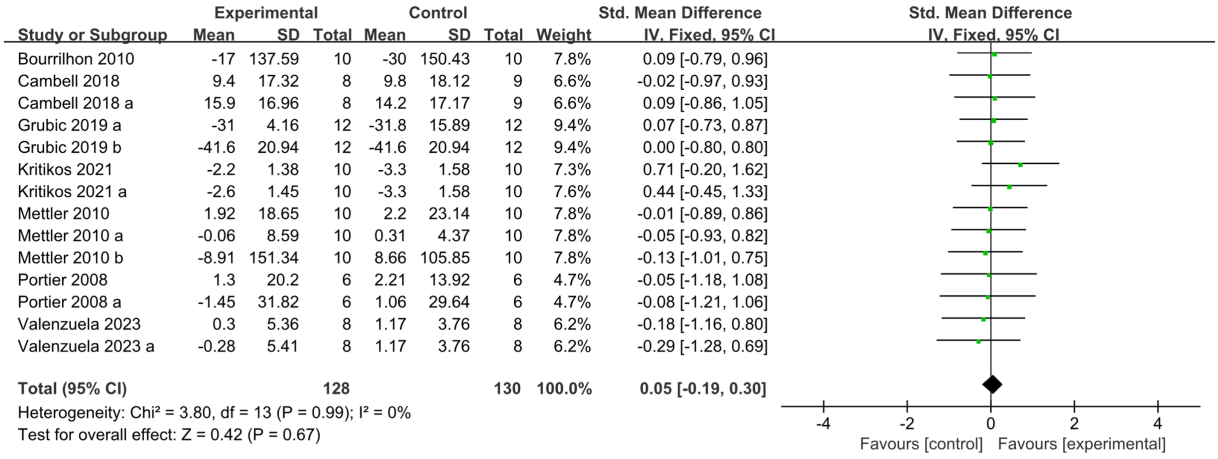

FIGURE 9
The forest plot of muscle strength (no protein group vs. protein group and change value).

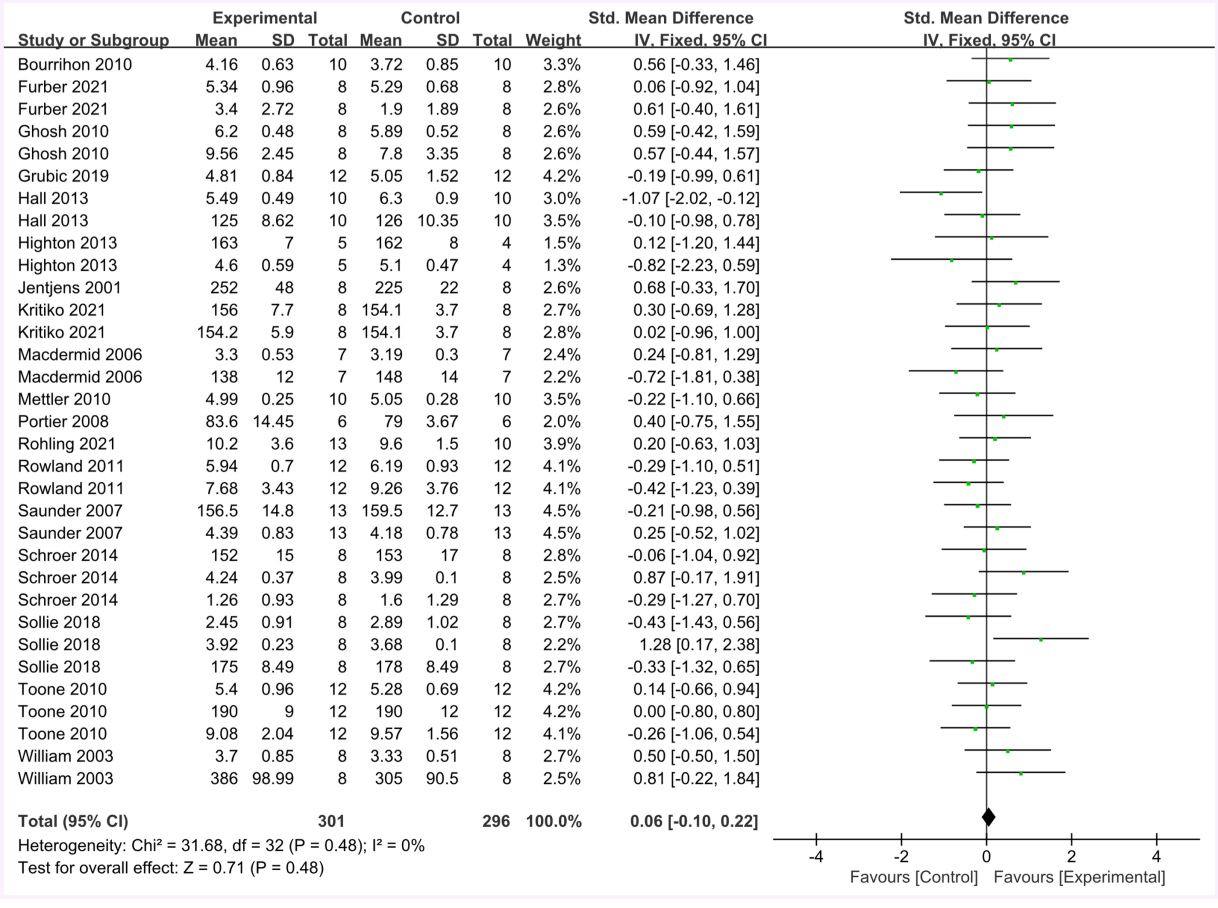

FIGURE 10
The forest plot of physiological indices (no protein group vs. protein group, final value difference).

high-intensity exercise, and they concluded that the amount of muscle glycogen was linked to high-intensity tolerance (64). As a result, adequate protein ingestion could improve muscle glycogen content, indirectly increasing athletes' tolerance for high intensity. The body first consumes muscle glycogen during high-intensity training, followed by blood glucose. Inadequate blood glucose can cause fatigue

TABLE 7 The summary of subgroup analysis based on protein supplementation strategy (final value).

Subgroup name	The types of athletic performance	SMD (95%CI)	*p*	*Z*	I^2
Protein plus carbohydrate	Endurance performance	0.36 [0.11, 0.61]	0.005	2.78	2%
High protein intake	Endurance performance	0.18 [−0.01, 0.37]	0.07	1.82	17%
High protein intake	Muscle strength	−0.08 [−0.37, 0.2]	0.56	0.58	0%

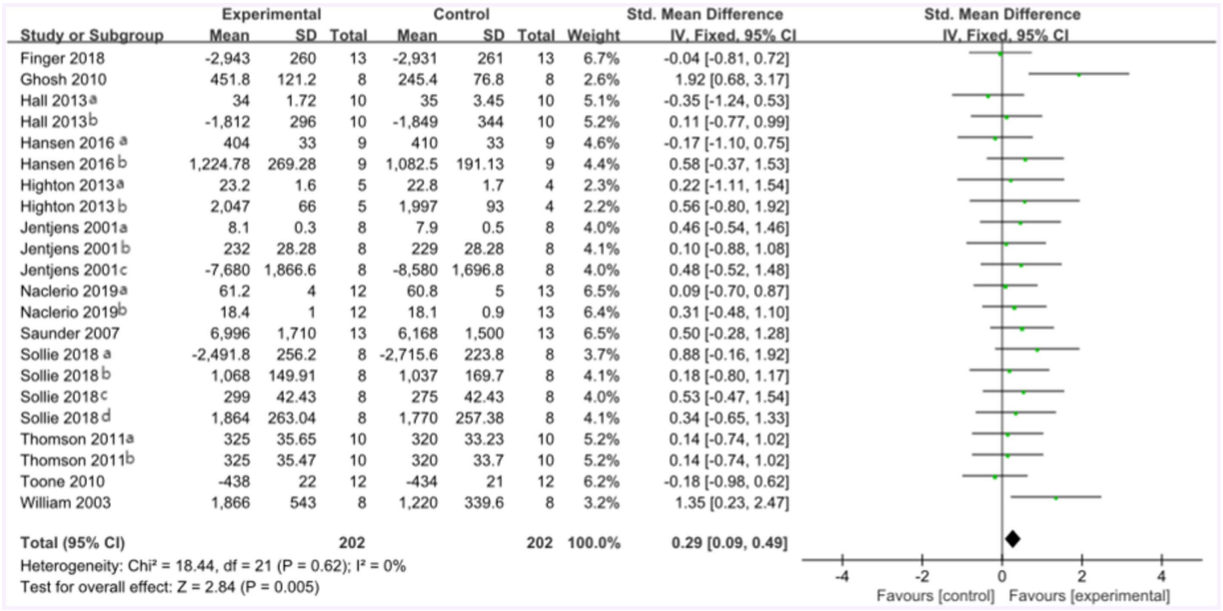

FIGURE 11
The forest plot of endurance performance after protein plus carbohydrate intervention (CHO group vs. PRO plus CHO group).

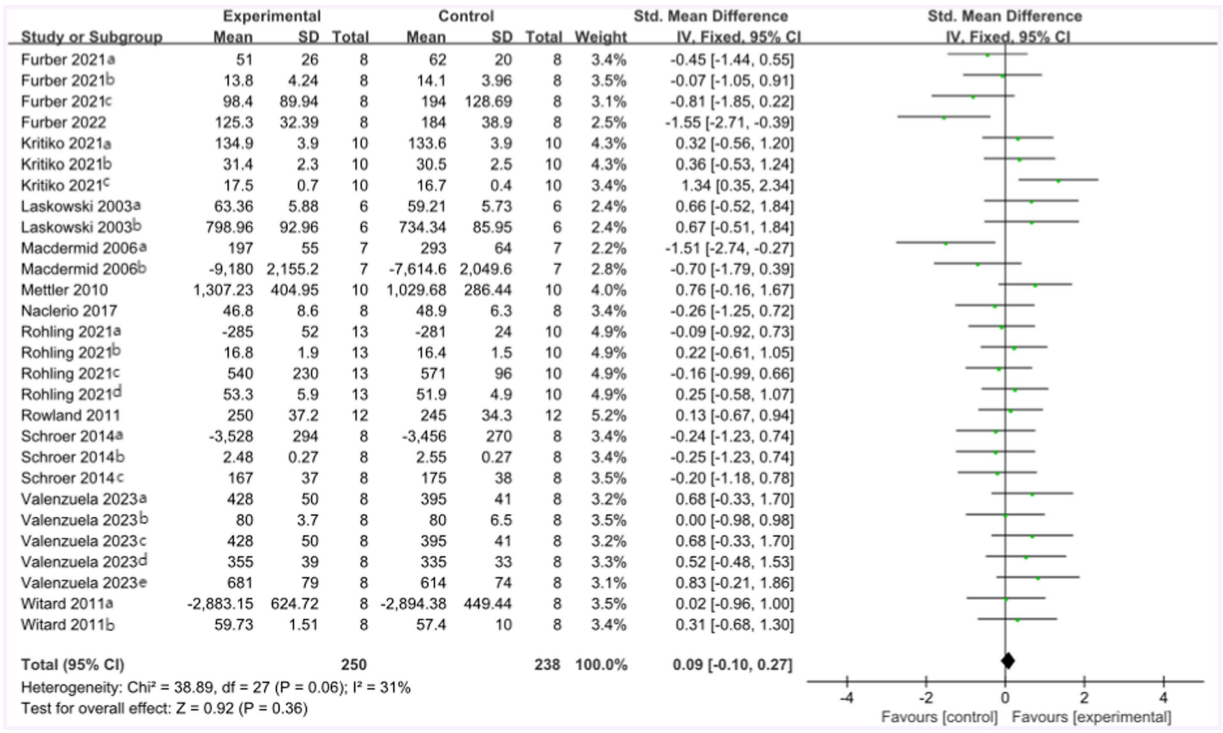

FIGURE 12
The forest plot of endurance performance after high protein intervention (no protein group vs. high protein group).

