frontiers | Research Topics

Functional foods, supplements, and dietary approaches in sports and clinical nutrition

Edited by
Heitor O. Santos, Scott Forbes and Mihnea-Alexandru Găman

Published in
Frontiers in Nutrition

ISSN 1664-8714
ISBN 978-2-8325-2482-4
DOI 10.3389/978-2-8325-2482-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Functional foods, supplements, and dietary approaches in sports and clinical nutrition

Topic editors

Heitor O. Santos — Federal University of Uberlandia, Brazil

Scott Forbes — Brandon University, Canada

Mihnea-Alexandru Găman — Carol Davila University of Medicine and Pharmacy, Romania

Citation

Santos, H. O., Forbes, S., Găman, M.-A., eds. (2023). *Functional foods, supplements, and dietary approaches in sports and clinical nutrition*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2482-4

Table of contents

05 Editorial: Functional foods, supplements, and dietary approaches in sports and clinical nutrition
Heitor O. Santos, Scott C. Forbes and Mihnea-Alexandru Găman

08 A Novel Plant-Based Protein Has Similar Effects Compared to Whey Protein on Body Composition, Strength, Power, and Aerobic Performance in Professional and Semi-Professional Futsal Players
Filipe J. Teixeira, Catarina N. Matias, João Faleiro, Rita Giro, Joana Pires, Helena Figueiredo, Raquel Carvalhinho, Cristina P. Monteiro, Joana F. Reis, Maria J. Valamatos, Vítor H. Teixeira and Brad J. Schoenfeld

20 Effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes mellitus: A randomized clinical trial
Masoud Khorshidi, Aliakbar Sayyari, Naheed Aryaeian, Beheshteh Olang, Mohammadreza Alaei, Mitra Khalili, Amirhossein Hosseini and Masoud Salehi

31 Effects of the 5:2 intermittent fasting diet on non-alcoholic fatty liver disease: A randomized controlled trial
Hamed Kord Varkaneh, Ammar Salehi sahlabadi, Mihnea-Alexandru Găman, Mohsen Rajabnia, Melahat Sedanur Macit-Çelebi, Heitor O. Santos and Azita Hekmatdoost

44 Low fat diet versus low carbohydrate diet for management of non-alcohol fatty liver disease: A systematic review
Hamed Kord Varkaneh, Faezeh Poursoleiman, Mohammad Khaldoun Al Masri, Kamar Allayl Alras, Yamen Shayah, Mohd Diya Masmoum, Fulwah Abdulaziz Alangari, Abd Alfatah Alras, Giulia Rinaldi, Andrew S. Day, Azita Hekmatdoost, Ahmed Abu-Zaid and Emad Kutbi

55 Effects of a natural nutritional supplement on immune cell infiltration and immune gene expression in exercise-induced injury
Feng Jiang, Rongfeng Yang, Diya Xue, Rong Li, Meiling Tan, Zhicong Zeng, Luhua Xu, Linling Liu, Yinzhi Song and Fengxia Lin

68 The effect of high-fructose corn syrup vs. sucrose on anthropometric and metabolic parameters: A systematic review and meta-analysis
Xiang Li, Yunqi Luan, Yuejin Li, Shili Ye, Guihui Wang, Xinlun Cai, Yucai Liang, Hamed Kord Varkaneh and Yunpeng Luan

80 Performance effects of internal pre- and per-cooling across different exercise and environmental conditions: A systematic review
Maria Roriz, Pedro Brito, Filipe J. Teixeira, João Brito and Vitor Hugo Teixeira

102 Evaluation of pre-workout and recovery formulations on body composition and performance after a 6-week high-intensity training program
Hannah E. Cabre, Amanda N. Gordon, Noah D. Patterson and Abbie E. Smith-Ryan

116 FruHis significantly increases the anti-benign prostatic hyperplasia effect of lycopene: A double-blinded randomized controlled clinical trial
Alireza Sadeghi, Ahmad Saedisomeolia, Leili Jalili-Baleh, Mehdi Khoobi, Mohammad Soleimani, Ali Mohammad Fakhr Yasseri, Mir Saeed Yekaninejad, Amirreza Farzin, Erfan Amini and Mohammad Reza Nowroozi

128 Effect of prebiotics, probiotics, and synbiotics on gastrointestinal outcomes in healthy adults and active adults at rest and in response to exercise—A systematic literature review
Christopher E. Rauch, Alice S. Mika, Alan J. McCubbin, Zoya Huschtscha and Ricardo J. S. Costa

176 The association between vitamin D status and inflammatory bowel disease among children and adolescents: A systematic review and meta-analysis
Somaye Fatahi, Naseem Alyahyawi, Naryman Albadawi, Farzaneh Mardali, Naghi Dara, Mohammad Hassan Sohouli, Kousalya Prabahar, Pejman Rohani, Nazanin Koushki, Aliakbar Sayyari, Amir Hossein Hosseini and Ahmed Abu-Zaid

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 05 May 2023
DOI 10.3389/fnut.2023.1203477

OPEN ACCESS

EDITED AND REVIEWED BY
David Christopher Nieman,
Appalachian State University, United States

*CORRESPONDENCE
Heitor O. Santos
✉ heitoroliveirasantos@gmail.com

RECEIVED 10 April 2023
ACCEPTED 19 April 2023
PUBLISHED 05 May 2023

CITATION
Santos HO, Forbes SC and Găman M-A (2023) Editorial: Functional foods, supplements, and dietary approaches in sports and clinical nutrition. *Front. Nutr.* 10:1203477. doi: 10.3389/fnut.2023.1203477

Editorial: Functional foods, supplements, and dietary approaches in sports and clinical nutrition

Heitor O. Santos[1]*, Scott C. Forbes[2] and Mihnea-Alexandru Găman[3,4]

[1]School of Medicine, Federal University of Uberlandia (UFU), Uberlandia, Minas Gerais, Brazil, [2]Faculty of Education, Department of Physical Education Studies, Brandon University, Brandon, MB, Canada, [3]Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [4]Department of Hematology, Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania

KEYWORDS

herbal medicines, functional food, supplements, cardiometabolic risk, sports nutrition, clinical nutrition

Editorial on the Research Topic
Functional foods, supplements, and dietary approaches in sports and clinical nutrition

Nutritional research includes the investigation of various dietary approaches, functional foods, and supplements, to guide personalized advice to enhance health and/or performance (1–8). Evidence-based decisions must be made based on the most recent advances in research. Therefore, we created a special issue entitled "*Functional Foods, Supplements, and Dietary Approaches in Sports and Clinical Nutrition*", which consisted of a number of leading experts. In the special issue, 7 papers were focused on health or clinical populations and 4 manuscripts had a sport or performance focus. In this editorial, we briefly highlight the key aspects of each manuscript.

Clinical studies

Intermittent fasting regimens have emerged as therapeutic tools to improve markers of non-alcoholic fatty liver disease and cardiovascular risk factors (9–15). Kord-Varkaneh, Salehi Sahlabadi et al. conducted a randomized controlled trial to assess the benefits of intermittent fasting 5:2 in patients with non-alcoholic fatty liver disease. The results of their research revealed that adherence to the 5:2 intermittent fasting regimen can decrease anthropometric indices (waist circumference, body weight, fat mass, and body mass index), several biochemical markers (alanine and aspartate aminotransferases, triglycerides, high-sensitivity C-reactive protein, and cytokeratin-18), as well as fibrosis and steatosis scores. Fasting plasma glucose, insulin, HOMA-IR, total cholesterol, low- and high-density lipoprotein cholesterol, as well as total antioxidant capacity levels, remained unaltered following the intervention (Kord-Varkaneh, Salehi Sahlabadi et al.).

Another evidence-based manuscript targeted the topic of non-alcoholic fatty liver disease. Kord-Varkaneh, Poursoleiman et al. performed a systematic review of the literature to assess the potential benefits of low-fat vs. low-carbohydrate diets in the aforementioned

metabolic disorder and concluded that both dietary interventions are effective in reducing anthropometric indices and metabolic biomarkers, however, it appears that a reduced intake of fats were more likely to drop liver enzyme concentrations.

Li et al. conducted a systematic review and meta-analysis to assessed the impact of high-fructose corn syrup compared to sucrose on anthropometric indices and metabolic biomarkers. Their findings suggest that the two compounds are similar in terms of their effects on anthropometric indices and metabolic parameters, however, consumption of the former was linked to higher concentrations of C-reactive protein (a marker of systemic inflammation) (Li et al.).

Omega-3 polyunsaturated fatty acid supplementation (*n*-3 PUFAs) are essential components that can improve the glycemic and cardiovascular profiles in different populations (16, 17). Khorshidi et al. evaluated the effects of *n*-3 PUFA supplementation on adolescents diagnosed with type 1 diabetes mellitus through a randomized controlled trial. The researchers demonstrated that the intervention enhanced flow-mediated dilatation, and reduced triglyceride concentrations, without any notable impact on other biomarkers of endothelial or vascular function or on metabolic parameters (Khorshidi et al.).

Micronutrients, including vitamin D, are essential for health and against diseases Proper screening for vitamin D status is of global interest to prevent and possibly treat many ailments diseases (18–21). Employing a systematic review and meta-analysis approach, Fatahi et al. investigated the association between serum vitamin D concentrations and inflammatory bowel disease in children and adolescents. Their research indicated a tendency toward children and adolescents with inflammatory bowel disease to display vitamin D deficiency (Fatahi et al.).

Natural compounds are purported to modulate hormones to enhance men's heath (22–24). In their randomized controlled trial, Sadeghi et al. investigated the action of FruHis, a natural compound derived from the combination of fructose and histidine. This product was investigated alone or in combination with lycopene in the setting of benign prostatic hyperplasia. According to their findings, the co-administration of FruHis and lycopene reduced IGF-1 levels, without statistically significant changes in other clinical or laboratory parameters (Sadeghi et al.).

Herbal medicines can complement dietary approaches possibly due to their anti-inflammatory properties (25–28). Jiang et al. explored the impact of supplementation with echinacoside, a natural compound used in traditional Chinese medicine and extracted from *Herba Cistanches*, a type of medicinal herb, on immune parameters and key genes involved in the immune response in a murine model of exercise-induced injury. Their research highlighted its anti-inflammatory properties. In addition, they employed genetic studies and artificial intelligence techniques to propose a new method of screening for the detection of natural products that could elicit health benefits in humans by targeting relevant genes involved in immunity (Jiang et al.).

Sport studies

Teixeira et al. investigated the effects of 8 weeks of protein supplementation with either a plant-based protein supplement or whey protein in high level futsal players. The futsal players were ingesting "sufficient" amounts of daily protein previously shown to optimize muscle performance (>1.6 g/kg/day) and therefore the supplements did provide any further benefit. These results are important for athletes and highlight that more protein is not always better and further corroborates that 1.6 g/kg/day of protein is optimal (Teixeira et al.).