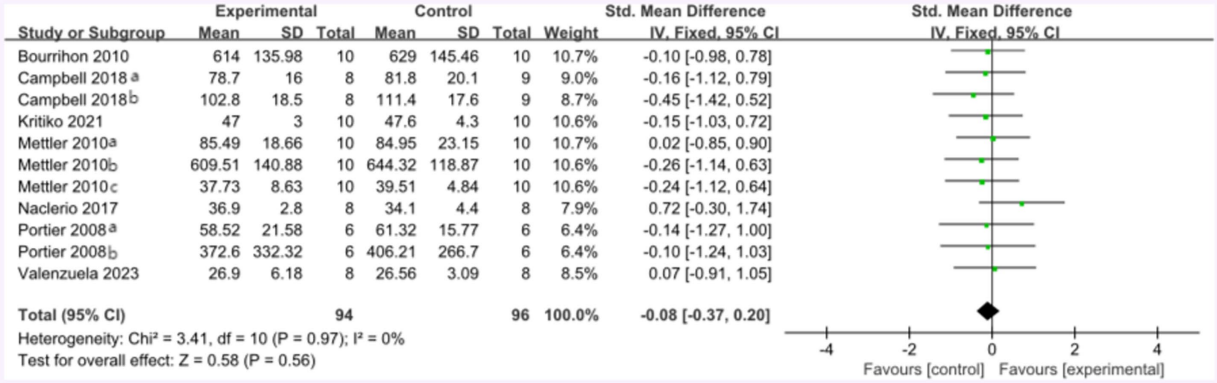

FIGURE 13
The forest plot of muscle strength after high protein ingestion (no protein group vs. high protein group).

TABLE 8 Subgroup analysis based on the types of specific athletic performance test and blood parameters (non-protein group vs. protein group, final value).

Subgroup name	Studies number	Participants number	SMD or MD (95% CI)	*p*	*Z*	I^2
Endurance performance						
Maximum speed	4	68	0.29 [−0.14, 0.72]	0.19	1.31	0%
Average speed	3	35	0.39 [0.05, 0.73]	0.02	2.24	0%
Wingate test (peak power)	3	50	0.65 [0.16, 1.15]	0.009	2.61	0%
Time to exhaustion (95% Vo_{2max})	2	32	−1.14 [−1.91, −0.37]	0.004	2.9	0%
Time to exhaustion ($Vo_{2max} \leq 90\%$)	3	29	1.03 [0.46, 1.60]	0.0004	3.53	52%
Vo_{2max}	6	116	0.13 [−0.25, 0.51]	0.5	0.68	0%
Cycling completed time	8	74	−0.11 [−0.45, 0.23]	0.53	0.62	0%
Cycling mean power	5	65	−0.30 [−0.7, 0.09]	0.13	2.16	0%
Muscle strength						
Maximum voluntary contraction (MVC)	3	42	−0.1 [−0.59, 0.39]	0.68	0.41	0%
Counter-movement jump (CMJ)	2	34	−0.14 [−0.66, 0.39]	0.61	0.51	0%
Jump height	4	66	−0.19 [−0.63, 0.25]	0.39	0.86	0%
1RM chest press	2	32	0.03 [−0.56, 0.62]	0.93	0.09	0%
1RM Squat	2	29	−0.07 [−0.68, 0.55]	0.83	0.21	0%
Physiological indices						
Blood glucose	14	153	0.11 [−0.14, 0.37]	0.39	0.86	35%
Muscle glycogen	2	16	0.74 [0.02, 1.47]	0.04	2.02	0%
Heart rate	9	89	−0.07 [−0.38, 0.23]	0.64	0.47	0%
Blood lactate	7	87	0.04 [−0.38, 0.30]	0.83	0.22	0%

TABLE 9 Subgroup analysis based on the energy matching or not between protein group and no protein group.

Subgroup name	Studies number	SMD (95% CI)	*p*	*Z*	I^2
Energy matching	20	0.11 (−0.03 to 0.26)	0.12	1.55	22%
Non-energy matching	6	0.07 (−0.17 to 0.31)	0.57	0.56	0%

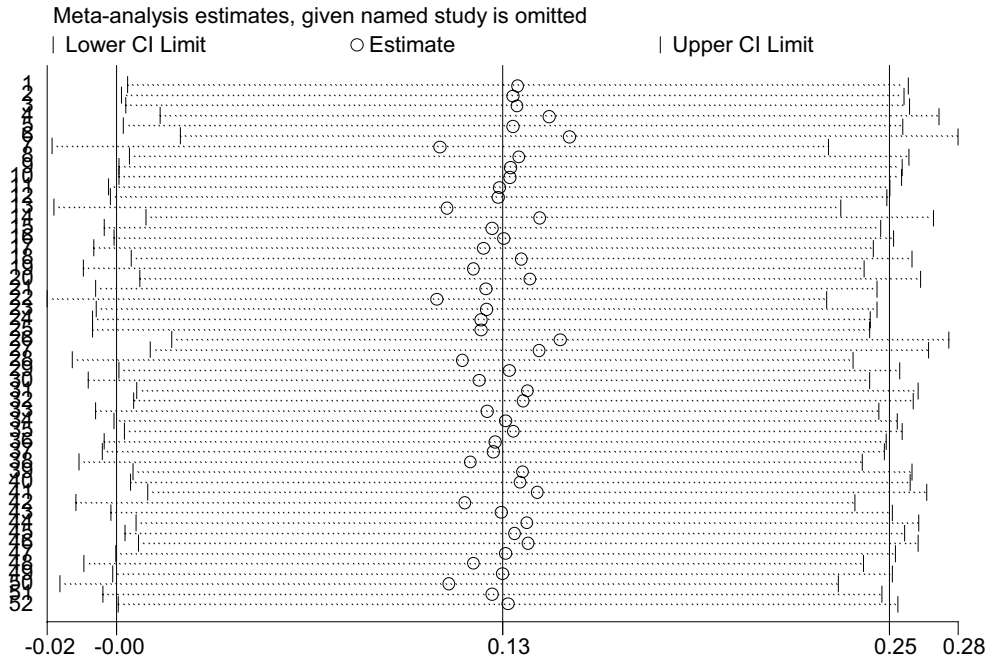

FIGURE 14
The sensitive analysis of athletic performance.

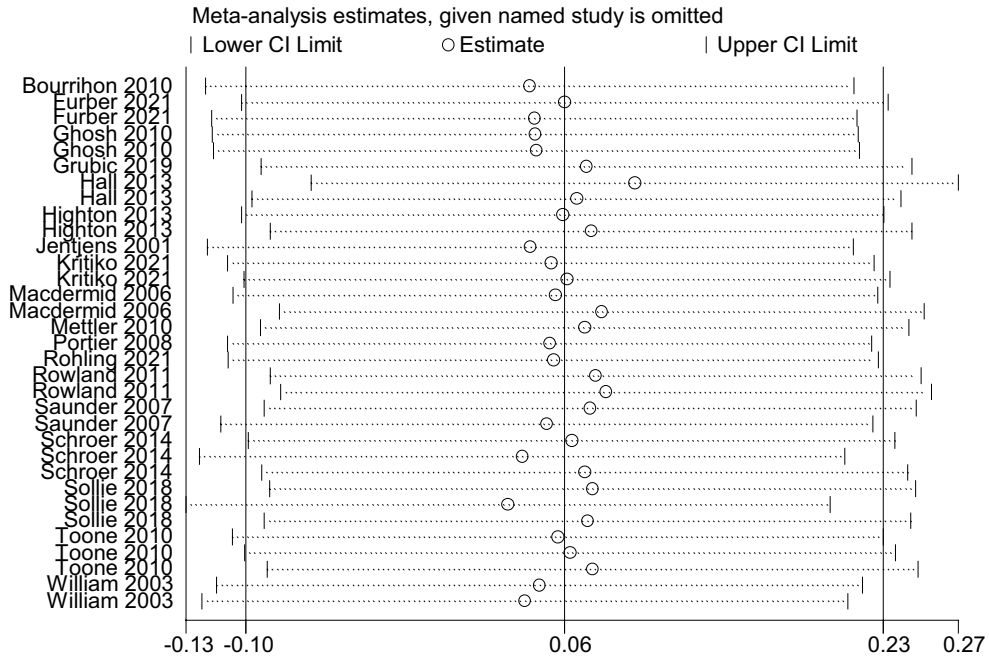

FIGURE 15
The sensitive analysis of physiological indices.

and hinder athletic performance, while a deficiency in muscle glycogen can have the same effect. Athletes' endurance performance has a strong correlation with their muscle glycogen stores. An increase in muscle glycogen concentration could indirectly improve endurance performance. Stearns et al. found solid evidence through the marathon match that protein ingestion could save muscle glycogen and blood glucose in the body (59). Manninen et al. concluded that whey protein hydrolysate appeared to enhance the effects of carbohydrate ingestion

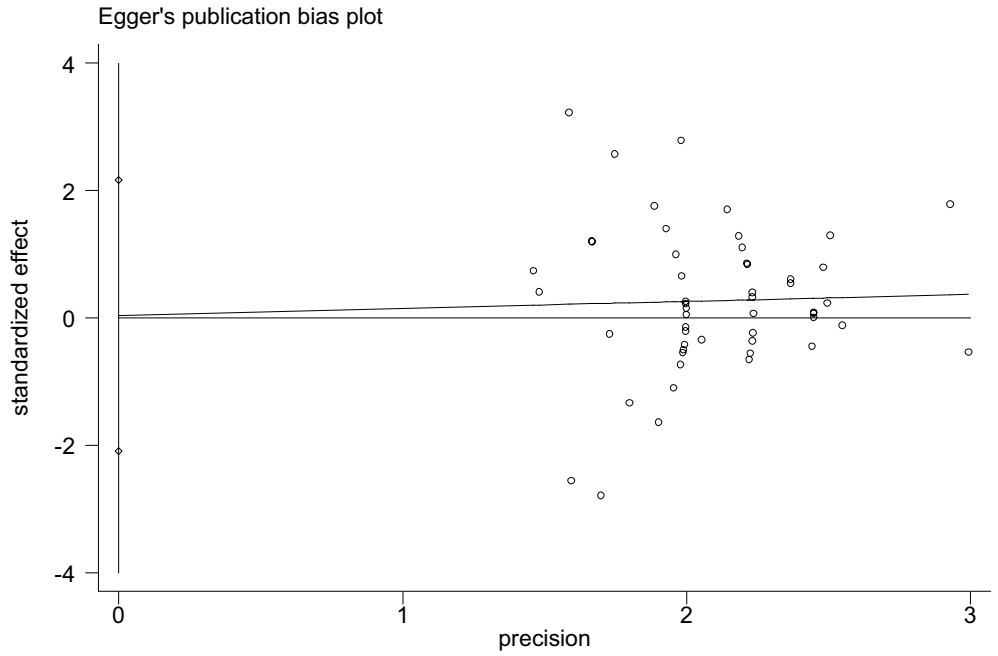

FIGURE 16
The funnel plot of studies in the athletic performance (Egger's test).