In another longitudinal study, Cabre et al. randomized healthy active participants to either a pre-post multi-ingredient supplement or placebo in conjunction with a high intensity resistance training and high intensity interval training program. Overall, the supplement was able to positively enhance lean mass and muscular strength (both upper and lower body) in both men and women (Cabre et al.).

Rauch et al. conducted a systematic review with an aim to investigate the effects of various supplementation protocols of pre, pro, and syn-biotics in healthy active adults on gastrointestinal outcomes at rest and in response to acute exercise. Overall, 1,204 participants were included from 37 manuscripts. The review highlights that prebiotics can alter gut microbial composition and short-chain fatty acids (SCFA) concentrations, while probiotics increase the supplemented species-strain with limited effect on SCFA and no effects on gastrointestinal status markers at rest. Further, probiotics and synbiotic supplementation did not influence epithelial injury and permeability, systemic endotoxin and inflammation cytokine profiles, or gastro-intestinal symptoms in response to exercise (Rauch et al.).

Exercising in a hot and humid environment stresses the cardiovascular system and may impair performance. Roriz et al. performed a systematic review aimed to examine whether ice, cold beverages or menthol solutions can alter performance when exercising in different environmental conditions. Menthol solution appeared to improved physical performance during continuous endurance exercise in the heat. In contrast, ice ingestion or cold beverages did not consistently increase performance. Menthol with or within ice drinks resulted in a synergistic effect on performance. Interestingly, even in environmental conditions that are not extreme, internal cooling strategies may be ergogenic (Roriz et al.).

Conclusion

This Special Issue included several high-quality original research articles and several rigorous systematic reviews that provided new insights for scientists and practitioners to help guide evidence based practice.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

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

Publisher's note

References

1. Santos HO, Cerqueira HS, Tinsley GM. The Effects of Dietary Supplements, Nutraceutical Agents, and Physical Exercise on Myostatin Levels: Hope or Hype? *Metabolites.* (2022) 12:146. doi: 10.3390/metabo12111146

2. de Moraes W, de Almeida FN, Dos Santos LEA, Cavalcante KDG, Santos HO, Navalta JW, et al. Carbohydrate Loading Practice in Bodybuilders: Effects on Muscle Thickness, Photo Silhouette Scores, Mood States and Gastrointestinal Symptoms. *J Sports Sci Med.* (2019) 18:772–9.

3. Santos HO, Genario R, Gomes GK, Schoenfeld BJ. Cherry intake as a dietary strategy in sport and diseases: a review of clinical applicability and mechanisms of action. *Crit Rev Food Sci Nutr.* (2021) 61:417–30. doi: 10.1080/10408398.2020.1734912

4. Macedo RCO, Santos HO, Tinsley GM, Reischak-Oliveira A. Low-carbohydrate diets: Effects on metabolism and exercise - A comprehensive literature review. *Clin Nutr ESPEN.* (2020) 40:17–26. doi: 10.1016/j.clnesp.2020.07.022

5. Sohouli MH, Fatahi S, Sharifi-Zahabi E, Santos HO, Tripathi N, Lari A, et al. The impact of low advanced glycation end products diet on metabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. *Adv Nutr.* (2021) 12:766–76. doi: 10.1093/advances/nmaa150

6. Santos HO, Penha-Silva N. Translating the advanced glycation end products (AGEs) knowledge into real-world nutrition strategies. *Eur J Clin Nutr.* (2022) 76:922–8. doi: 10.1038/s41430-021-01028-8

7. Delpino FM, Figueiredo LM, Forbes SC, Candow DG, Santos HO. Influence of age, sex, and type of exercise on the efficacy of creatine supplementation on lean body mass: A systematic review and meta-analysis of randomized clinical trials. *Nutrition.* (2022) 103:111791. doi: 10.1016/j.nut.2022.111791

8. Lari A, Sohouli MH, Fatahi S, Cerqueira HS, Santos HO, Pourrajab B, et al. The effects of the Dietary Approaches to Stop Hypertension (DASH) diet on metabolic risk factors in patients with chronic disease: A systematic review and meta-analysis of randomized controlled trials. *NMCD.* (2021) 31:2766–78. doi: 10.1016/j.numecd.2021.05.030

9. Kord-Varkaneh H, Salehi-Sahlabadi A, Tinsley GM, Santos HO, Hekmatdoost A. Effects of time-restricted feeding (16/8) combined with a low-sugar diet on the management of non-alcoholic fatty liver disease: A randomized controlled trial. *Nutrition.* (2022) 105:111847. doi: 10.1016/j.nut.2022.111847

10. Santos HO, Genario R, Tinsley GM, Ribeiro P, Carteri RB, Coelho-Ravagnani CF, et al. scoping review of intermittent fasting, chronobiology, and metabolism. *Am J Clin Nutr.* (2022) 115:991–1004. doi: 10.1093/ajcn/nqab433

11. Meng H, Zhu L, Kord-Varkaneh H, Tinsley GM, Fu P. Effects of intermittent fasting and energy-restricted diets on lipid profile: A systematic review and meta-analysis. *Nutrition.* (2020) 77:110801. doi: 10.1016/j.nut.2020.110801

12. Wang X, Yang Q, Liao Q, Li M, Zhang P, Santos HO, et al. Effects of intermittent fasting diets on plasma concentrations of inflammatory biomarkers: A systematic review and meta-analysis of randomized controlled trials. *Nutrition.* (2020) 80:110974. doi: 10.1016/j.nut.2020.110974

13. Kord Varkaneh H, Salehi Sahlabadi A, Găman MA, Rajabnia M, Sedanur Macit-Çelebi M, Santos HO, et al. Effects of the 5:2 intermittent fasting diet on non-alcoholic fatty liver disease: A randomized controlled trial. *Front. Nutr.* (2022) 9:948655. doi: 10.3389/fnut.2022.948655

14. Santos HO. Intermittent Fasting and Fat Mass: What Is the Clinical Magnitude? *Obesities.* (2022) 2:1–7. doi: 10.3390/obesities2010001

15. Santos HO, Macedo RCO. Impact of intermittent fasting on the lipid profile: Assessment associated with diet and weight loss. *Clin Nutr ESPEN.* (2018) 24:14–21. doi: 10.1016/j.clnesp.2018.01.002

16. Delpino FM, Figueiredo LM, da Silva BGC, da Silva TG, Mintem GC, Bielemann RM, et al. Omega-3 supplementation and diabetes: A systematic review and meta-analysis. *Crit Rev Food Sci Nutr.* (2022) 62:4435–48. doi: 10.1080/10408398.2021.1875977

17. Santos HO, Price JC, Bueno AA. Beyond Fish Oil Supplementation: The Effects of Alternative Plant Sources of Omega-3 Polyunsaturated Fatty Acids upon Lipid Indexes and Cardiometabolic Biomarkers-An Overview. *Nutrients.* (2020) 12:429. doi: 10.3390/nu12103159

18. Santos HO, Tinsley GM, da Silva GA, Bueno AA. Pharmaconutrition in the clinical management of COVID-19: a lack of evidence-based research but clues to personalized prescription. *J Pers Med.* (2020) 10:145. doi: 10.3390/jpm10040145

19. Dahma G, Neamtu R, Nitu R, Gluhovschi A, Bratosin F, Grigoras ML, et al. The Influence of Maternal Vitamin D Supplementation in Pregnancies Associated with Preeclampsia: A Case-Control Study. *Nutrients.* (2022) 14:3008. doi: 10.3390/nu14153008

20. Zhang Y, Tan H, Tang J, Li J, Chong W, Hai Y, et al. Effects of vitamin D supplementation on prevention of type 2 diabetes in patients with prediabetes: a systematic review and meta-analysis. *Diabetes Care.* (2020) 43:1650–8. doi: 10.2337/dc19-1708

21. Santos HO, Howell S, Nichols K, Teixeira FJ. Reviewing the evidence on vitamin D supplementation in the management of testosterone status and its effects on male reproductive system (testis and prostate): mechanistically dazzling but clinically disappointing. *Clin Ther.* (2020) 42:e101–14. doi: 10.1016/j.clinthera.2020.03.016

22. Santos HO, Cadegiani FA, Forbes SC. Nonpharmacological Interventions for the Management of Testosterone and Sperm Parameters: A Scoping Review. *Clin Ther.* (2022) 44:1129–49. doi: 10.1016/j.clinthera.2022.06.006

23. Santos HO, Howell S, Teixeira FJ. Beyond tribulus (Tribulus terrestris L): The effects of phytotherapics on testosterone, sperm and prostate parameters *J Ethnopharmacol.* (2019) 235:392–405. doi: 10.1016/j.jep.2019.02.033

24. Santos HO. Teixeira F. Use of medicinal doses of zinc as a safe and efficient coadjutant in the treatment of male hypogonadism. *Aging Male.* (2020) 23:669–78. doi: 10.1080/13685538.2019.1573220

25. Zhu C, Yan H, Zheng Y, Santos HO, Macit MS, Zhao K. Impact of Cinnamon Supplementation on cardiometabolic Biomarkers of Inflammation and Oxidative Stress: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Complement Ther Med.* (2020) 53:102517. doi: 10.1016/j.ctim.2020.102517

26. Santos HO, da Silva GAR. To what extent does cinnamon administration improve the glycemic and lipid profiles? *Clin Nutr ESPEN.* (2018) 27:1–9. doi: 10.1016/j.clnesp.2018.07.011

27. Santos HO, Bueno AA, Mota JF. The effect of artichoke on lipid profile: A review of possible mechanisms of action. *Pharmacol Res.* (2018) 137:170–8. doi: 10.1016/j.phrs.2018.10.007

28. Santos HO, Macedo RCO. Cocoa-induced (Theobroma cacao) effects on cardiovascular system: HDL modulation pathways. *Clin Nutr ESPEN.* (2018) 27:10–5. doi: 10.1016/j.clnesp.2018.06.001

frontiers | Frontiers in Nutrition

CLINICAL TRIAL
published: 19 July 2022
doi: 10.3389/fnut.2022.934438

A Novel Plant-Based Protein Has Similar Effects Compared to Whey Protein on Body Composition, Strength, Power, and Aerobic Performance in Professional and Semi-Professional Futsal Players

Filipe J. Teixeira[1,2,3†], Catarina N. Matias[1,4†], João Faleiro[1,5], Rita Giro[1], Joana Pires[6], Helena Figueiredo[7], Raquel Carvalhinho[1,8], Cristina P. Monteiro[3,9], Joana F. Reis[3,9], Maria J. Valamatos[3,10], Vítor H. Teixeira[11,12,13,14] and Brad J. Schoenfeld[15]*

[1] Bettery Lifelab, Bettery S.A., Lisboa, Portugal, [2] Atlântica, Instituto Universitário, Fábrica da Pólvora de Barcarena, Barcarena, Portugal, [3] Interdisciplinary Center for the Study of Human Performance, Universidade de Lisboa, Cruz-Quebrada, Portugal, [4] Centro de Investigação em Desporto, Educação Física, Exercício e Saúde, Universidade Lusófona, Lisbon, Portugal, [5] Athletic Club Oulu Football Club, Oulu, Finland, [6] Grupo de Ativistas em Tratamentos, Lisboa, Portugal, [7] José de Mello Saúde, Lisboa, Portugal, [8] Departamento de Saúde do Futebol Clube do Porto, Porto, Portugal, [9] Laboratory of Physiology and Biochemistry of Exercise, Faculdade de Motricidade Humana, Universidade de Lisboa, Cruz-Quebrada, Portugal, [10] Neuromuscular Research Lab, Faculdade de Motricidade Humana, Universidade de Lisboa, Estrada da Costa, Cruz-Quebrada, Portugal, [11] Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal, [12] Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sports, University of Porto, Porto, Portugal, [13] Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal, [14] Futebol Clube do Porto, Porto, Portugal, [15] Health Sciences Department, Lehman College, City University of New York, Bronx, NY, United States

Introduction: The effects of dietary protein on body composition and physical performance seemingly depend on the essential amino acid profile of the given protein source, although controversy exists about whether animal protein sources may possess additional anabolic properties to plant-based protein sources.