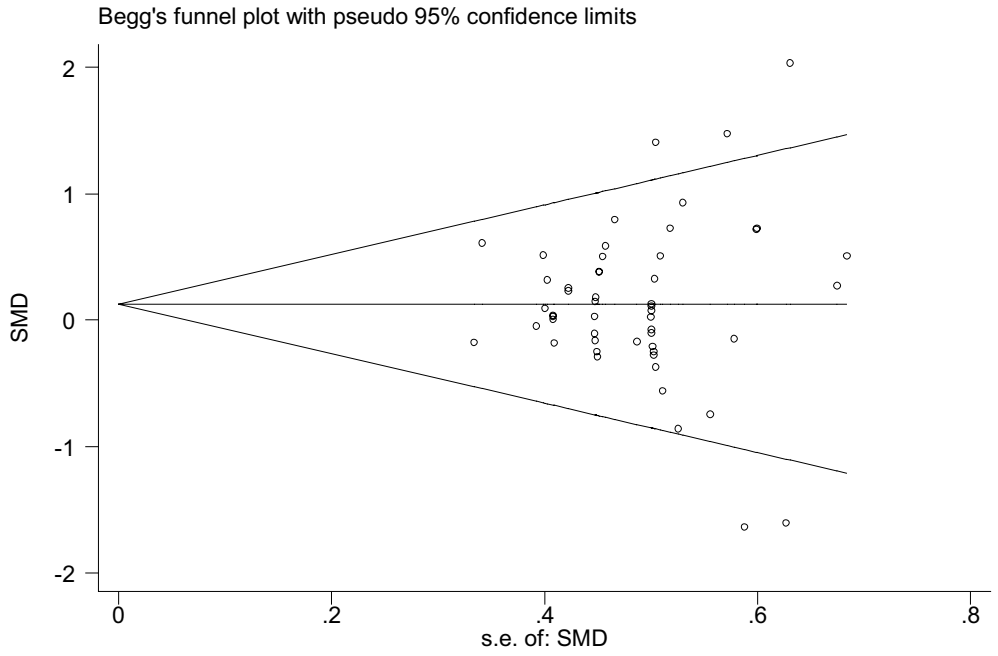

FIGURE 17
The funnel plot of studies in athletic performance (Begg's test).

on post-exercise muscle glycogen resynthesis (65). Thus, protein intake could accelerate the body's increase in protein oxidation, which in turn reduces the speed at which athletes' bodies consume sugar and preserves muscle glycogen and blood glucose. The subgroup analysis of muscle glycogen, blood lactate, blood glucose, and heart rate did not reveal a statistically significant difference between the protein and control groups. Furthermore, protein intake did not reduce blood lactate levels following exercise, implying that athletes' feelings of muscle soreness and fatigue might not alter with sufficient protein intake.

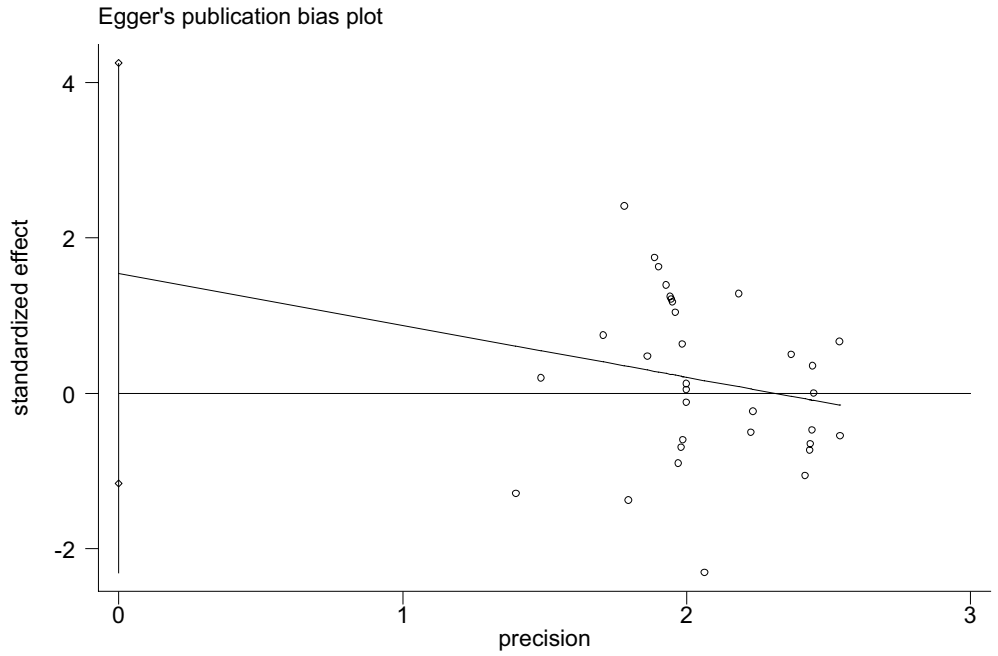

FIGURE 18
The funnel plot of studies in the physiological index (Egger's test).

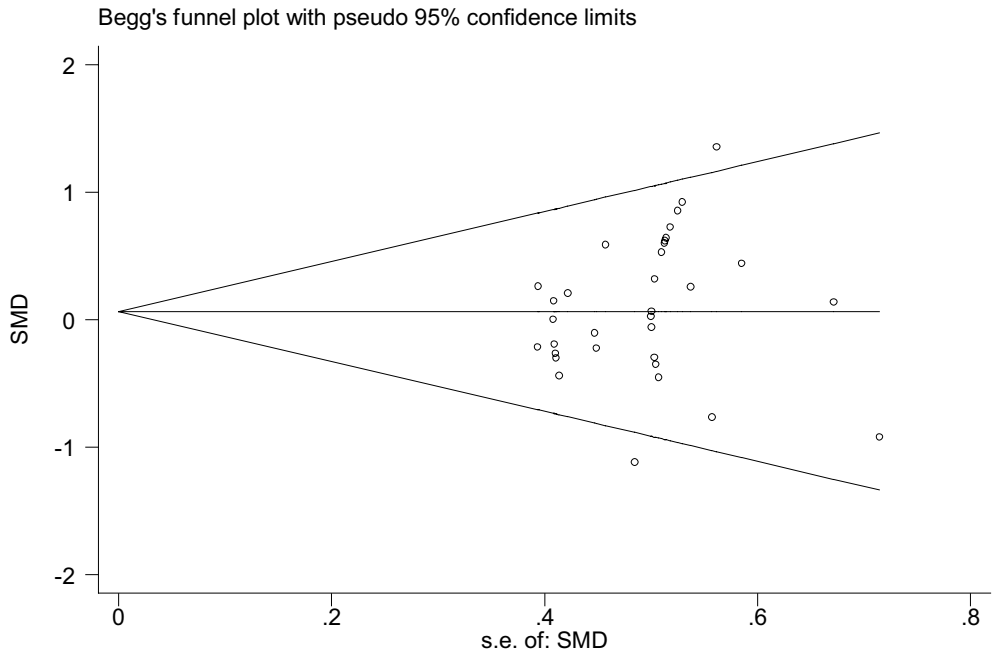

FIGURE 19
The funnel plot of studies in the physiological index (Begg's test).

4.4 High protein ingestion versus protein plus carbohydrate co-ingestion in athletes

The possibility that the carbohydrate-protein treatment enhances carbohydrate availability has been offered as a potential explanation for enhanced performance or endurance (30, 38, 39). Protein can provide athletes with additional energy, extending their time to exhaustion. The NSCA guide to sport and exercise nutrition mentions that CHOs with protein in a 4:1 ratio before or after prolonged exercise can facilitate greater performance (1). In the subgroup

analysis, protein plus carbohydrate co-ingestion found statistical significance in endurance performance, but the high protein ingestion group did not. The benefits of high protein intake were negligible for athletes. Many researchers mentioned the advantage of protein plus carbohydrate co-ingestion. Kloby et al. mentioned that compared with carbohydrate, carbohydrate plus protein co-ingestion appeared to enhance the time to exhaustion (TTE) performance and time trials (TT) performance, and the participants included in their study were healthy adults, both male and female (66). Furthermore, co-ingesting protein with CHOs outperformed carbohydrate ingestion alone, and the subgroup analysis of the Wingate test and TTE revealed that athletes' endurance performance improved after co-ingesting protein and CHOs compared to the carbohydrate group. Similarly, Stearns et al. concluded that co-ingestion of protein with CHOs during exercise had a benefit (9%) on performance compared to carbohydrate alone, and protein ingestion was not statistically significant in the time-trial studies. In contrast, the time-to-exhaustion studies revealed a significant improvement (59). It has provided robust evidence to support the benefits of protein-carbohydrate co-ingestion.

4.5 Limitation

Several limitations must be addressed. First, studies involve different types of proteins, such as soy protein, whey protein, and so on. Plant protein seems to have a different effect on athletic performance than other types of protein. However, due to a lack of studies, we are unable to conduct a meta-analysis based solely on plant protein. We only found two studies that compared the effects of plant protein and whey protein on athletic performance in athletes, and the lack of a comparison group prevents us from incorporating it into our research. It is a new and vague area that deserves more attention. Second, the meta-analysis's results, derived from several trials with a small sample size of approximately 10 participants in treatment arms, resulted in a low-quality outcome. Therefore, we need high-quality, large-scale studies to validate the current study's findings. Third, in the subgroup analysis, the samples and studies included in the forest plot were few, like the muscle glycogen. The forest plot of muscle glycogen only included two studies from 2001 and 2003, indicating that while the protein group showed statistical significance, it lacks credibility and requires further studies to bolster its findings in the future. Finally, it appears that when adequate amounts of CHOs are consumed, protein supplements do not further increase aerobic performance (42, 53), and it cannot be investigated through this meta-analysis.

5 Conclusion

This systematic review and meta-analysis included 28 RCT studies involving 373 athletes. While the overall meta-analysis of athletic performance did not find statistically significant effects, subgroup analysis showed that protein ingestion had a beneficial effect on endurance performance and muscle glycogen levels, suggesting that athletes may benefit more from co-ingesting protein with CHOs rather than relying solely on high protein intake.

Protein intake appears to support protein oxidation, which helps preserve muscle glycogen and indirectly enhances endurance performance. However, protein ingestion, whether alone or combined with CHOs, did not improve muscle strength in athletes. Additionally, different types of proteins may vary in their effectiveness on athletic performance. Future research should focus on the efficacy of plant protein on athletic performance.

Further studies should incorporate physical tests, particularly focused on measuring muscle glycogen levels. Additionally, future research should ensure the blinding of outcome assessment during the experiment to reduce potential bias. The small sample size of athletes in existing studies has contributed to low-quality outcomes, highlighting the need for larger, well-designed studies to provide more robust and convincing evidence to support these findings.

Data availability statement

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

Author contributions

SZ: Conceptualization, Data curation, Investigation, Resources, Software, Writing – original draft, Writing – review & editing. HZ: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. YX: Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. JL: Resources, Writing – review & editing. SD: Resources, Supervision, Validation, Writing – review & editing. ZN: Conceptualization, Data curation, Formal analysis, Investigation, Resources, 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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From semi-starvation to the stage: a case report on indicators of low energy availability in a drug-free bodybuilder during contest preparation and peak week

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Natural bodybuilding competitions involve periods of low energy availability (EA) combined with resistance training and high-protein diets to achieve extreme leanness. This study tracked a drug-free bodybuilder adopting evidence-based nutrition practices during 18 weeks of contest preparation. We measured endocrine function, resting energy expenditure, respiratory exchange ratio, body composition, resting heart rate, oral temperature, mood, and strength performance. Endocrine function was remeasured after 2 days of energy repletion. From baseline to week 18, free triiodothyronine (T3) and total testosterone (TT) fell into clinically low (2.7 pmol/L^{-1}) and sub-clinically low (9.1 nmol/L^{-1}) ranges. Resting energy expenditure decreased by −519 kcal (REE_{ratio} 0.78), and respiratory exchange ratio decreased from 0.95 to 0.85. Body mass reduced by −5.1 kg, with a sum of eight skinfold loss of −15.7 mm. Correlations were observed between body mass and decreases in oral temperature ($r = 0.674$, $p = 0.002$) and resting heart rate ($r = 0.560$, $p = 0.016$). Mood remained stable until the final 2 weeks and relative one-repetition maximum decreased in the squat (−5.4%), bench (−2.6%), and deadlift (−3.6%). Following 2 days of modest energy repletion, free T3 increased (18.5%), returning to sub-clinically low values (3.2 pmol/L^{-1}), whereas TT fell (−20.9%), reaching clinically low values (7.2 nmol/L^{-1}). These results offer insight into the dynamics of T3 and TT following a short-term period of modest energy repletion and further information on indicators of low EA during chronic energy restriction.