Purpose: To compare the effects of a novel plant-based protein matrix and whey protein supplementation on body composition, strength, power, and endurance performance of trained futsal players.

Methods: Fifty male futsal players were followed during 8 weeks of supplementation, with 40 completing the study either with plant-based protein ($N = 20$) or whey protein ($N = 20$). The following measures were assessed: bone mineral content, lean body mass, and fat mass; muscle thickness of the rectus femoris; total body water; blood glucose, hematocrit, C-reactive protein, aspartate aminotransferase, alanine aminotransferase, creatine kinase, creatinine, and estimated glomerular filtration rate; salivary cortisol; maximal strength and 1-RM testing of the back squat and bench press exercises; muscle power and countermovement jump; VO_{2max} and maximal aerobic speed. Subjects were asked to maintain regular dietary habits and record dietary intake every 4 weeks through 3-day food records.

OPEN ACCESS

Edited by:
Heitor O. Santos, Federal University of Uberlandia, Brazil

Reviewed by:
Francesco Campa, University of Padua, Italy
Ezequiel Gonçalves, State University of Northern Paraná, Brazil

***Correspondence:**
Filipe J. Teixeira
filipe.teixeira@betterylife.com

† These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

Received: *02 May 2022*
Accepted: *23 June 2022*
Published: *19 July 2022*

Citation:
Teixeira FJ, Matias CN, Faleiro J, Giro R, Pires J, Figueiredo H, Carvalhinho R, Monteiro CP, Reis JF, Valamatos MJ, Teixeira VH and Schoenfeld BJ (2022) A Novel Plant-Based Protein Has Similar Effects Compared to Whey Protein on Body Composition, Strength, Power, and Aerobic Performance in Professional and Semi-Professional Futsal Players. Front. Nutr. 9:934438. doi: 10.3389/fnut.2022.934438

Results: No differences in any variable were observed between groups at baseline or pre- to post-intervention. Moreover, no time*group interaction was observed in any of the studied variables, and a time effect was only observed regarding fat mass reduction.

Conclusions: Supplementing with either a novel plant-based protein matrix or whey protein did not affect any of the variables assessed in high-level futsal players over 8 wks. These results suggest that whey protein does not possess any unique anabolic properties over and above those of plant-based proteins when equated to an essential amino acid profile in the population studied. Furthermore, when consuming a daily protein intake >1.6 g/kg BW.day^{-1}, additional protein supplementation does not affect body composition or performance in trained futsal players, regardless of protein type/source.

Keywords: supplementation, protein, athletes, lean body mass, power, aerobic capacity

INTRODUCTION

Futsal is a demanding team sport involving strenuous high-intensity bouts of running accelerations and decelerations along with kicking, tackling, turning, changes of direction, and repeated sprinting (1, 2). Although similar to football (soccer), futsal has different features i.e., unlimited number of substitutions, use of a smaller ball with less bounce, lower number of players (only four outfield players and one goalkeeper), smaller goals (with 2 × 3 m), shorter match duration (two equal periods of only 20 min with clock stoppage for fouls, etc.) (3, 4). Due to these specific features, research indicates that high-level futsal players require high levels of agility (5), muscle power (5–7), repeated sprint ability (2, 8, 9), jumping (10), and aerobic performance (3, 11). In fact, 5 to 10% of the distance covered during a futsal match is performed while sprinting (12–14), which when associated with other tasks (i.e., kicking, turning, etc.) requires high strength and power development of the lower limbs (15). Additionally, like other small-sided games, high level futsal requires elevated levels of aerobic capacity (VO_2max 55.2–62.8 ml.kg^{-1}.min^{-1}) (3).

Despite its growing popularity (12), research studies regarding dietary supplements in futsal athletes are scant. While evidence indicates that protein supplementation enhances muscle strength and hypertrophy, it may also play a role in muscle repair and recovery from endurance exercise (16), and further improve VO_2peak with endurance training (17). Current protein recommendations for endurance athletes and those engaged in team sports range from 1.2 to 2.0 g/kg BW.day^{-1} (18–20), with earlier recommendations for football (soccer) ranging between 1.4 and 1.7 g.kg BW.day^{-1} (21) and more recent recommendations indicating potential benefits from higher amounts 1.6–2.2 g/kg BW.day^{-1} (22).

To optimize muscle hypertrophy in conjunction with regimented resistance exercise training (RET), a protein should provide 6–15 g of essential amino acids per serving (23, 24), including 1.7–3.5 g of leucine (24, 25), with a total daily protein intake of ∼1.6 g/kg BW.day^{-1}; timing, dose, and protein source appear to play only a minor role in the process (16). Several research studies have compared whey protein with plant-based proteins such as rice (26, 27), pea (28), and soy (29, 30), with no differences in body composition or strength observed between groups. Conversely, other research has found superior results with whey or milk protein when compared to soy (31, 32). No research has been performed comparing a plant-based protein to whey regarding team sports, i.e., futsal. Thus, the purpose of this study was to compare supplementation with whey vs. a protein matrix combining multiple plant-based protein sources fortified with branched-chain amino acids (BCAA) (33) on measures of body composition, strength, power, and aerobic performance in futsal players. Given that both protein sources provided identical amounts of protein and essential amino acids, we hypothesized that changes would be similar between conditions.

METHODS

Ethics

This investigation was approved by the Faculty of Human Kinetics Institutional Review Board (approval number 37/2021) and conformed to all standards of human research set out in the declaration of Helsinki (34). The trial was registered at clinicaltrials.gov as NCT05228236 (https://clinicaltrials.gov/ct2/show/NCT05228236). Prior to engaging in any of the study procedures, the purpose and design of the study, the data collection methodologies, and all potential risks and benefits were explained to potential research participants. All participants gave their verbal and written informed consent before enrolling.

Participants

Fifty male futsal players, not engaged in RET, at the time of the investigation, volunteered to participate in this 8-week study. From the initial sample, forty completed the investigation. Participants were between the ages of 18 and 35 y and were recruited from national level futsal sports clubs. They were randomly assigned to one of two groups: novel plant-based protein (PB) or whey protein (WP). For details, please refer to the CONSORT flow diagram (**Figure 1**). Baseline measures were performed pre-season while wk 4 and 8 were assessed during the competitive season, in the first half of the regular phase of the championship.

The final sample was comprised of both professional and semi-professional players. The professional players ($n = 16$) participated in the Major Portuguese National Futsal League

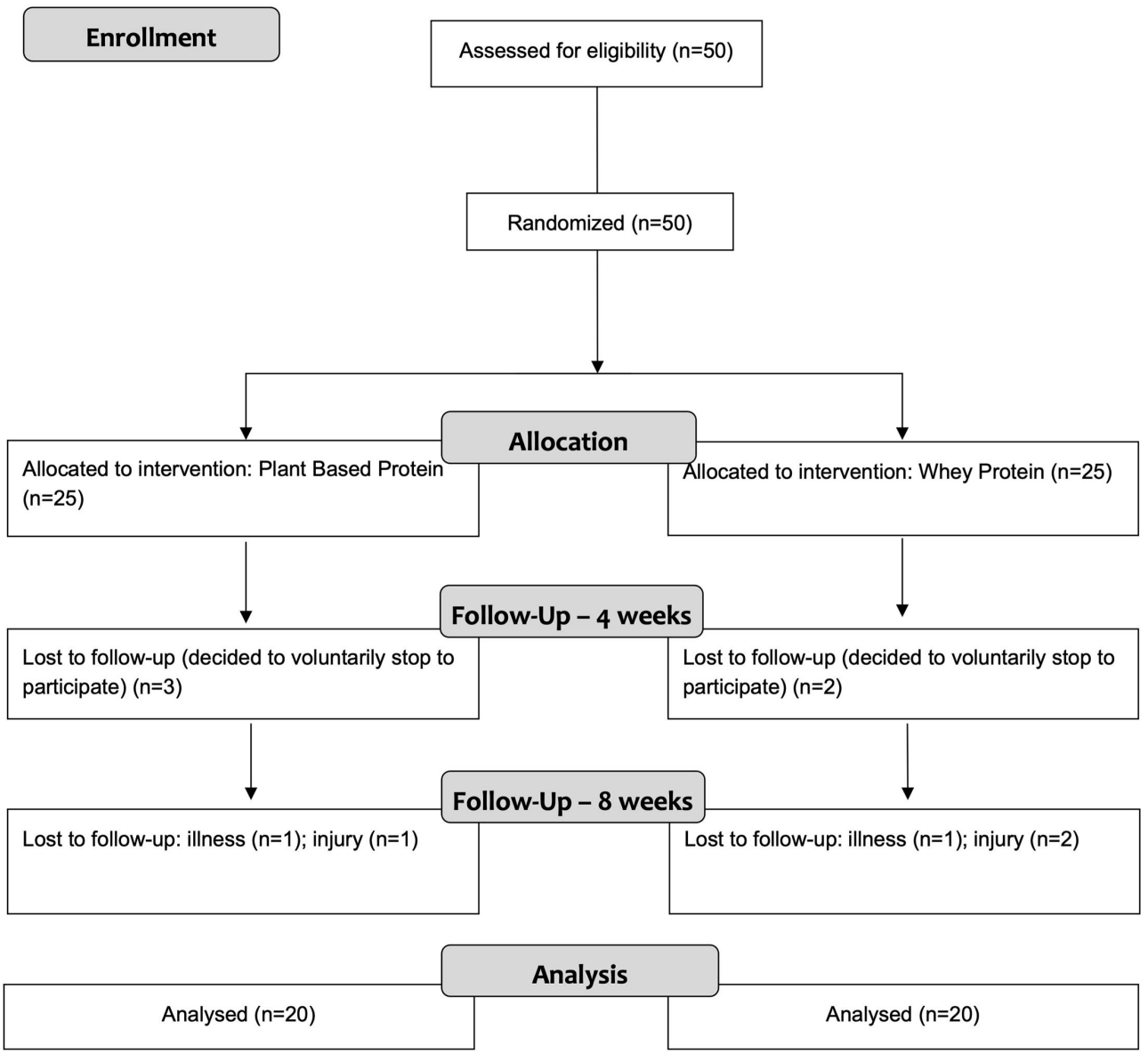


FIGURE 1 | CONSORT diagram of the randomization and flow of participants through the study.