KEYWORDS

natural bodybuilding, relative energy deficiency in sport, endocrine function, IOC REDs CAT2, case report, total testosterone, free triiodothyronine

1 Introduction

Natural bodybuilding is a sport that blends physical skill with artistic presentation (1). Bodybuilding competitions are judged on an athlete's muscular size, symmetry, definition, and posing skills (2). To obtain the desired muscle definition, athletes undergo a period of calorie restriction and increased activity energy expenditure to lose fat mass, coupled with nutrition and resistance training practices to preserve skeletal muscle (3). After an extended period of energy restriction, athletes often increase their calorie, carbohydrate, and sodium intake

several days before competition to increase muscle glycogen stores and intracellular fluid to accentuate muscle volume, known as "peaking" (4). Collectively, these strategies encompass a bodybuilder's contest preparation ("contest prep").

Natural bodybuilding is unique in that athletes willingly subject themselves to semi-starvation to achieve their contest condition, cognizant of the disruptions to physiological and psychological function, which are largely "adaptable" states of low energy availability (EA; transient, reversible signs of body system suppression with minimal long-term adverse health effects) (5, 6), but can manifest into "problematic" low EA (signs of body system suppression with long-term adverse health effects) in some bodybuilders (7) and other physique competitors (8).

Since the first prospective studies associated low EA with reduced triiodothyronine (T3) and luteinizing hormone (LH) dysregulation in healthy, sedentary women three decades ago (9, 10), mirroring observations seen in amenorrhoeic female athletes (11, 12), a growing body of research has surfaced on indicators of low EA in athletes. Consolidating low EA research with athlete testing, validation, and usability, the IOC REDs Clinical Assessment Tool Version 2 (IOC REDs CAT2) was developed to detect signs of problematic low EA in athletes (13).

Two primary indicators of low EA in Step 2 of the IOC REDs CAT2 for males are clinically low and sub-clinically low (within the lowest 25% quartile of the reference range) total or free T3 and testosterone (14). During chronic states of energy restriction in natural bodybuilders, T3 and testosterone levels reach clinically low values (5, 6); however, both can recover to within normal physiological ranges and baseline values in 1–3 months following an increase in energy intake and body mass (BM) post contest prep (5, 6). Experimental research in a male combat athlete and soldiers following a chronic state of energy restriction has shown that energy repletion that far exceeds total daily energy expenditure rates restores T3 and testosterone levels to within normal physiological ranges and baseline values within several days to a week, albeit with pronounced increases in BM (15, 16).

Although the diagnostic measures of T3 and testosterone serve as primary indicators of problematic low EA in male athletes, the measurements themselves adapt quickly to changes in energy intake and EA. While substantial energy repletion intakes and rapid increases in BM are practiced by combat and physique athletes following the termination of a phase of energy restriction (16–18) they do not reflect the acute energy repletion ("refeed") practices of physique athletes during continued energy restriction (19, 20) nor the modest fluctuations in energy balance and EA of other athletes between training (and matches) and rest days (21–24).

A lack of research exists on the transient nature of T3 and testosterone levels after short-term, modest energy repletion following a period of energy restriction. Understanding how T3 and testosterone levels respond to acute changes in energy intake following a phase of energy restriction and low EA would clarify their responsiveness and assist physicians in accurately timing and interpreting these diagnostic measurements of problematic low EA in certain athletes. In addition, such insights would help discern whether the dietary practice of refeeding (2 days) in bodybuilding is efficacious for attenuating endocrine adaptations during ongoing energy restriction (25).

Reductions in T3 have been shown to coincide with stepwise suppressions in REE and menstrual disturbance (26), the latter being a primary marker of low EA in women (27). The assessment of low EA using REE is commonly depicted as a REE_{ratio} of measured REE (mREE) via indirect calorimetry to a predictive estimate of REE (pREE) with a cut-off ratio of <0.90 (mREE/pREE) (27). However, REE remains a "potential" marker in the IOC REDs CAT2 assessment model due to a lack of consistency in the literature (13). This discrepancy may be partly due to predictive equations for determining the REE_{ratio} being non-specific to the population studied, exercise being performed close to measurement, and the lack of weight stabilisation before an intervention (27).

Therefore, this case study serves as a lens to assess the effects of two primary indicators of low EA in a male bodybuilder during chronic energy restriction and 2 days of modest energy repletion in the athlete's peaking phase. It also provides further information on REE and REE_{ratio} during chronic energy restriction using an athlete-specific predictive equation and adopting recommended pre-assessment procedures. Lastly, we offer insight into field-based measurements accessible to the research-active practitioner on aspects of physiology, psychology, and strength performance during a chronic phase of energy restriction. Collectively, our findings offer further empirical evidence to contribute to the shortage of research on low EA in male athletes and assist in contextualizing low EA markers using tools such as the IOC REDs CAT2.

2 Case description and assessment methods

2.1 Case presentation

The athlete (25 years, BMI = 26.2 $kg.m^2$) was a drug-free Caucasian male amateur bodybuilder competing in his fourth natural bodybuilding competition in the Natural Physique Association. The athlete adhered to a varied, flexible diet with no food restrictions. As a qualified Personal Trainer and competitive powerlifter, the athlete was aware of the biases, typical errors and standardization requirements with tracking their dietary intake and BM and had ample experience after maintaining a detailed dietary/BM record during previous contest preps and powerlifting competitions. The athlete tracked their dietary intake using MyFitnessPal (MyFitnessPal Inc. CA, USA) and BM daily throughout the 18-week intervention. The athlete was experienced with testing their one-repetition maximum (1RM) on the three multi-joint exercises: back squat, bench press, and deadlift. The athlete was not taking any prescribed medication. He was a non-smoker and supplemented with creatine monohydrate, omega 3 (fish oil), vitamin D3, whey protein, casein, citrulline malate, and beta-alanine.

2.2 Metabolic assessment

An overview of assessments is shown in the intervention timeline in Figure 1a. Resting energy expenditure (REE) and respiratory exchange ratio (RER) were determined using an online gas analysis system (MetaLyzer 3B, Cortex) at baseline and beginning of week 18 (Table 1). The gas analyzer was calibrated before testing, and environmental conditions during testing were 23.8 ± 1.3 degrees Celsius. For the REE and RER assessments, the athlete was required to lie still on a bed in a supine position for 30 min before the

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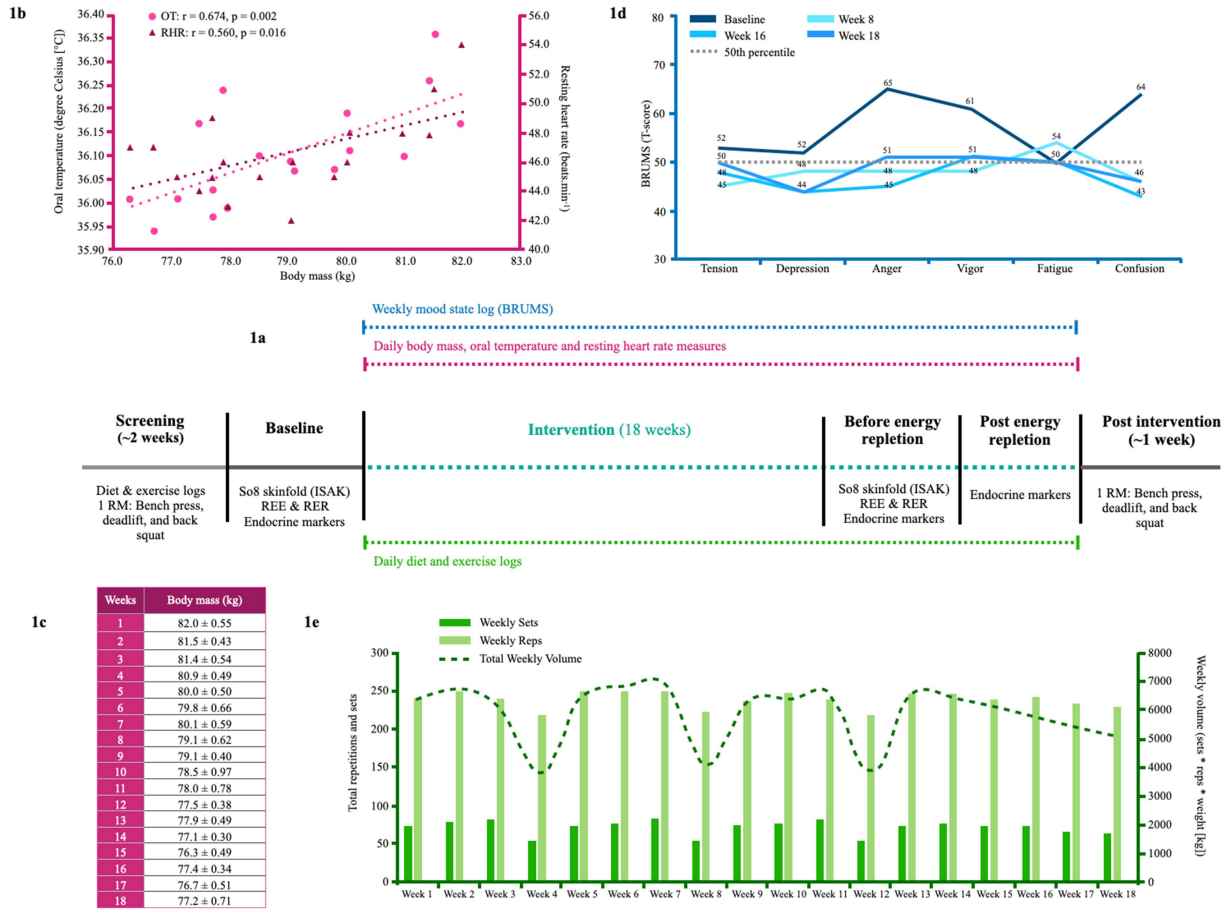

Weeks	Body mass (kg)
1	82.0 ± 0.55
2	81.5 ± 0.43
3	81.4 ± 0.54
4	80.9 ± 0.49
5	80.0 ± 0.50
6	79.8 ± 0.66
7	80.1 ± 0.59
8	79.1 ± 0.62
9	79.1 ± 0.40
10	78.5 ± 0.97
11	78.0 ± 0.78
12	77.5 ± 0.38
13	77.9 ± 0.49
14	77.1 ± 0.30
15	76.3 ± 0.49
16	77.4 ± 0.34
17	76.7 ± 0.51
18	77.2 ± 0.71

FIGURE 1
(a) The intervention timeline. **(b)** The relationship between oral temperature, RHR and body mass from week 1 to week 18. **(c)** Weekly body mass averages in kilograms taken at the athlete's home. **(d)** BRUMS scale at baseline, week 8, week 16, and week 18. The horizontal dashed line represents the normative mean. **(e)** Overview of weekly resistance volume and weekly sets and reps completed from week 1 to week 18. 1RM, one repetition maximum; BRUMS, Brunel mood scale; OT, oral temperature; REE, resting energy expenditure; RER, respiratory exchange rate; RHR; resting heart rate; So8, sum of eight.

assessment. There was no visual or auditory stimulation throughout the measurement period. The athlete was requested to abstain from strenuous physical activity the day before or the morning of the test. For the initial assessment, the athlete maintained a stable BM (≤1% deviation) for 2 weeks before the assessment. The athlete's REE_{ratio} was calculated as the division of measured REE (mREE) and predictive REE (pREE). We chose a validated prediction equation that best aligned with the physical characteristics and sport of the athlete, specifically, the bodyweight-based predictive equation derived from physique athletes (28). Although the equation was determined in a mixed cohort of users and non-users of anabolic-androgenic steroids, the sub-analysis of self-reported non-users ($n = 20$) showed a strong positive agreement (r = 0.93; 29 kcal.d) with the equation.