"LIGA PLACARD" and had a sport-specific training frequency of 5 times per week (5×/wk) and a weekend game frequency of 1×/wk. Each training session lasted ∼1.5 h.

The semi-professional players ($n = 34$) participated in either the second ($n = 17$) or third ($n = 17$) Portuguese National Futsal League. Training sessions lasted ∼1.5 h for both teams and athletes had a training frequency of 3×/wk and a weekend game frequency of 1×/wk.

Participants represented the top five teams of each division and included players from the current and former national team. Although the players were from different teams, they had similar training schemes, where sport-specific training regimen was normally comprised of mobility and injury prevention exercises for ∼20 min, followed by technical and tactical components for ∼30 min. The remainder of the training session was dedicated to open exercises, mimicking game conditions. RET was not part of the coaches' training prescription at the initial phase of the season, nevertheless, three players reported that they occasionally went to the gym on their own to perform RET.

Sample Size and Study Design

Most previous research comparing plant-based whey proteins show similar effects on body composition and performance (26–28, 35), assuming equal amounts of protein and essential amino acids are provided. Anticipating similar results and bearing in mind that when there are no differences between the arms of

TABLE 1 | Supplementation nutritional composition.

	PB	WP
Dose per serving (g)	32	34
Energy (kcal)	130	135
Fat (g)	2.7	2.4
Saturated fats	0.6	1.5
Carbohydrates (g)	0.6	2.7
Sugars	<0.5	2.1
Protein (g)	25	24.8
Essential amino acids (g)		
Histidine	0.6	0.4
Isoleucine*	1.1	1.4
Leucine*	2.2	2.4
Lysine	1.7	1.8
Methionine	0.3	0.3
Phenylalanine	1.1	0.8
Threonine	0.9	1.2
Tryptophan	0.2	0.1
Valine*	1.1	1.0
∑ Essential Amino acids (g)	9.2	9.4
∑ BCAA* (g)	4.4	4.8

*BCAA, branched-chain amino acids; PB, plant-based protein; WP, whey protein.

the study, a significant effect size does not exist, we employed a non-inferiority trial. Nevertheless, the only study that found significant differences between a plant-based protein (soy) and whey protein reported an 80% power at an α-level of 5%, with a 0.5 kg difference in lean body mass (LBM) (32). Assuming similar conditions but 85% power, an alpha of 0.05, and a 0.5 effect size (36), GPower (version 3.1, Dusseldorf, Germany) calculated a required sample of 36 participants. Considering a 25% dropout, a sample of 48 participants would be required and we recruited 50 participants as a buffer against attrition.

Supplementation and Randomization Protocol

All protein powders presented a third-party certificate of analysis regarding their nutritional composition (**Table 1**) and were tested for doping-controlled substances (LGC group, Middlesex, UK). Additionally, the protein content of both supplements was re-assessed at our laboratory using a Dumatherm automatic Nitrogen analyzer (C. Gerhardt GmbH, Germany) according to the manufacturer's guidelines. More specifically, a sample within 90–140 mg was weighted in an aluminum foil (Dumafoil, C. Gerhardt GmbH, Germany) using an analytical scale (Kern model ABS 320-4N, GmbH, Germany). The sample was then compacted and inserted into the device. The results were analyzed considering a conversion factor of 6.25 (for PB) and 6.38 (for WP) per N gram, an O_2 factor of 1.8 mL/mg and a 300 ml/min of O_2 flow rate.

The PB supplement was comprised of a proprietary blend (BETTPRO®) containing pea protein isolate (85% PRO concentration), yeast protein (80% PRO concentration), and BCAA 4:1:1, currently, patent-pending (Bettery S.A., Oeiras, Portugal), while the WP supplement was comprised of a whey protein concentrate yielding an 80% protein concentration (Ewalco AB, Gothenburg, Sweden). The proprietary blend was mostly comprised of pea protein (>70%) with the yeast and BCAA included to complement and match the essential amino acid profile of whey. Both supplements required the addition of fat-reduced cocoa powder, emulsifiers, thickeners, and sweeteners to ensure similar taste and texture. The PB required the use of masking excipients to neutralize the typical flavor of the pea protein. Both supplements were weighted in single-dose (see **Table 1** for supplement composition) individual plastic bags using a digital scale model EMB 5200 G 5 (Kern, Balingen, Germany). The participants were instructed to dissolve the content of each bag within 250 mL of water. A 2-week supply was delivered to each player; a researcher collected the empty plastic bags to assess adherence while delivering a new supply. Participants received the packaged supplements and instructions in a double-blinded fashion. Supplementation compliance (a minimum of 90% compliance was deemed acceptable), possible side effects, and subjectively perceived fatigue and muscle recovery were assessed by questionnaire at the end of each 4-week block of supplementation.

Participants were randomized, within each competitive level, using a covariate adaptative randomization method based on age, grip strength, DXA-measured fat (FM), and LBM. Accordingly, at baseline, there were no statistically significant differences between groups regarding all the reported variables (**Tables 2**, **3**). The investigator responsible for the sample randomization and supplement distribution was not directly involved in participants' eligibility interviews or data collection. All other investigators and all the participants were blind to the supplementation conditions and randomization sequence.

Assessments

Athletes' anthropometry, body composition, strength, power, aerobic performance, nutritional intake, health, and safety biochemical parameters were assessed at baseline and week 8. A partial evaluation was performed in week 4 that included anthropometry, body composition, strength, power, and nutritional intake assessment. All evaluations of the participants, including blood and saliva samples collection, were performed in our laboratory facilities, early in the morning (7 a.m.) after a 12-h fast and without consumption of alcohol, caffeine/stimulant beverages, and at least 12 h from the last exercise session. Biofluids and body composition assessments were performed fasting, while the performance evaluations were made in a fed state, with the consumption of a meal replacement bar (nutritional composition: 231 kcal, 14 g fat, 12 g carbohydrate, and 13 g protein).

Anthropometry

Participants had their weight and height measured wearing minimal clothing and without shoes to the nearest 0.1 kg and 0.1 cm, respectively, with a scale and a stadiometer (Seca, Hamburg, Germany) using standardized procedures as reported elsewhere (37).

TABLE 2 | Energy and macronutrient intake at baseline and after 8 weeks of intervention.

	PB ($n = 20$)			WP ($n = 20$)		
	Baseline	**Post 4 weeks**	**Post 8 weeks**	**Baseline**	**Post 4 weeks**	**Post 8 weeks**
Energy (kcal)	2,057.3 ± 350.3	2,073.5 ± 309.9	2,064.2 ± 366.7	2,055.5 ± 242.1	2,093.5 ± 246.3	2,080.5 ± 255.0
Energy (kcal/kg FFM)	36.3 ± 8.1	36.1 ± 9.5	35.8 ± 7.1	36.1 ± 6.7	36.2 ± 6.0	37.1 ± 6.4
Protein (g)	113.6 ± 26.6	117.7 ± 21.5	121.1 ± 21.4	118.2 ± 27.2	118.6 ± 28.0	121.7 ± 20.9
Protein (g/kg BW.day^{-1})	1.6 ± 0.5	1.7 ± 0.2	1.7 ± 0.3	1.6 ± 0.5	1.7 ± 0.4	1.7 ± 0.4
Fat (g)	84.9 ± 17.4	84.6 ± 18.8	84.1 ± 16.3	85.5 ± 22.5	83.2 ± 15.0	84.2 ± 18.4
Carbohydrate (g)	203.9 ± 51.9	202.7 ± 54.4	198.8 ± 58.0	188.5 ± 32.9	201.5 ± 33.8	200.8 ± 38.2

BW, body weight; PB, plant-based protein; WP, whey protein.

TABLE 3 | Body composition, performance, and hematological and biochemical markers at baseline and after 4 and 8 weeks of intervention.