2.3 Anthropometric assessment

The athlete underwent two body composition assessments at baseline and at the beginning of week 18. Height (Stadiometer, Seca, UK), BM (Seca, UK), skinfolds (Harpenden® calipers) and girth measurements (Lufkin® steel tape) were performed by two certified anthropometrist's (Level 1) by the International Society for the Advancement of Kinanthropometry (ISAK) using the same calibrated equipment. Level 1 ISAK anthropometrists' must not exceed a technical error of measurement (TEM) of 7.5% for any skinfold nor 1.5% for any other measure to obtain their certification. Skinfold and girth measurements were performed in duplicate or triplicate, depending on whether the difference of the first and second measurements exceeded 5% (skinfold) and 1% (all other measurements), following which a mean value was obtained (results shown in Table 1). Due to large discrepancies in body fat predictive equations when using skinfold thickness measurements (29), the athlete's values were presented as a sum of eight sites; this also prevented the study from directly quantifying the athlete's EA. Throughout the intervention, the athlete tracked his weight daily in minimal clothing to the nearest tenth of a pound (converted to kilograms in this manuscript) immediately upon waking using a standard calibrated electrical scale (Tanita HD 386 Super Compact Digital Scale) (Figure 1c).

2.4 Blood parameters

Endocrine markers (Table 2) were obtained at baseline, beginning of week 18 and 2 days following energy repletion.

TABLE 1 Anthropometric and metabolic measurements at baseline and week 18.

Athlete characteristics	Baseline	Week 18	Absolute change (%)
Body mass (kg)	81.8	76.7	−5.1 (−6.2%)
BMI (kg.m^2)	26.2	24.6	−1.6 (−6.1%)
Waist/hip ratio	0.84	0.79	−0.05 (−6.0%)
ISAK sum of 8 skinfolds (mm)	50.8 (z-score: −2.3)	35.1 (z-score: −2.6)	−15.7 (−30.9%)
REE (measured; kcal.d^{-1})	2015	1,496	−519 (−25.8%)
REE (predicted; kcal.d^{-1})	2039	1912	−127 (−6.2%)
mREE/pREE ratio	0.99	0.78	−0.21 (−21.2%)
RER	0.95	0.85	−0.10 (−10.5%)

All data presented are measurements taken at baseline (before the intervention) and at the beginning of week 18 (before the peaking phase). The sum of skinfolds is the total of eight skinfold sites (tricep, subscapular, bicep, iliac crest, supraspinale, abdominal, front thigh [straight leg], and medial calf) taken in accordance with the standards of the International Society for the Advancement of Kinanthropometry (ISAK). All skinfold measurements were performed directly after the athlete's REE and RER measurements. REE, resting energy expenditure; RER, respiratory exchange ratio; m, measured; p, predicted.

Measurements were taken by venipuncture of the antecubital vein of the left arm at 0900-0920 following a 12-h overnight fast at each time point. Endocrine markers were conducted using the electrochemiluminescence immunoassay (ECLIA) method on the cobas e 602 analyzer (Roche Diagnostics). Measurements were performed by The Doctors Laboratory (TDL, London, UK). The inter assay coefficient of variation (CoV) and sensitivity (lowest detection limit) for all endocrine markers were sourced from Roche Diagnostics validation reports, as replicate measurements were not performed internally; thus, precision was determined based on the manufacturer's reported CoV% for similar mean concentrations (see Supplementary Table 1).

2.5 Strength assessment

Strength was assessed using absolute and relative one repetition maximum (1RM) determination for three multi-joint exercises: back squat, bench press, and deadlift. 1 RM testing was performed at baseline and post-competition at the same time of day and followed a progressive loading format, with an inverse relationship between repetitions and load until the athlete reached failure on their maximal load for one repetition. Considering the athlete's competitive powerlifting experience, the athlete did not undergo familiarisation with the 1RM test before the intervention. Table 3 shows the baseline and post-competition results (week 19).

2.6 Psychological assessment

As per a previous case study on a physique athlete (30), the Brunel Mood Scale (BRUMS) was used to assess mood state and completed at baseline and every Saturday morning by the athlete until the competition date (31). Baseline, week 8, week 16, and week 18 results are shown in Figure 1d.

TABLE 2 Endocrine markers at baseline, week 18 and 2 days of energy repletion (peaking).

Endocrine markers	Baseline	Beginning of week 18	Post 2days of energy repletion
Follicular stimulating hormone (IU/L^{-1}) Ref values: 1.5–12.4	6.7	6.0	5.7
Luteinizing hormone (IU/L^{-1}) Ref values: 1.7–8.6	3.8	3.6	2.9
DHEA sulphate (µmol/L^{-1}) Ref values: 4.34–12.2	10.3	9.3	9.7
Total testosterone (nmol/L^{-1}) Ref values: 8.6–29.0 Lowest 25% quartile of the ref values: 8.6–13.7 nmol/L	14.4	9.1*	7.2**
SHBG (nmol/L^{-1}) Ref values: 18.3–54.1	27	28	26
Testosterone/SHBG ratio Ref values: 24–104	53.3	32.5	27.7
Cortisol (nmol/L^{-1}) Ref values: 166–507 Morning 6–10 am	482	449	454
Free triiodothyronine (pmol/L^{-1}) Ref values: 3.1–6.8 Lowest 25% quartile of the ref values: 3.1–4.0 pmol/L	4.3	2.7**	3.2*
Free thyroxine (pmol/L^{-1}) Ref values: 12.0–22.0	18.4	16.6	16.6

All data presented are values retrieved at baseline (before the intervention) at the beginning of week 18 and following 2 days of energy repletion. Each endocrine marker's reference values were derived from the assay manufacturer (Roche Diagnostics) validation reports. SHBG, sex hormone-binding globulin; Ref, reference.
*Within the lowest 25% quartile of the reference value.
**Below the reference value.

2.7 Oral temperature

The athlete was provided with two oral temperature thermometers (Digital Thermometer iProven) and advised to measure his temperature in triplicate immediately upon waking every morning before consuming fluids. Weekly averages are shown in Figure 1b.

2.8 Resting heart rate

The athlete tracked his resting heart rate (RHR) using a Samsung Gear 2 Smart Watch immediately upon waking every morning for 18 weeks. Weekly averages are shown in Figure 1b.

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TABLE 3 One repetition maximum determination for three multi-joint exercises at baseline and week 19.

Exercises	Baseline (weight 81.8 kg) Absolute 1 RM (1RM relative to BM)	Week 19 (weight: 77.8 kg) Absolute 1RM (1RM relative to BM)	Absolute change (%)	Relative change (%)
Back squat (kg)	200.0 (2.44)	180.0 (2.31)	−20.0 (−10%)	−0.13 (−5.4%)
Bench press (kg)	135.0 (1.65)	125.0 (1.61)	−10.0 (−7.4%)	−0.04 (−2.6%)
Deadlift (kg)	240.0 (2.93)	220.0 (2.83)	−20.0 (−8.3%)	−0.11 (−3.6%)

2.9 Dietary intake

The athlete tracked every item of food and drink consumed using a digital scale and added their data to the app MyFitnessPal. During the first week of the intervention, the accuracy of their dietary report was verified by manually replicating the athlete's dietary intake into Nutritics (Nutritics Ltd., Dublin, Ireland). The lead author assessed the data every Wednesday and Saturday throughout the intervention period to ensure compliance. Furthermore, the lead author retrospectively cross-referenced the athlete's dietary intake with more than 50 video blogs recorded by the athlete during contest prep to ensure accuracy.

2.10 Intervention

The athlete's REE (REE_{ratio} 0.99) and endocrine markers (all within physiological reference ranges) at baseline indicated sufficient EA (see Tables 1, 2). In addition, the athlete's self-reported BM oscillated ≤1% 2 weeks before the start of the intervention; thus, the athlete's self-reported off-season calorie intake was used as the foundation to program their contest prep diet. At baseline, the athlete was assigned an undulated intake of 2,100 kcal.d^{-1} for five consecutive days and 2,600 kcal.d^{-1} for 2 days (arithmetic mean reported intake 2,276 ± 246 kcal [week 2]). We aimed to sustain a rate of BM loss of ~0.5% per week to maximise muscle retention in accordance with evidence-based recommendations (3). By week nine, the athlete's dietary intake was adjusted to 2,100 kcal.d^{-1} for six consecutive days, with one higher intake of 2,600 kcal.d^{-1} (arithmetic mean reported intake 2,173 ± 196 kcal [week 9]). By the final month, the athlete's daily energy intake was reduced by a further 100 kcal (arithmetic mean reported intake 2066 ± 193 kcal [week 17]). During the athlete's three-day energy repletion period before competition ("peaking"), the athlete was assigned a calorie intake of 2,450 kcal (arithmetic mean reported intake 2,458 ± 4 kcal; see Supplementary Table 2).

The athlete's protein intake was 2.4 g.kg^{-1}.d^{-1}, which remained until week 17, when it was reduced to 2.1 g.kg^{-1}.d^{-1} to accommodate an increase in carbohydrates during energy repletion ("peaking"). The athlete's carbohydrate intake was 2.9 g.kg^{-1}.d^{-1} until week 18, when it increased to 4.8 g.kg^{-1}.d^{-1} for three consecutive days during energy repletion, with an increase in sodium and water on the day of competition to accentuate vascularity and muscle volume (32). The athlete consumed five protein-rich meals (0.25–0.5 g.kg^{-1} per meal) spaced evenly throughout the day to optimize muscle anabolism and protein balance (33). Before training, the athlete consumed a carbohydrate and protein-rich meal (~2 h before) to increase carbohydrate availability and a further 0.5 g.kg^{-1} of protein within 90 min of training finishing to potentiate the post-exercise muscle protein synthetic response (34). Furthermore, the athlete consumed a protein snack of a non-whey source ~2 h before bedtime to increase overnight protein balance (35).

Figure 1e presents the athlete's weekly training volume. The athlete completed four resistance training sessions weekly, focusing on each muscle group 2–4 times per week, with an average weekly set volume of 71 ± 9 sets and a rep volume of 238 ± 10 reps per week. The athlete incorporated blood flow restriction training at the end of workouts as an alternative training stimulus for the final 4 weeks before the competition. The athlete performed two "350 kcal target" long-intensity steady-state (LISS) cardiovascular sessions per week at baseline. By week 6, the athlete increased their LISS training three times per week; by week 13, this increased to four times per week.

3 Statistics

Data are presented as arithmetic means and standard deviations (±). Data was analyzed using Microsoft Excel (version 2021, Microsoft Corporation, WA, USA). Normality was determined using the Shapiro–Wilk test. Pearson's correlation (normal distribution) and Spearman's rank correlation (non-normal distribution) were used to test associations for oral temperature, resting heart rate and BM. Statistical significance was set at an alpha of $p < 0.05$. As internal replicate measurements of all endocrine markers were not performed, the inter-assay CoV% provided by Roche Diagnostics for the cobas e 602 analyzer was used as a proxy measure. The typical error (TE) was estimated to determine the variability of each endocrine measure and calculated as the inter-assay CoV% multiplied by the mean measurements by the assay manufacturer divided by 100. The TE multiplied by 2 was used to discern a value indicative of a meaningful change.

4 Results

4.1 Endocrine, metabolic, psychological, and performance parameters

Between weeks 1–18, the athlete's free T3 and TT levels fell by −37.2% and −36.8%, falling into clinically low and sub-clinically low ranges, respectively. Follicular stimulating hormone (FSH), LH, and free thyroxine (T4) trended downwards but remained within their reference ranges, whereas sex hormone binding globulin (SHBG) remained largely unchanged. After 2 days of modest energy repletion (2,458 ± 4 kcal.d^{-1}, 4.8 g.kg^{-1} BM.d^{-1} of CHO), free T3 increased by 18.5%, returning to within reference range, albeit within the lowest 25% quartile. TT levels decreased by a further −20.9%, reaching clinically low levels in accordance with a − 19.4% decrease in LH (see Table 2 for measured values). The athlete's REE decreased from baseline by −519 kcal (REE_{ratio} 0.99 to 0.78), and RER decreased from

0.95 to 0.85 by week 18. From week 1 to 17, the athlete averaged a weekly BM loss of −0.30 kg (−0.38% of BM loss per week). The athlete's sum of eight skinfolds was reduced by −15.7 mm (50.8 mm to 35.1 mm). The athlete's BRUMs assessment for confusion, depression, and tension remained below average throughout the intervention. Fatigue increased above average at week 8, and vigor and anger were slightly above average during weeks 16–18. The athlete demonstrated decreases in absolute 1 RM in the squat, bench, and deadlift from baseline by −10.0%, −7.4%, and −8.3%, respectively, while their relative 1 RM decreased by −5.4%, −2.6%, and −3.6%.

4.2 Physiological associations with reductions in body mass

Considering previous associations between metabolic rate, oral temperature, and energy restriction (36) and reports of a reduction in resting heart rate during contest prep in natural bodybuilders (6, 30), we tested for associations between oral temperature, resting heart rate and BM during the 18-week intervention. As shown in Figure 1b, the athlete's oral temperature ($r = 0.674$, $p = 0.002$) and RHR ($r = 0.560$, $p = 0.016$) tended to decrease with BM throughout the intervention period.

5 Discussion

This case study assessed the nature of two primary markers of low EA (free T3 and TT) following 2 days of modest energy repletion. Moreover, we provide further insight into endocrine, physiological, psychological, and strength performance outcomes following a prolonged period of BM loss. The athlete's free T3 and TT fell into clinically low (2.7 pmol/L^{-1}) and sub-clinically low (9.1 nmol/L^{-1}) ranges by week 18, respectively. Previous research on male bodybuilders, a combat athlete and military personnel during chronic phases of energy restriction have demonstrated similar reductions (5, 6, 15, 16). Considering the secretion of LH regulates the production of testosterone in males via the anterior pituitary gland secondary to the pulsatile release of gonadotropin-releasing hormone (GnRH) within the hypothalamus, it was surprising that LH, albeit trending downwards, remained largely unchanged between baseline and week 18 compared to TT. Similarly, we anticipated free T4 production to decrease per free T3 and REE and SHBG production to increase per a reduction in TT; however, neither demonstrated meaningful changes. Discrepancies between LH, SHBG and testosterone have been previously documented in male athletes (37, 38) and could have been due to the small number of measurements taken. Moreover, discrepancies in T4 with decreases in T3 have also been reported in the literature (9, 39). However, LH levels from baseline to the athlete's third measurement (2 days of energy repletion) fell by −23.7%, in conjunction with a −50.0% reduction in TT, suggesting that a reduction in GnRH mediated the decrease in TT.

After 2 days of modest energy repletion, free T3 increased by 18.5%, whereas TT levels continued to decrease by −20.9%. In a recent study of a male combat athlete, a two-day *ad-libitum* increase in calories (64–89 kcal.kg FFM^{-1} $BM.d^{-1}$) led to clinically low testosterone levels increasing more than two-fold, returning close to baseline values in a week (16). Similar increases in free testosterone have been demonstrated in explorers 3 days after completing an 850-km cross-country skiing expedition to the North Pole. While the explorer's energy repletion intake was not measured in the 3 days following the expedition, a 5 kg weight regain at 5 days indicates a pronounced calorie increase above energy balance (40). The discrepancy between our results and those mentioned is likely due to both studies' energy repletion intake exceeding total daily energy expenditure requirements and thus reaching optimal EA values to support the restoration of endocrine function. In contrast, if we consider the athlete's pronounced reduction in REE, our intake likely achieved close to energy balance but below adequate EA to support the restoration of the hypothalamic–pituitary-gonadal axis. Thus, we can speculate that the continued reduction in TT following 2 days of modest energy repletion could suggest that the hypothalamic–pituitary-gonadal axis requires a greater energy and/or carbohydrate intake to respond during periods of short-term energy repletion following energy restriction compared to the hypothalamic–pituitary-thyroid axis; however, due to the study design and limitations described below, this warrants further study.

A reduction in REE appears to be a consistent finding in case studies during contest prep, with reductions in REE ranging from −179 kcal to roughly ~1,000 kcal (5, 6, 20, 30) and a decrease in RER (30). While the use of a predictive equation specific to physique athletes provided a close REE_{ratio} at baseline (0.99) and detected energy deficiency at week 18 (0.78) with the cut-off ratio of <0.90 (mREE/pREE) (27), due to the substantial decrease in the athlete's REE, common predictive equations used in athletes such as the Harris-Benedict (41) (0.81) and Ten-Haaf (42) (0.75) equations also produced an mREE/pREE ratio of <0.90. From week 1–17, the athlete averaged a weekly BM loss of −0.30 kg, which is lower than the average (−0.42 kg) of other male physique athletes during contest prep (43) but aligns with current evidence-based recommendations (32). Notably, the athlete's lowest weight was recorded at week 15 (76.3 kg); however, during weeks 16–18, the athlete complained of gastrointestinal (GI) symptoms (bloating and constipation), which likely contributed to the athlete's lack of BM loss in the final 2 weeks. Upon highlighting these GI complaints, a review of the athlete's dietary intake was performed to see if any potential dietary culprits of GI distress could be identified (e.g., uncharacteristically high amounts of FODMAP-rich foods) (44), but no abnormal changes from the athlete's previous 15 weeks of dietary intake had occurred. GI disturbance has been implicated in the REDs physiological model and reported in male athletes with secondary exercise dependence and disordered eating (14, 45); however, further studies are warranted in male athletes. In the final weeks leading up to the competition, the athlete moved house while maintaining his case study, video blogging, and work commitments. In the athlete's video blog during this time, he mentions stress may have been the cause of his GI disturbance, which is empirically supported (46).

The athlete's oral temperature decreased throughout the intervention period in accordance with BM losses ($r = 0.674$, $p = 0.002$; Figure 1b), as per previous research during a period of energy restriction (36). A similar relationship was demonstrated with the athlete's RHR ($r = 0.560$, $p = 0.016$), which was reduced by 9 beats. min^{-1} by week 16, akin to previous case reports during contest prep (6, 30). As shown in Figure 1d, the athlete's mood state remained largely undisturbed during contest prep. However, vigor and anger increased slightly above average in the final 2 weeks. Mood disturbances have been previously reported during contest prep (7), with pronounced increases reported in one physique athlete in the

final stage before competition (6). While a heightened preoccupation with food could have been partly causal of the athlete's mood disturbance, per previous research (7), it may have also been a result of changes in his personal life noted earlier, and the emotional toll on his relationships—both of which were mentioned in the athlete's video blogs. The athlete demonstrated decreases in absolute 1 RM in the squat, bench, and deadlift from baseline by −10.0%, −7.4%, and −8.3%, while their relative 1 RM decreased by −5.4%, −2.6%, and −3.6%, respectively. Notably, these changes are within the CoV (0.5–12.1%) of a recent systematic review on the retest reliability of the 1RM test (47); thus, some of the reduction in strength could simply be a product of day-to-day variability.

While our study measured indicators of EA, we did not directly measure EA due to discrepancies in skinfold body fat prediction equations (29) and dietary energy intake and exercise energy expenditure both being highly susceptible to error (48, 49). Moreover, it is likely that these measurements alone are not a true reflection of an athlete's EA, considering non-exercise activity thermogenesis is not factored into the equation and can surpass exercise activity expenditure values in certain athletes (50). Several limitations are worthy of note with this research, which the reader must consider when evaluating the finding's validity and translatability to practice. The athlete was recruited for the intervention just 2 weeks before its commencement, which prevented test re-test reliability measurements for oral temperature, morning RHR, BRUMS, and 1RM testing. Furthermore, the athlete's baseline BRUMS assessment was performed on just 1 day, which could have been influenced by transient external factors and the reason for the discrepancy between scores at baseline and during the intervention. Internal quality control metrics of the endocrine measurements were not performed; thus, the inter-assay CoV was derived from the assay manufacturer as a proxy measure. The Metallyzer 3B (Cortex) system used to assess RER at rest in our study has been shown to overestimate carbohydrate oxidation by 53% and underestimate the energy derived from fat by 25%, albeit in an exercise context (51). While our results indicate a shift towards greater fat oxidation similar to previous research on a physique athlete during contest prep (30), caution is advised when interpreting the RER values due to the potential for measurement inaccuracies. Furthermore, the athlete's free T3 levels were assessed using the immunoassay method, which, compared to the gold-standard method of liquid chromatography–tandem mass spectrometry, frequently overestimates free T3 levels at the low reference interval (52). Lastly, the athlete's TT levels were measured at three *single* time points, when it is recommended to take two consecutive measures at each time point due to day-to-day variation (53).

6 Practical perspective

Our case study offers insight into the nature of two primary indicators of low EA in males (free T3 and TT) as per the IOC REDs CAT2 during chronic energy restriction and a short-term period of modest energy repletion. Moreover, this study offers further insight into the effects of "potential indicators" of low EA (e.g., REE) during 18 weeks of contest prep (13). The athlete adopted evidence-based weight-loss recommendations for athletes yet incurred declines in metabolic and reproductive hormones, resulting in a risk status of "Moderate to High" (Orange) according to the IOC REDs CAT2 Severity/Risk Assessment Tool (13, 14). The extent to which these alterations resulted from the athlete's low EA load or degree of leanness was not determined and requires further insight; however, the results are pertinent to practitioners working with athletes adopting similar weight-loss approaches. The implications of the transient nature of free T3 and the unresponsiveness of TT following a 2 days modest energy repletion warrant further study and could have a meaningful impact on assessing low EA in males. We successfully documented endocrine, physiological, psychological, and performance indicators while the athlete adopted evidence-based guidelines for contest prep. Importantly, our case study was undertaken using assessment methods accessible to many research-active practitioners, which we hope will allow more practitioners to report their inductions from practice to inform future research, bridging the gap between science and practice (54).

7 Case perspective

The athlete felt he achieved his best contest prep condition and won his physique category. Reflecting on the intervention the day before the competition, the athlete questioned whether 18 weeks of energy restriction, necessitating constraints on social events, travel, and causing a strain on his close relationships, coupled with an ongoing heightened fixation with food, was worth the reported changes in body composition. Several days after the show, the athlete mentioned that his motivation, as a result of no longer working towards the competition, had noticeably reduced. A week post-contest prep, the athlete increased his weight by ~1.5 kg but was not preoccupied with the rise in BM, unlike previous post-contest prep experiences. He credited this change in perspective to viewing his increased BM as an indicator of returning to a condition commensurate with achieving his best lifting performances.

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 case report was approved by the Institute of Performance Nutrition. The study was conducted in accordance with the local legislation and institutional requirements. The participant provided their written informed consent to participate in this study. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

AR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. LM: Formal analysis, Writing – review & editing. JA: Formal analysis, Writing – review & editing. LB: Formal analysis, Investigation, Supervision, Writing – review & editing.

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Conflict of interest

LM was employed by Bodyrecomposition Ltd.