	PB ($n = 20$)			WP ($n = 20$)		
	Baseline	**Post 4 weeks**	**Post 8 weeks**	**Baseline**	**Post 4 weeks**	**Post 8 weeks**
Body composition						
Body mass (kg)	70.8 ± 9.2	70.5 ± 9.3	69.9 ± 9.0	71.6 ± 10.2	70.6 ± 7.7	69.7 ± 7.7
TBW (L)	43.1 ± 4 4	43.0 ± 4.3	42.9 ± 4.4	43.7 ± 4.3	43.1 ± 3.7	42.8 ± 4.0
Muscle thickness rectus femoris (mm)	26.7 ± 3 6	27.2 ± 2.7	28.0 ± 3.2	26.4 ± 3.4	27.0 ± 2.9	27.9 ± 2.7
Bone mineral content (kg)	2.93 ± 0.39	2.96 ± 0.38	2.97 ± 0.43	3.06 ± 0.45	3.06 ± 0.46	3.07 ± 0.42
Lean body mass (kg)	57.7 ± 5.8	57.9 ± 5.4	58.5 ± 6.3	58.0 ± 5.9	58.2 ± 5.3	58.9 ± 5.5
Lean soft tissue (kg)	54.6 ± 5.5	55.0 ± 5.1	55.3 ± 5.9	54.8 ± 5.5	55.1 ± 4.97	55.7 ± 5.1
Fat mass (kg)	13.1 ± 5.0	12.1 ± 5.0	11.4 ± 4.1$^{\#}$	13.6 ± 5.4	11.5 ± 3.5	10.9 ± 3.2$^{\#}$
Fat mass (%)	18.1 ± 4.9	17.6 ± 4.8	16.1 ± 4.2	18.5 ± 4.8	17.7 ± 3.7	15.4 ± 3.2
Visceral fat area (cm)	61.9 ± 21.5	59.6 ± 20.0	57.1 ± 21.3	57.1 ± 18.3	53.1 ± 11.9	51.2 ± 10.4
Muscle strength						
Handgrip dominant hand (N)	471.2 ± 91.2	483.4 ± 93.8	486.4 ± 89.3	461.3 ± 75.9	470.5 ± 74.0	475.4 ± 73.7
Back squat 1 RM (kg)	76.6 ± 14.3	78.4 ± 13.8	85.5 ± 15.6	80.2 ± 14.7	81.0 ± 13.8	85.9 ± 13.1
Bench press 1 RM (kg)	54.6 ± 13.0	55.3 ± 16.2	56.9 ± 9.1	56.8 ± 8.6	57.4 ± 9.4	59.1 ± 9.7
Counter movement jump (cm)	33.5 ± 4.2	34.7 ± 4.5	38.0 ± 4.7	33.5 ± 3.9	36.9 ± 4.0	38.7 ± 3.4
Anaerobic and aerobic performance						
Anaerobic peak power (W/kg)	12.9 ± 4.50	–	11.4 ± 3.2	12.4± 4.8	–	11.5 ± 4.2
Anaerobic average power (W/kg)	8.5 ± 1.0	–	8.4 ± 0.7	8.3 ± 0.9	–	8.1 ± 0.9
Anaerobic power drop (%)	62.7 ± 15.4	–	58.5 ± 11.1	64.0 ± 16.9	–	62.8 ± 14.0
VO_{2max} (mL/kg/min)	49.7 ± 7.1	–	50.6 ± 7.6	51.2 ± 7.0	–	51.1 ± 3.3
VO_{2max} (mL/min)	3,545.8 ± 436.8	–	3,544.8 ± 380.9	3,719.1 ± 410.8	–	3,582.7 ± 294.8
MAS (km/h)	16.0 ± 1.6	–	16.3 ± 1.9	15.0 ±1.9	–	16.2 ± 1.2
Hematological and biochemical markers						
C Reactive protein (mg/L)	10.0 ± 5.0	–	9.4 ± 1.2	8.9 ± 2.2	–	8.3 ± 0.7
Hematocrit (%)	44.2 ± 3.3	–	43.6 ± 2.6	44.4 ± 2.4	–	44.3 ± 2.4
Creatine kinase (U/L)	254.4 ± 126.3	–	201.0 ± 68.9	272.5 ± 114.1	–	195.5 ± 55.6
Alanine aminotransferase (U/L)	22.8 ± 15.4	–	19.0 ± 6.1	21.0 ± 9.6	–	20.2 ± 6.9
Aspartate aminotransferase (U/L)	25.3 ± 16.9	–	20.5 ± 6.9	19.3 ± 7.4	–	19.7 ± 5.9
Glucose (mg/dL)	77.8 ± 13.1	–	70.1 ± 11.6	76.6 ± 13.6	–	67.6 ± 12.4
Creatinine (mg/dL)	1.4 ± 0.2	–	1.5 ± 0.2	1.4 ± 0.3	–	1.5 ± 0.2
Estimated glomerular filtration rate (mL/min)	82.1 ± 17.3	–	80.8 ± 12.6	83.4 ± 20.2	–	79.6 ± 20.1
Salivary cortisol (μg/dL)	1.2 ± 0.6	–	1.2 ± 0.4	0.9 ± 0.6	–	0.7 ± 0.5

PB, plant-based protein; WP, whey protein; TBW, Total body water; MAS, maximal aerobic speed.
$^{\#}$*Time effect from baseline (ANCOVA).*
Results are presented as mean ± SD.

Body Composition

Body composition was determined by three methodologies:

(a) Dual energy X-ray absorptiometry (DXA) (Horizon Wi, Hologic, Waltham, USA) where participants underwent a whole-body DXA scan according to the procedures recommended by the manufacturer. The same technician positioned the patient, performed the scan, and executed the analyses. The DXA measurements included whole-body measurements of bone mineral content (BMC, g), LBM (kg), as well as absolute and percentage fat mass (FM, kg, and %). Within FM, visceral adipose tissue was distinguished using DXA software (38).

(b) B-mode ultrasonography for muscle thickness (MT) measurement of the rectus femoris (RF) (39) using a 7.5 MHz linear-array transducer (model WED-180 HL, Welld, Shenzhen, China). Longitudinal and transversal scans were obtained at the muscles' mid-belly at 56% of the distance from the proximal edge of the patella to the anterior superior iliac spine (40). Participants were positioned in a seated position with their knees flexed at 90° (0° being a full extension), participants' legs were supported during the scan and their muscles relaxed. To ensure that repeated scans (weeks 4 and 8) were taken from the same site, scanning locations were mapped with a malleable transparent plastic sheet at the baseline measurement, along with other distinguishing surface landmarks (e.g., border of the patella, tattoos, scars, moles). We defined MT as the perpendicular distance between the subcutaneous adipose tissue-muscle interface and intermuscular interface. Averaged values from three measurements were considered for further analysis. All measures were collected and digitally analyzed by the same operator who was blinded to group allocation. The test-retest CV in 29 participants for the MT in RF using ultrasonography in our laboratory is 2.1%.

(c) Bioelectrical impedance analysis (BIA) is a phase sensitive device, from which whole-body resistance (R) and reactance (Xc) are obtained using a single frequency of 50 kHz (BIA 101 BIVA®PRO, Akern S.R.L., Pisa, Italy). Device calibration was performed according to the procedures recommended by the manufacturer. Assessments were obtained after a 10-min rest period in a supine position following the guidelines for athletes stated elsewhere (41). From the raw data R and Xc, total body water (TBW) was determined using Akern Software (version 1.19.2). The test-retest CV in 15 participants for R and Xc in our laboratory is 3.5 and 1.5%, respectively.

Hematological and Biochemical Analysis

Saliva and whole blood were collected into salivettes and EDTA tubes by standard procedures. Blood measurements included the assessment of glucose, hematocrit (Hct), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and creatinine, using photometry techniques in automated equipment (Vario Photometer II DP310, Diaglobal Gmbh, Berlin, Germany; Nycocard reader II, Abbott, Chicago, Illinois, EUA). Creatinine values were used to calculate the estimated glomerular filtration rate (eGFR) (42). Cortisol was evaluated in saliva through Enzyme-Linked Immunosorbent Assay (ELISA) commercial kits (Salimetrics, PA, USA) in an automated reader (800 TS Absorbance Reader, Biotek, Vermont, USA).

Performance Tests

Muscle Strength

Maximal isometric handgrip strength of the dominant hand was determined using a Jamar® hydraulic hand dynamometer (Jamar, Sammons Preston, Inc, Bolingbrook, IL). The participants were tested in a standing position with an elbow in full extension (43). The participant was asked to squeeze the dynamometer at maximal effort for three trials, with a 30-s break between each trial. The best of the three trials was considered for data analysis.

Maximum strength (1-RM) was predicted based on load-velocity relationship for the back squat and bench press exercises on a Multipower machine. For both back squat and bench press, participants performed one set of 8 repetitions at a self-selected load, having been instructed to perform the concentric phase as fast as possible. A linear encoder (Vitruve Encoder, Madrid, Spain) was attached to the barbell during the tests, measuring its vertical displacement and velocity. The higher mean propulsive velocity along the 8 repetitions was used to predict the 1-RM (44). The determination of 1RM was directly supervised by an NSCA-certified strength and conditioning specialist.

Jump height was assessed by a countermovement jump which was performed on a contact-mat jump system controlled by an open-source hardware and software model (Chronojump, Barcelona, Spain). The displacement of center of gravity (jump height h) during the flight was estimated by means of flight time (t) through a standardized kinematic equation $h = t2.g/8$, where g is gravity (45). The best attempt out of three was considered for analysis.

Participants were familiarized with the muscle strength tests prior to our investigation.

Anaerobic and Aerobic Performance

Anaerobic power performance was assessed *via* a supramaximal cycling test—Wingate, performed on a cycle ergometer (Monark ergomedic 894 E, Monark Exercise AB, Vansbro, Sweden). Participants were instructed to cycle as fast as possible against a predetermined resistance (7.5% of the participant's body mass)for 30 s (46). Variables collected at the end of the test included anaerobic peak power, anaerobic average power, and anaerobic drop power, calculated as the percentage drop between peak and minimum power of the test.

Aerobic performance was assessed *via* VO_{2max} and maximal aerobic speed (MAS), determined by a breath-by-breath gas analyzer (Quark, Cosmed, Italy) in an incremental treadmill test. After a 3-min warm-up at 5 $km \cdot h^{-1}$, participants began the test at 6 $km \cdot h^{-1}$ and 2% grade. Each minute the speed increased 1 $km \cdot h^{-1}$ until volitional exhaustion so that fatigue would be induced within 8–12 min (47). Standardized verbal encouragement was given throughout the test. The MAS was considered as the speed of the last stage completed (48) while VO_{2max} was considered the highest 30 s average value of VO_2 and assumed when at least 2 of the following criteria were met: respiratory quotient >1.10; heart rate (HR) equal to or >95%

of predicted maximal HR (calculated as 208–0.7× age); and increments in VO_2 below 2 ml·kg^{-1} · min^{-1} despite an increase in speed (49).

Diet Control and Supplementation

Three-day food records (2 weekdays and 1 weekend day) were requested to characterize the food intake of the participants at baseline, weeks 4 and 8. Individuals were instructed to maintain their normal dietary intake during the 8-week study period. Food records were then analyzed by software [Nutritics Research Edition (v5.09), Dublin, Ireland] for total energy and macronutrient consumption, by a registered dietitian.

After evaluations, each participant received either PB or WP. Participants were only aware that these were protein supplements and that compounds were distributed in a double-blinded manner. Both groups consumed the protein supplements 30–60 min after exercise on training days and 30–60 min before bedtime on non-training days.

Statistical Analysis

IBM SPSS Statistics version 25.0, 2012 (IBM, Chicago, Illinois, USA) was used to analyze the data. Basic descriptive statistics were run to characterize the study participants. All variables were assessed for normality using the Kolmogorov–Smirnov test. Independent sample *T*-tests were used to compare means between groups at baseline. *Post-hoc* intention to treat (ITT) analysis was applied to investigate the effects of intervention over time within and between groups through generalized estimated equations (GEE) analyses. Time and time-by-group interactions were analyzed by analysis of covariance (ANCOVA), using baseline measurements as the covariate. The equality of the matrix of variance and sphericity were explored with the Levene F-test and Mauchly's test, respectively. Significance for α was set at $p \leq 0.05$.

RESULTS

Data from 40 participants were considered for final analysis (PB = 20, WP=20). Sample losses were due to personal reasons ($N = 5$) or due to injury during practice ($N = 5$), therefore an intervention adherence of 80% was observed. No differences were observed between groups at baseline nor after 8 weeks of supplementation. Analysis of food records showed no differences between groups at baseline nor pre- to post-study regarding energy and macronutrient intake (**Table 2**).

Participants were compliant with the supplementation, taking 93 ± 1% of supplements with no adverse effects reported throughout the study. No differences were found between groups regarding general perceived side effects of the supplementation. Additionally, perceived gastrointestinal disturbances were 18% higher in the WP group.