The remaining 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.2024.1465001/full#supplementary-material

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Are very high rates of exogenous carbohydrate ingestion (>90 g/hr) sufficient or indeed necessary to run a sub-2hr marathon? An analysis of the model predictions of Lukasiewicz and colleagues

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KEYWORDS

carbohydrate oxidation, fat oxidation, muscle glycogen, carbohydrate ingestion, sub-2hr marathon

Introduction

Lukasiewicz et al. (1) have published a model which predicts that to run a sub-2hr marathon (sub2hrM), prospective male and female runners will need to oxidize ingested (exogenous) carbohydrate (CHO) at respective rates of 93 and 108 g/hr during that race. Their predictions are dependent on the assumptions of their model.

The ability to run at 21.1 km/hr for 2 h requires high rates of (obligatory) CHO oxidation

Their first assumption is that to run at 21.1 km/hr for 2 h requires a high rate of CHO oxidation, intitially from muscle glycogen. But once muscle glycogen concentrations fall below 32% of the starting value, to prevent any slowing of pace thereafter, essentially all the runners' energy must come from the oxidation of ingested (exogenous) CHO with a small contribution from liver glycogen. Table 1 details their core predictions, in particular rates of exogenous CHO oxidation required to run a sub2hrM. These predictions raise a number of interesting points.

Estimations of muscle glycogen use during the first 90–120 min of a sub2hrM attempt in CHO-adapted athletes

Based on the reported range associated with performance decrements, the model predicts that exercise performance deteriorates once the muscle glycogen content falls below 32% of its starting value which, accordingly to the calculated endogenous glycogen stores remaining at the point of slowing, would leave 221 and 160 grams in males and females respectively (Table 1, Row F).

TABLE 1 Modeled rates of total energy expenditure (A); energy from CHO (B, C) and fat oxidation (D); muscle glycogen content at capacity (start of exercise) (E); muscle glycogen use during exercise (F, G); model predicted additional CHO oxidation from blood glucose and exogenous CHO to balance total CHO requirement (H); model predicted CHO contribution from liver glucose disappearance (I); corrected model predicted rates of exogenous CHO contribution to balance total CHO requirement if liver glucose disappearance is 0 g (J); model predicted rates of exogenous CHO oxidation to balance total CHO oxidation (K); model predicted rates of exogenous CHO oxidation required to balance total CHO requirements for runners of different weights (L).

		Male	Female
A	Total energy expenditure (kJ/min) [Calculated from Calculated caloric cost, kcal – Table 2 in Lukasiewicz et al. (1)]	88.3	75.7
B	Model predicted energy from CHO oxidation (g/min) [Table 2 in Lukasiewicz et al. (1)]	5.1	4.4
C	Model predicted total CHO oxidation (g) during 120 min exercise. (Row B × 120)	612	528
D	Model predicted energy from fat oxidation (g/min) [Table 2 in Lukasiewicz et al. (1)].	0.07	0.06
E	Muscle glycogen (g) at capacity (start of exercise) [Table 3 in Lukasiewicz et al. (1)]	690	499
F	Muscle glycogen (g) remaining if exercise terminates (1) when muscle glycogen concentration falls to 32% or (2) to 20% of the starting concentration	1. 221 2. 138 Difference: 83g	1. 160 2. 100 Difference: 60g
G	Total muscle glycogen use (g) if exercise terminates (1) at 32% or (2) at 20% of starting muscle glycogen concentration.	1. 469 2. 552	1. 339 2. 399
H	Model predicted additional CHO requirement from blood glucose and exogenous CHO (g) contribution to balance total CHO requirement according to the authors' model that exercise terminates when muscle glycogen concentration drops (1) below 32% or (2) 20% of the starting concentration (Row C minus Row G)	1. 143 2. 60	1. 189 2. 129
I	Model predicted CHO contribution from liver glucose (g) disappearance [Figures 4A, B in Lukasiewicz et al. (1)]	68	49
J	Corrected model predicted total exogenous CHO oxidation (g) required to balance total CHO requirement if hepatic glucose disappearance is 0 g (not the values listed in Row I) (Row H plus Row I).	1. 211 2. 128	1. 238 2. 178
K	Model predicted exogenous CHO (g/hr) required to balance CHO requirement [Figure 6A in Lukasiewicz et al. (1)].	90	106
L	Model predicted rates of exogenous CHO oxidation (g/min) required to balance total CHO requirements for runners of different weights [From Figure 6C in Lukasiewicz et al. (1)].	70 (50 kg) 82 (54 kg) 105 (58 kg) 120 (62 kg)	90 (42 kg) 103 (46 kg) 114 (50 kg) 128 (54 kg)

Rows A–D: Model predicted CHO and fat oxidation rates. Row E: Muscle glycogen content at capacity (start of exercise). Rows F–G: Contribution of muscle glycogen disappearance to total CHO oxidation if fatigue develops at (1) 32% or (2) 20% of starting muscle glycogen concentration. Row H: Model predicted CHO in addition to that from muscle glycogen oxidation required to balance the total required CHO oxidation under two conditions of muscle glycogen disappearance. The (2) in Rows F–H, and J refers to values if fatigue occurs when muscle glycogen is depleted to 20% of the starting concentration. Row I: Predicted CHO contribution from liver glucose disappearance. From Figures 4A, B in Lukasiewicz et al. (1). Row J: Corrected model predicted rates of exogenous CHO oxidation needed to balance total CHO demand if liver glucose disappearance provides 0g to total CHO requirement. Row K: Model predicted rates of exogenous CHO oxidation required to balance CHO requirement [Figure 6A in Lukasiewicz et al. (1)]. Row L: Model predicted exogenous CHO oxidation rates to balance the total CHO requirement for runners of different ages and either sex. From Figure 6C in Lukasiewicz et al. (1).

The authors also argue that "*running activates only approximately 68% of the total lower limb muscle (sic);*" thus fatigue develops when glycogen depletion occurs in those 68% of all lower limb muscle fibers.

But is it possible to run at >90% VO_2max (2) whilst recruiting just 68% of all the quadriceps muscle fibers (as opposed to 68% of all the muscle fibers in the lower limb)? This is relevant because most studies measure exercise-induced changes in muscle glycogen concentrations in that muscle, rather than in all the lower limb muscles. The authors derive their value of 68% from a study (3) of young female physical education students, described as recreational runners with a mean VO_2max of 49 ml/kg/min. Yet Sale (4) has calculated that 100% of the quadriceps muscle fibers are activated when cycling at >85%VO_2max [Figure 2; page 99 in Sale (4)]. Perhaps the value of 68% is not realistic for world class male and female athletes competing in the sub2hrM attempt. However, as we show, which ever percentage is chosen, it has little or no influence on the model's predictions.

In the original study (5), on which this model of Lukasiewietz et al. (1) is ultimately based—specifically that there is an obligatory role for CHO oxidation to sustain endurance exercise performance (6)—subjects terminated exercise when their muscle glycogen concentrations were 13%, 10%, and 20% of their starting concentrations following 3 different diets. If subjects in this model terminated exercise with muscle glycogen concentrations reduced to 20% and not 32% of starting concentrations, an additional 83 and 60 g CHO become available for the male and female athletes respectively (Table 1, Row F) leaving a residual 60 g CHO (30 g/hr) in males and 129 g CHO (64.5 g/hr) in females (Lines 2 in Row H,

Table 1) to be provided by liver glucose release and exogenous CHO oxidation.

Estimated CHO contribution from liver glucose disappearance

Panels A and B in the authors' Figure 4 (1) predict that liver glucose disappearance contributes 68 and 49 g CHO to total CHO oxidation in males and females respectively (Table 1, Row I). This is likely overestimated since high rates of CHO ingestion suppress liver glucose disappearance (7–9).

When modified for these two contestable calculations, the predicted rate of exogenous CHO oxidation needed to run a sub2hrM would be 128–211 g CHO/2hr (64–105.5 g CHO/hr) in males and 178–238 CHO/2 hr (80–119 g CHO/hr) in females (Table 1, Row J) compared to rates of 90 and 106 g CHO/hr predicted in the original model (Table 1, Row K). These calculations support the general accuracy of the authors' original conclusions.

Estimated rates of fat oxidation during exercise at >90% VO_2max

The authors' second presumption is that during a sub2hrM, the rate of fat oxidation would be 2–3 kJ/min (0.07 and 0.06 g/min for men and women respectively; Row D; Table 1) providing <3% of the total energy. However, even at exercise intensities >85% VO_2max, some well-trained but recreational athletes oxidized fat at rates >1.5 g/min (57 kJ/min) (10) potentially supplying 65%−75% of the 76–89 kJ/min required for males and female athletes to run a sub2hrM (Table 1, Row A). Other studies report high rates of fat oxidation even at moderate to high exercise intensities (11).

Figure 1 shows the rates of endogenous CHO (muscle glycogen and blood/liver glucose), fat and exogenous CHO oxidation in male (Row A) and female (Row B) athletes running a sub2hrM according to the calculations of Lukasiewicz et al. (1). Increasing the rate of fat oxidation to 0.5 g/min (18 kJ/min) reduces the CHO deficit required from exogenous CHO oxidation to 25 g/hr for males (Row C) and 46 g/min for females (Row D). Increasing the fat oxidation rate to 0.7 g/min in males (Row E) and to 0.9 g/min in females (Row F) removes any requirement for exogenous CHO oxidation to fuel a sub2hrM in either sex.

Thus the model's predictions are critically dependent on the value given to rates of fat oxidation. At present there are few data of rates of fat oxidation in elite athletes exercising at 90% VO_2max or higher. Furthermore, those data that are available, are usually from studies of athletes habituated to high CHO diets which produce submaximal "peak" rates of fat oxidation (11).

Rates of total exogenous CHO oxidation calculated from instantaneous rates of exogenous CHO oxidation during exercise

The third presumption is that ingesting CHO at 90–120 g/min will produce equivalent exogenous CHO rates of 90–120 g/min for the full duration of the sub2hrM. Figure 2 depicts results from the two laboratory studies (12, 13) that have reported the highest rates of exogenous CHO oxidation during exercise. Figure 2A shows instantaneous exogenous CHO oxidation rates in these studies; Figure 2B the cumulative amounts of CHO ingested and oxidized, including the cumulative amounts remaining unoxidized when these experiments concluded.

The study of Jentjens et al. (12) found that the total amount of exogenous CHO oxidized during 2 h of exercise from the optimum solution of glucose, fructose and sucrose was 137 g or 48% of the total ingested amount of 288 g (Figure 2B) leaving 151 g (52%) unoxidized (Figure 2B). Fifty three and 70% of the ingested CHO from the two other tested solutions remained unoxidized after 2 h exercise (Figure 2B) (12, 13).

These data predict that the maximum amount of exogenous CHO oxidation during a sub2hrM in which subjects ingest CHO even at 144 g/hr, would likely be only 137 g (68.5 g/hr). This is substantially less than the rates of 106 g/hr for females and 90 g/hr for males [Figures 6A, B; Lukasiewicz et al. (1)] that the model predicts are required to complete a sub2hrM. Thus the model overpredicts the available total exogenous CHO oxidation amounts by a minimum of 43.6 g/2hr in males and of 75 g/2hr in females.

Rates of exogenous CHO oxidation fall short of values required to run a sub2hrM

The result is that this model predicts that it is not possible for any athlete to run a sub2hrM relying exclusively on CHO metabolism, even when CHO is ingested at rates of 120–144 g/hr. Rather a reasonable contribution from fat oxidation might make it possible for some athletes to achieve the sub2hrM even whilst ingesting either none or quite small amounts of CHO during the race (Figure 1).

Is there a time penalty associated with drinking frequently when running at 21.2 km/hr during the sub2hrM attempt?

The fourth assumption is that during the sub2hrM attempt, runners can ingest CHO at very high rates without losing time as a result of frequent drinking. With one exception (14), studies of high rates of CHO ingestion during exercise come from tightly controlled laboratory studies of carbohydrate-adapted cyclists (12, 13, 15–24). The presumption is that results from controlled laboratory experiments of cyclists can be extrapolated to runners competing in an uncontrolled real-world environment, frequently disturbed by the presence of other competitors. For example, intra-abdominal pressures are substantially higher during running than during cycling (25, 26), a difference that will likely influence the ability to replace CHO at high rates when running at >90% VO_2max for 2 h.