No differences were observed between conditions at baseline regarding all variables (see **Table 3**; **Figures 2**, **3**). Moreover, no differences were observed between groups from baseline to week 8 ($p > 0.05$). No time*group interaction was observed in any of the studied variables, and a time effect was only observed for a reduction in FM. Similar results were observed when the analysis was carried out using ITT.

DISCUSSION

Body Composition

We found no differences between groups regarding all body composition variables when comparing PB and WP in professional and semi-professional futsal athletes. These findings are in line with previous populations (26–30, 35) but not with others (31, 32, 50, 51). Of note, a study by Babault et al. (28) that compared pea protein with whey used 25 g 2 times a day (50 g daily) on untrained subjects. Compared to the present study, this investigation (28) was of longer duration (12 wks), used only three exercises (involving elbow flexor and extensor muscles), and involved a higher daily protein supplementation in untrained men. Moreover, Babault et al. (28) did not control the dietary intake and the pea protein supplement offered a lower amount of leucine per serving than in our study (1.6 vs. 2.2 g). Another study compared pea protein with whey protein, in trained subjects (35), performed a combination of high-intensity functional training (HIFT) and no differences were found between groups after 8 wks of HIFT, with only a time effect being reported regarding 1-RM squat and deadlift. Limitations in that study included a small sample size ($n = 15$) and the use of a BIA model with questionable accuracy (52, 53).

Studies from Joy et al. (26) and Moon et al. (27) that compared rice protein with whey protein also presented several limitations. As acknowledged by the authors, sample sizes were relatively small ($n = 24$) and the Moon et al. (27) work lacked training supervision. Moreover, neither study employed a direct measure of hypertrophy, which may have limited the ability to detect subtle longitudinal changes in muscle mass (54). The sample in our study was comprised of professional and semi-professional futsal players without RET exposure, which explains the absence of a time effect pertaining MT and fat free mass (FFM) gains, even when consuming at least 1.6 g/kg BW.day^{-1} of protein (16). Still, the FFM gains in both groups are in line with the values reported in some meta-analysis (16, 55) and with the works by Joy et al. (26) and Moon et al. (27) using rice protein. Our results pertaining to MT are also consistent with the work by Banazsek et al. (35), who found no differences between pea protein and whey protein after 8 wks of HIFT.

In contrast, Volek et al. (32) reported superior increases in FFM favoring WP vs. soy protein in untrained subjects. It should be noted that the supplements used by Volek et al. (32) also provided carbohydrates and the soy protein supplement provided a lower amount of leucine than the PB in our study (1.4 vs. 2.2 g). Furthermore, the amount of leucine provided from both supplements in Volek et al. (32) differed greatly (1.4 g of leucine in the soy protein group vs. 2.2 g in the WP), which is particularly important given the recommended leucine dosage for maximizing muscle protein synthesis (1.7–3.5 g per meal) (24, 25). Other studies that reported conflicting findings with ours did not compare isolated protein sources (50), and assessed dairy protein vs. soy protein in elderly participants (31).

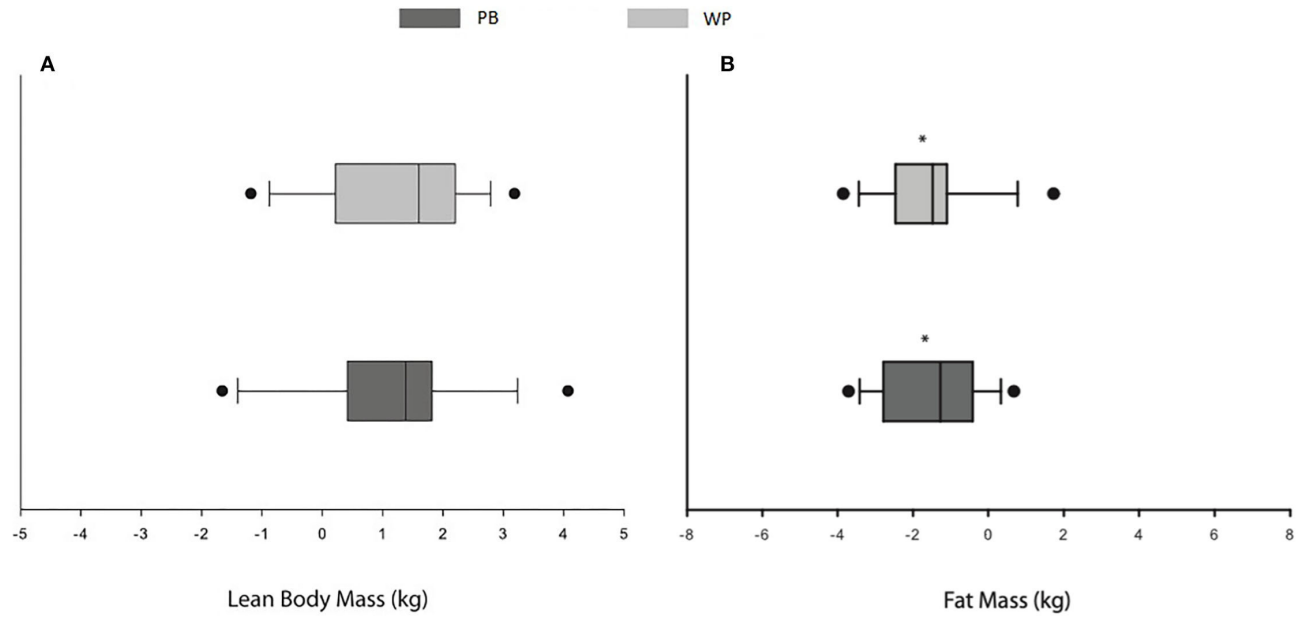


FIGURE 2 | Changes (Δ baseline-week 8) in lean body mass **(A)**, fat mass **(B)** during the 8-week supplementation protocol. Data are shown as box and whisker plots where whiskers are the maximum and minimum and the box represents the interquartile range, the line the group median. Dots represent outliers. *Represents an observed time effect. PB, plant-based protein; WP, whey protein.

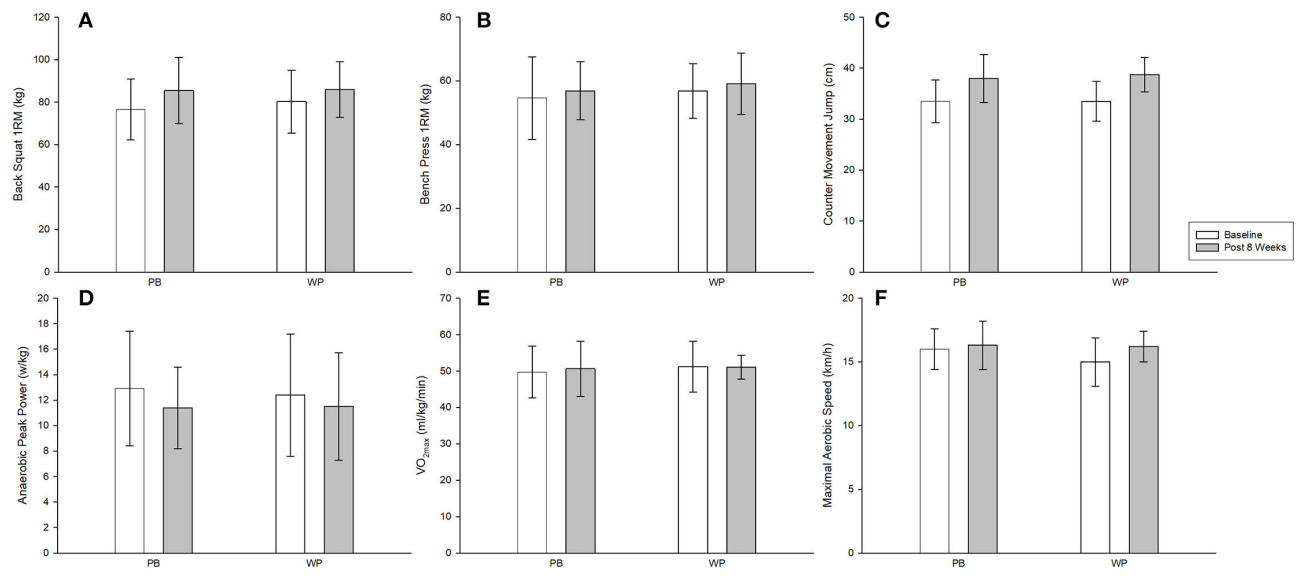


FIGURE 3 | Performance markers at baseline and post 8 weeks: back squat **(A)**, bench press **(B)**, counter movement jump **(C)**, anaerobic peak power **(D)**, VO2max **(E)** and maximal aerobic speed **(F)**. PB, plant-based protein; WP, whey protein.

In our study, a time effect was noted regarding FM loss, with no differences between groups. These results are in agreement with some studies (26, 27, 32) but not others (28, 35). This might be explained by the reduced energy availability in both arms of our study (PB: 35.8 ± 7.1; WP: 37.1 ± 6.4 kcal/kg FFM), which seems more appropriate for fat loss (56). Moreover, research indicates a higher protein intake might mitigate FFM loss during periods of energy restriction (57, 58), which might explain the FM reduction while maintaining FFM. Taken together, the absence of a RET protocol and the reduced energy availability in our trial might explain the lack of a time effect pertaining to FFM gains and the reduction of FM in both conditions. Furthermore, since the dietary intake did not change throughout the study, one might speculate that a natural increase pertaining to in-season

training volume might have led to the reported FM reduction in both groups.

Muscle Strength

No differences were found between groups regarding both muscle strength markers, nor did we observe a time effect. These results are in contrast with protein supplement research performed in combination with RET (26–28, 32, 35). Since our sample was comprised of futsal players not engaged in RET, these results are not surprising. A meta-analysis by Morton et al. (16), found that protein supplementation has little effect on strength when compared to RET when individuals consume >1.6 g/kg BW.day^{-1} of protein. Similar results have been reported regarding protein supplementation on muscle force production with both acute and chronic concurrent training (59). Still, when comparing our results with the study from Banazsek et al. (35), which used a sample closer to ours (HIFT), our results are in line with all findings except for 1-RM. It could be hypothesized that protein supplementation might improve performance *via* enhanced recovery. However, a recent study showed no differences between a high intake of pea protein or WP when compared with water intake on post-exercise delayed onset muscle soreness (60), casting doubt on the hypothesis.

Anaerobic and Aerobic Performance

No between-group differences or time effects were found regarding anaerobic or aerobic performance. Few studies have investigated the effects of protein supplementation on anaerobic (59) and aerobic performance (61–63). Regarding protein supplementation on muscle power Camera et al. (59) showed similar results to ours. Also, it seems that when adequate amounts of carbohydrates are consumed, protein supplementation does not further increase aerobic performance (24). Moreover, our results agree with a recent systematic review showing that protein supplementation increases myofibrillar but not mitochondrial protein synthesis (64), consequently failing to enhance whole-body aerobic power (i.e., VO_{2max}).