Rowe et al. (14) reported high rates of CHO ingestion (90 g/hr) and total exogenous CHO oxidation (132 g; 66 g/hr) in runners during 2 h of treadmill running but at much lower exercise intensities than are achieved during the sub2hrM. Subjects ingested

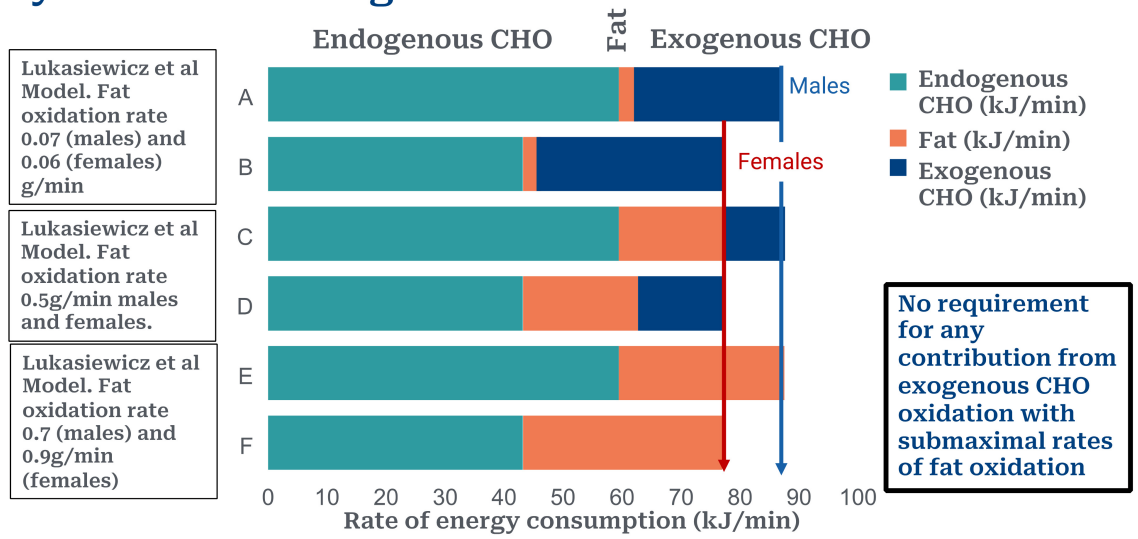

FIGURE 1
Contributions of endogenous (muscle glycogen), fat and exogenous CHO to total energy expenditure in males and females wishing to run a sub2hrM. Rows A and B according to model of Lukasiewicz et al. (1). Rows C and D when rate of fat oxidation is increased to 0.5 g/min; Rows E and F when rate of fat oxidation is increased to 0.7 g/min for males and 0.9 g/min for males. These higher rates of fat oxidation remove the need for any contribution from exogenous CHO oxidation. Liver glucose disappearance likely makes little or no contribution to energy production at such high rates of CHO ingestion (7–9).

fluid at a rate of 400 ml/hr from a 22.5% CHO drink and oxidized 73% of the ingested CHO. Cyclists in the study of King et al. (19) achieved similar rates of exogenous CHO oxidation and CHO ingestion (112.5 g/hr) from a much less concentrated CHO solution (11%) but at a much higher fluid ingestion rate (1 L/hr). Hawley et al. (24) reported similarly high rates of CHO ingestion (120 g/hr) by cyclists who ingested a 15% CHO solution at 800 ml/hr. In that study total exogenous CHO oxidation was 45 g/hr, leaving 150 g unoxidized at the end of exercise.

These scientists seem to have discovered that athletes have substantially greater difficulty ingesting fluid at high rates when running than when cycling, even under laboratory conditions. Additionally the movements of the upper body are quite different in running and cycling. The upper body is essentially static during cycling, but rotates substantially when running, increasing with running speed, contributing to increased running efficiency (27). Repeated drinking whilst attempting to ingest CHO at high rates must potentially impairing running performance. Clearly the possible time-wasting effects of frequent drinking during the sub2hrM need to be quantified.

High rates of CHO ingestion during exercise may have metabolic effects not considered in this model

The final assumption is that ingesting CHO will not produce metabolic effects that could impair running performance. Hawley et al. (24) found that the ingestion of 120 g CHO/hr during exercise at 70% VO_2max raised blood insulin concentrations, reducing the rate of fat oxidation from ~0.94 to ~0.17 g/min. Total muscle glycogen use during 125 min of exercise was 140 g, 67% greater than in a control group who received enough CHO by infusion to maintain euglycemic blood levels. Similarly the intravenous infusion of 252 g of glucose during 2 h of cycling exercise (126 g/hr) increased total muscle glycogen use by 40 g (28).

Discussion

The authors have gone to extraordinary lengths to develop a model of the rates of exogenous carbohydrate (CHO) ingestion required to run a sub2hrM. We argue that their model contains two potential flaws that might reverse their conclusions.

First, their model requires that fat oxidation makes no significant contribution to energy use during exercise at >90%VO_2max. Whilst this is the accepted doctrine for the past century or so, more recent studies find that athletes chronically adapted to low-CHO diets can achieve high rates of fat oxidation even during exercise at >85% VO_2max (10, 11). We show that rates of fat oxidation of 0.7 g/min in males and 0.9 g/min in females would, according to the predictions of this model, allow athletes of either sex to run a sub2hrM without requiring any additional energy from exogenous CHO oxidation.

Second, the authors assume that high rates of ingested CHO produce equivalent high rates of total exogenous CHO oxidation during exercise. However, studies of high rates of CHO ingestion during exercise show that, at best, only 50% of the ingested CHO is oxidized during the first 2 h of exercise (Figure 2B).

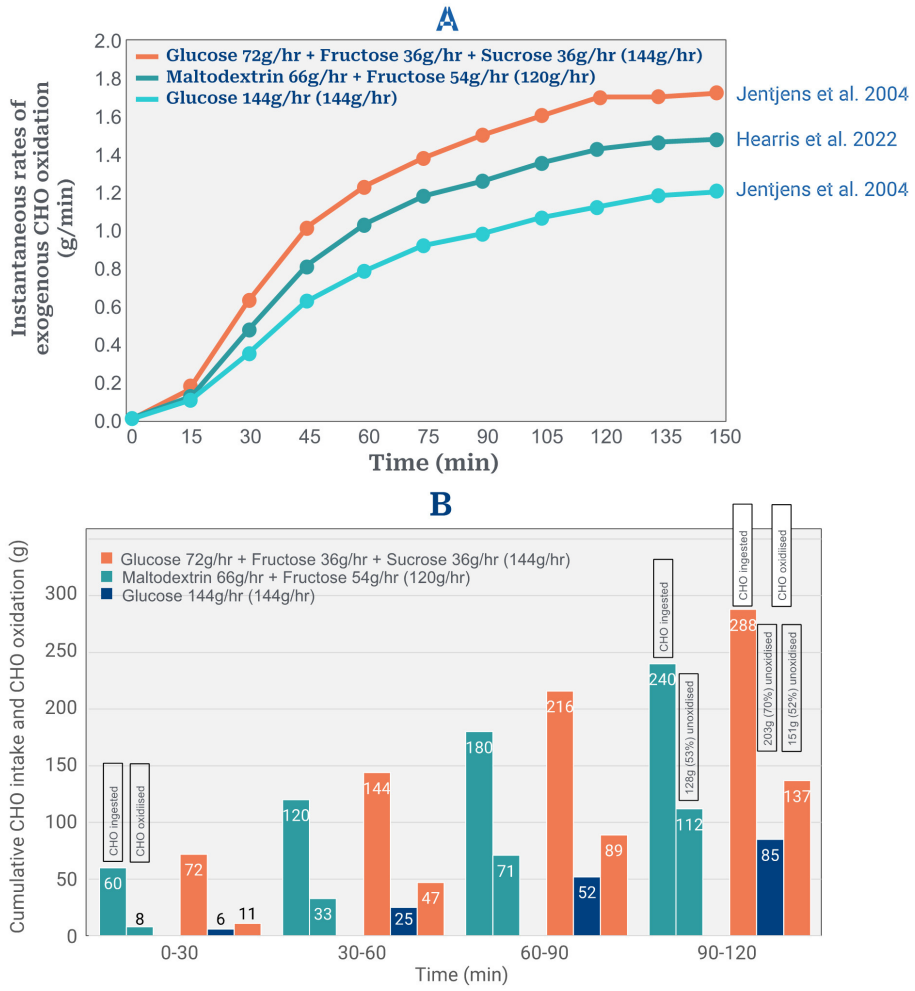

FIGURE 2
(A) Instantaneous rates of exogenous CHO oxidation from three different solutions ingested during 2 h of exercise. Data reproduced from references (12, 13). **(B)** Cumulative amounts of ingested CHO oxidized every 30 min from 3 different solutions. Cumulative amounts of ingested CHO oxidized were estimated from average rates of exogenous CHO oxidation every 30 min using data in **(A)**. Cumulative amounts of unoxidized CHO every 30 min were calculated as amounts ingested minus the average amounts oxidized in that 30 min period.

When this latter correction is made to this model, the prediction must be that it will not be possible for a male or female athlete to run a sub2hrM relying purely on CHO metabolism. But with a reasonable contribution from fat oxidation, the sub2hrM might be possible in some athletes even with little or no contribution from the oxidation of exogenous CHO during exercise.

A limitation of this discussion is that it remains theoretical in nature; therefore, further empirical investigation is necessary to validate and substantiate the proposed concepts. Future experimental research should aim to test these hypotheses and explore their practical implications in greater depth. Additionally, it should be acknowledged that no cellular or tissue-level data measurements were obtained from either group, potentially limiting the direct applicability of our findings. Moreover, our calculations did not account for the contributions of amino acids and ketone bodies, which may have influenced the metabolic outcomes considered.

Author contributions

TN: Writing – original draft, Writing – review & editing. PP: Writing – original draft, Writing – review & editing.

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Conflict of interest

TN is author of low-carbohydrate nutrition books. TN book royalties are donated to The Noakes Foundation which contributes to the Eat Better South Africa Campaign.

The remaining author declares 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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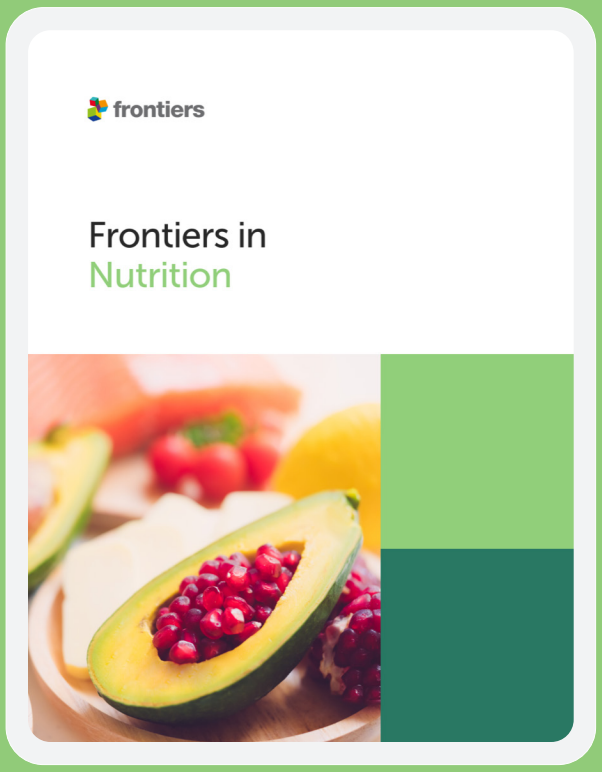

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