Hematological and Biochemical Markers

No effects of time or group were detected regarding all hematological and biochemical markers. Only one previous study by Nieman et al. (60) compared pea protein vs. WP after a 90-min eccentric exercise bout regarding proxy markers of muscle damage (CK) in non-athletic, non-obese males. Results showed that WP delayed the elevation of CK to a greater extent than pea protein or water. These results conflict with ours since we did not find any differences between groups. Discrepancies in findings can potentially be explained by the fact that we studied a different population (trained futsal players) and did not include a specific muscle damage-inducing protocol. Moreover, we used a novel protein/BCAA matrix that combined not only pea protein but also yeast protein and BCAA as well, yielding an equal amino acid profile to whey protein.

Regarding ALT and AST (proxy markers of liver health) our null findings align with those of Nieman et al. (60). The lack of changes in eGFR and creatinine in our study is consistent with a plethora of research showing that relatively high protein diets do not have negative effects on renal health in healthy individuals (65–67) or even in overweight/obese individuals with mild kidney function impairment (68). In brief, both proteins displayed a good safety profile after 8 wks of supplementation, with no changes in inflammatory markers (CRP), red blood cell profile (Hct), liver (ALT/AST), glucose or kidney function (creatinine, eGFR), being observed between groups.

Strengths and Limitations

This is the first study to compare a plant-based protein vs. whey in professional and semi-professional team sports athletes (i.e., futsal). Few (or no) studies have compared the effects of a protein source when provided as supplemental protein outside RET or strength/power sports (61–63), with only one comparative study being performed in collegiate female basketball players (whey vs. casein) (69). Furthermore, we supplemented with an iso-energetic, iso-nitrogenous, iso-EAA novel plant-based protein/BCAA mixture that matched whey protein, using yeast protein for the first time. Moreover, we assessed not only body composition using multiple gold standard methods but also anaerobic and aerobic performance, as well as several hematological and biochemical markers, thus providing a complete longitudinal view of the effects of both proteins. While this study provides novel insights, it nevertheless presents some limitations. For one, the inclusion of a placebo/control group would have added important information to the findings. In addition, we did not assess the effectiveness of the blinding and thus cannot be completely sure that there was not a residual placebo effect. Additionally, the participants spontaneously reduced their protein intake from other sources, when supplementing with either protein. Albeit not interfering with their usual diet adds ecological validity to our study, it precludes any conclusions from an increase in dietary protein. Moreover, extending the study length to 12 wks or more and employing a RET protocol in both groups would have provided additional insights into the effects of the proteins on measures of strength and body composition. It is also important to bear in mind that DXA, as well as BIA-derived parameters, have different instrumental sensitivities thus not allowing for direct comparisons between studies that measure R, Xc, and/or water compartments with different technologies or sampling frequencies (70, 71). As previously discussed, although not changing the diet of the participants adds ecological validity to this study, this might have influenced some performance outcomes (i.e., aerobic capacity) due to reduced energy and carbohydrate intake.

CONCLUSION

In summary, supplementing with either a novel plant-based protein matrix or whey protein did not affect any of the variables assessed in high-level futsal players over 8 wks. These results suggest that whey protein does not possess any unique anabolic properties over and above those of plant-based proteins when equated for essential amino acid profile in the studied population. Furthermore, when consuming a daily protein intake >1.6 g/kg BW.day^{-1}, additional protein supplementation does not affect

body composition or performance in trained futsal players, regardless of protein type/source.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the portuguese data protection law does not allow to share data of the participants. The database can only be accessed by the investigation team. Requests to access the datasets should be directed to filipe.teixeira@betterylife.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Faculty of Human Kinetics Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FJT, CNM, CPM, JFR, MJV, and VHT: substantial contributions to the conception and design of the work. FJT, CNM, JF, RG, JP, HF, RC, and BJS: acquisition, analysis, and/or interpretation of data for the work. BJS: statistical expertise. FT and CNM: significant manuscript writer. FJT, CNM, CPM, JFR, MJV, VHT, JF, RG, JP, HF, RC, and BJS: manuscript revising critically for important intellectual content and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

FUNDING

The publishing cost of this work was supported by the Interdisciplinary Center for the Study of Human Performance—CIPER (Unit 447) under grant UIDB/00447/2020.

ACKNOWLEDGMENTS

A special thank you to the athletes and technical team that took time out of their busy schedules to participate. This work was dedicated to the memory of Prof. Kevin D. Tipton who recently passed away. Dr. Tipton was a pioneer in protein research and a soccer fan, his contributions to the field will live on eternally.

REFERENCES

1. Makaje N, Ruangthai R, Arkarapanthu A, Yoopat P. Physiological demands and activity profiles during futsal match play according to competitive level. *J Sports Med Phys Fitness.* (2012) 52:366–74.
2. Torres-Torrelo J, Rodríguez-Rosell D, Mora-Custodio R, Pareja-Blanco F, Yañez-García JM, González-Badillo JJ. Effects of resistance training and combined training program on repeated sprint ability in futsal players. *Int J Sports Med.* (2018) 39:517–26. doi: 10.1055/a-0596-7497
3. Alvarez JC, D'Ottavio S, Vera JG, Castagna C. Aerobic fitness in futsal players of different competitive level. *J Strength Cond Res.* (2009) 23:2163–6. doi: 10.1519/JSC.0b013e3181b7f8ad
4. López-Segovia M, Vivo Fernández I, Herrero Carrasco R, Pareja Blanco F. Preseason injury characteristics in Spanish professional futsal players: the LNFS project. *J Strength Cond Res.* (2022) 36:232–7. doi: 10.1519/JSC.0000000000003419
5. Ramos-Campo DJ, Rubio-Arias JA, Carrasco-Poyatos M, Alcaraz PE. Physical performance of elite and subelite Spanish female futsal players. *Biol Sport.* (2016) 33:297–304. doi: 10.5604/20831862.1212633
6. Vieira LH, de Souza Serenza F, de Andrade VL, de Paula Oliveira L, Mariano FP, Santana JE, et al. Kicking performance and muscular strength parameters with dominant and nondominant lower limbs in brazilian elite professional futsal players. *J Appl Biomech.* (2016) 32:578–85. doi: 10.1123/jab.2016-0125
7. Ribeiro JN, Gonçalves B, Coutinho D, Brito J, Sampaio J, Travassos B. Activity profile and physical performance of match play in elite futsal players. *Front Psychol.* (2020) 11:1709. doi: 10.3389/fpsyg.2020.01709
8. Naser N, Ali A, Macadam P. Physical and physiological demands of futsal. *J Exerc Sci Fit.* (2017) 15:76–80. doi: 10.1016/j.jesf.2017.09.001
9. Illa J, Fernandez D, Reche X, Serpiello FR. Positional differences in the most demanding scenarios of external load variables in elite futsal matches. *Front Psychol.* (2021) 12:625126. doi: 10.3389/fpsyg.2021.625126
10. Paz-Franco A, Rey E, Barcala-Furelos R. Effects of 3 different resistance training frequencies on jump, sprint, and repeated sprint ability performances in professional futsal players. *J Strength Cond Res.* (2017) 31:3343–50. doi: 10.1519/JSC.0000000000001766
11. Baroni BM, Leal Junior EC. Aerobic capacity of male professional futsal players. *J Sports Med Phys Fitness.* (2010) 50:395–9.
12. Barbero-Alvarez JC, Soto VM, Barbero-Alvarez V, Granda-Vera J. Match analysis and heart rate of futsal players during competition. *J Sports Sci.* (2008) 26:63–73. doi: 10.1080/02640410701287289
13. Castagna C, D'Ottavio S, Granda Vera J, Barbero Alvarez JC. Match demands of professional Futsal: a case study. *J Sci Med Sport.* (2009) 12:490–4. doi: 10.1016/j.jsams.2008.02.001
14. De Oliveira Bueno MJ, Caetano FG, Pereira TJ, De Souza NM, Moreira GD, Nakamura FY, et al. Analysis of the distance covered by Brazilian professional futsal players during official matches. *Sports Biomech.* (2014) 13:230–40. doi: 10.1080/14763141.2014.958872
15. Meylan C, Malatesta D. Effects of in-season plyometric training within soccer practice on explosive actions of young players. *J Strength Cond Res.* (2009) 23:2605–13. doi: 10.1519/JSC.0b013e3181b1f330
16. Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med.* (2018) 52:376–84. doi: 10.1136/bjsports-2017-097608
17. Lin YN, Tseng TT, Knuiman P, Chan WP, Wu SH, Tsai CL, et al. Protein supplementation increases adaptations to endurance training: a systematic review and meta-analysis. *Clin Nutr.* (2021) 40:3123–32. doi: 10.1016/j.clnu.2020.12.012
18. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine Joint Position Statement. *Nutr Athlet Perform Med Sci Sports Exer.* (2016) 48:543–68. doi: 10.1249/MSS.0000000000000852
19. Tarnopolsky M. Protein requirements for endurance athletes. *Nutrition.* (2004) 20:662–8. doi: 10.1016/j.nut.2004.04.008
20. Packer JE, Wooding DJ, Kato H, Courtney-Martin G, Pencharz PB, Moore DR. Variable-intensity simulated team-sport exercise increases daily protein requirements in active males. *Front Nutr.* (2017) 4:64. doi: 10.3389/fnut.2017.00064
21. Lemon PW. Protein requirements of soccer. *J Sports Sci.* (1994) 12:S17–22. doi: 10.1080/02640414.1994.12059274
22. Collins J, Maughan RJ, Gleeson M, Bilsborough J, Jeukendrup A, Morton JP, et al. UEFA expert group statement on nutrition in elite football. *Curr Evid Inform Pract Recommend Guide Fut Res.* (2021) 55:416. doi: 10.1136/bjsports-2019-101961

23. Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR, Kevin D, et al. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol.* (1999) 276 (4 Pt 1):E628–34. doi: 10.1152/ajpendo.1999.276.4.E628
24. Jäger R, Kerksick CM, Campbell BI, Cribb PJ, Wells SD, Skwiat TM, et al. International Society of sports nutrition position stand: protein and exercise. *J Int Soc Sports Nutr.* (2017) 14:20. doi: 10.1186/s12970-017-0177-8
25. Moore DR. Maximizing post-exercise anabolism: the case for relative protein intakes. *Front Nutr.* (2019) 6:147. doi: 10.3389/fnut.2019.00147
26. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Wilson SM, et al. The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J.* (2013) 12:86. doi: 10.1186/1475-2891-12-86
27. Moon JM, Ratliff KM, Blumkaitis JC, Harty PS, Zabriskie HA, Stecker RA, et al. Effects of daily 24-gram doses of rice or whey protein on resistance training adaptations in trained males. *J Int Soc Sports Nutr.* (2020) 17:60. doi: 10.1186/s12970-020-00394-1
28. Babault N, Paizis C, Deley G, Guerin-Deremaux L, Saniez MH, Lefranc-Millot C, et al. Pea proteins oral supplementation promotes muscle thickness gains during resistance training: a double-blind, randomized, Placebo-controlled clinical trial vs. Whey protein. *J Int Soc Sports Nutr.* (2015) 12:3. doi: 10.1186/s12970-014-0064-5
29. Denysschen CA, Burton HW, Horvath PJ, Leddy JJ, Browne RW. Resistance training with soy vs whey protein supplements in hyperlipidemic males. *J Int Soc Sports Nutr.* (2009) 6:8. doi: 10.1186/1550-2783-6-8
30. Mobley CB, Haun CT, Roberson PA, Mumford PW, Romero MA, Kephart WC, et al. Effects of whey, soy or leucine supplementation with 12 weeks of resistance training on strength, body composition, and skeletal muscle and adipose tissue histological attributes in college-aged males. *Nutrients.* (2017) 9:972. doi: 10.3390/nu9090972
31. Thomson RL, Brinkworth GD, Noakes M, Buckley JD. Muscle strength gains during resistance exercise training are attenuated with soy compared with dairy or usual protein intake in older adults: a randomized controlled trial. *Clin Nutr.* (2016) 35:27–33. doi: 10.1016/j.clnu.2015.01.018
32. Volek JS, Volk BM, Gomez AL, Kunces LJ, Kupchak BR, Freidenreich DJ, et al. Whey protein supplementation during resistance training augments lean body mass. *J Am Coll Nutr.* (2013) 32:122–35. doi: 10.1080/07315724.2013.793580
33. van Vliet S, Burd NA, van Loon LJ. The skeletal muscle anabolic response to plant- versus animal-based protein consumption. *J Nutr.* (2015) 145:1981–91. doi: 10.3945/jn.114.204305
34. World Medical A. World medical association declaration of helsinki: ethical principles for medical research involving human subjects. *JAMA.* (2013) 310:2191–4. doi: 10.1001/jama.2013.281053
35. Banaszek A, Townsend JR, Bender D, Vantrease WC, Marshall AC, Johnson KD. The effects of whey vs. pea protein on physical adaptations following 8-weeks of high-intensity functional training (HIFT): a pilot study. *Sports.* (2019) 7:12. doi: 10.3390/sports7010012
36. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Academic Press (1969).
37. Lohman T, Roche A, Martorell R. *Anthropometric Standardization Reference Manual*. Champaign, IL: Human Kinetics Publishers (1988).
38. Gadekar T, Dudeja P, Basu I, Vashisht S, Mukherji S. Correlation of visceral body fat with waist-hip ratio, waist circumference and body mass index in healthy adults: A cross sectional study. *Med J Armed Forces India.* (2020) 76:41–6. doi: 10.1016/j.mjafi.2017.12.001
39. Teixeira FJ, Matias CN, Monteiro CP, Valamatos MJ, Reis JF, Tavares F, et al. Leucine metabolites do not enhance training-induced performance or muscle thickness. *Med Sci Sports Exerc.* (2019) 51:56–64. doi: 10.1249/MSS.0000000000001754
40. Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed *in vivo*. *J Anat.* (2006) 209:289–310. doi: 10.1111/j.1469-7580.2006.00619.x
41. Campa F, Toselli S, Mazzilli M, Gobbo LA, Coratella G. Assessment of body composition in athletes: a narrative review of available methods with special reference to quantitative and qualitative bioimpedance analysis. *Nutrients.* (2021) 13:1620. doi: 10.3390/nu13051620
42. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* (1976) 16:31–41. doi: 10.1159/000180580
43. Spruit MA, Sillen MJ, Groenen MT, Wouters EF, Franssen FM. New normative values for handgrip strength: results from the UK Biobank. *J Am Med Direct Assoc.* (2013) 14:775 e5-11. doi: 10.1016/j.jamda.2013.06.013
44. González-Badillo JJ, Sánchez-Medina L. Movement velocity as a measure of loading intensity in resistance training. *Int J Sports Med.* (2010) 31:347–52. doi: 10.1055/s-0030-1248333
45. Pueo B, Penichet-Tomas A, Jimenez-Olmedo JM. Reliability and validity of the Chronojump open-source jump mat system. *Biol Sport.* (2020) 37:255–9. doi: 10.5114/biolsport.2020.95636
46. Maud PJ, Shultz BB. Norms for the Wingate anaerobic test with comparison to another similar test. *Res Q Exerc Sport.* (1989) 60:144–51. doi: 10.1080/02701367.1989.10607429
47. Buchfuhrer MJ, Hansen JE, Robinson TE, Sue DY, Wasserman K, Whipp BJ. Optimizing the exercise protocol for cardiopulmonary assessment. *J Appl Physiol Respir Environ Exerc Physiol.* (1983) 55:1558–64. doi: 10.1152/jappl.1983.55.5.1558
48. Berthoin S, Pelayo P, Lensel-Corbeil G, Robin H, Gerbeaux M. Comparison of maximal aerobic speed as assessed with laboratory and field measurements in moderately trained subjects. *Int J Sports Med.* (1996) 17:525–9. doi: 10.1055/s-2007-972889
49. Howley ET, Bassett DR Jr., Welch HG. Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc.* (1995) 27:1292–301. doi: 10.1249/00005768-199509000-00009
50. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, et al. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *Am J Clin Nutr.* (2007) 86:373–81. doi: 10.1093/ajcn/86.2.373
51. Wilkinson SB, Tarnopolsky MA, Macdonald MJ, Macdonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. *Am J Clin Nutr.* (2007) 85:1031–40. doi: 10.1093/ajcn/85.4.1031
52. Matias CN, Campa F, Santos DA, Lukaski H, Sardinha LB, Silva AM. Fat-free mass bioelectrical impedance analysis predictive equation for athletes using a 4-compartment model. *Int J Sports Med.* (2021) 42:27–32. doi: 10.1055/a-1179-6236
53. Campa F, Gobbo LA, Stagi S, Cyrino LT, Toselli S, Marini E, et al. Bioelectrical impedance analysis versus reference methods in the assessment of body composition in athletes. *Eur J Appl Physiol.* (2022) 122:561–89. doi: 10.1007/s00421-021-04879-y
54. Phillips SM. The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab.* (2016) 13:64. doi: 10.1186/s12986-016-0124-8
55. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr.* (2012) 96:1454–64. doi: 10.3945/ajcn.112.037556
56. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. *J Sports Sci.* (2011) 29:S7–15. doi: 10.1080/02640414.2011.588958
57. Walberg JL, Leidy MK, Sturgill DJ, Hinkle DE, Ritchey SJ, Sebolt DR. Macronutrient content of a hypoenergy diet affects nitrogen retention and muscle function in weight lifters. *Int J Sports Med.* (1988) 9:261–6. doi: 10.1055/s-2007-1025018
58. Longland TM, Oikawa SY, Mitchell CJ, Devries MC, Phillips SM. Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial. *Am J Clin Nutr.* (2016) 103:738–46. doi: 10.3945/ajcn.115.119339
59. Camera DM. Evaluating the effects of increased protein intake on muscle strength, hypertrophy and power adaptations with concurrent training: a narrative review. *Sports Med.* (2021) 52:441–61. doi: 10.1007/s40279-021-01585-9
60. Nieman DC, Zwetsloot KA, Simonson AJ, Hoyle AT, Wang X, Nelson HK, et al. Effects of whey and pea protein supplementation on post-eccentric exercise muscle damage: a randomized trial. *Nutrients.* (2020) 12:2382. doi: 10.3390/nu12082382

61. Macdermid PW, Stannard SR. A whey-supplemented, high-protein diet versus a high-carbohydrate diet: effects on endurance cycling performance. *Int J Sport Nutr Exerc Metab.* (2006) 16:65–77. doi: 10.1123/ijsnem.16.1.65
62. D'Lugos AC, Luden ND, Faller JM, Akers JD, McKenzie AI, Saunders MJ. Supplemental protein during heavy cycling training and recovery impacts skeletal muscle and heart rate responses but not performance. *Nutrients.* (2016) 8:550. doi: 10.3390/nu8090550
63. Knuiman P, van Loon LJC, Wouters J, Hopman M, Mensink M. Protein supplementation elicits greater gains in maximal oxygen uptake capacity and stimulates lean mass accretion during prolonged endurance training: a double-blind randomized controlled trial. *Am J Clin Nutr.* (2019) 110:508–18. doi: 10.1093/ajcn/nqz093
64. Hartono FA, Martin-Arrowsmith PW, Peeters WM, Churchward-Venne TA. The effects of dietary protein supplementation on acute changes in muscle protein synthesis and longer-term changes in muscle mass, strength, and aerobic capacity in response to concurrent resistance and endurance exercise in healthy adults: a systematic review. *Sports Med.* (2022) 52:1295–328. doi: 10.1007/s40279-021-01620-9
65. Antonio J, Ellerbroek A, Silver T, Orris S, Scheiner M, Gonzalez A, et al. A high protein diet (34 g/kg/d) combined with a heavy resistance training program improves body composition in healthy trained men and women – a follow-up investigation. *J Int Soc Sports Nutr.* (2015) 12:39. doi: 10.1186/s12970-015-0100-0
66. Antonio J, Ellerbroek A, Silver T, Vargas L, Tamayo A, Buehn R, et al. A high protein diet has no harmful effects: a one-year crossover study in resistance-trained males. *J Nutr Metab.* (2016) 2016:1–5. doi: 10.1155/2016/9104792
67. Devries MC, Sithamparapillai A, Brimble KS, Banfield L, Morton RW, Phillips SM. Changes in kidney function do not differ between healthy adults consuming higher- compared with lower- or normal-protein diets: a systematic review and meta-analysis. *J Nutr.* (2018) 148:1760–75. doi: 10.1093/jn/nxy197
68. Truche A-S, Bailly S, Fabre O, Legrand R, Zaoui P. A specific high-protein weight loss program does not impair renal function in patients who are overweight/obese. *Nutrients.* (2022) 14:384. doi: 10.3390/nu14020384
69. Wilborn CD, Taylor LW, Outlaw J, Williams L, Campbell B, Foster CA, et al. The effects of pre- and post-exercise whey vs. casein protein consumption on body composition and performance measures in collegiate female. *Athletes J Sports Sci Med.* (2013) 12:74–9.
70. Stratton MT, Smith RW, Harty PS, Rodriguez C, Johnson BA, Dellinger JR, et al. Longitudinal agreement of four bioimpedance analyzers for detecting changes in raw bioimpedance during purposeful weight gain with resistance training. *Eur J Clin Nutr.* (2021) 75:1060–8. doi: 10.1038/s41430-020-00811-3
71. Dellinger JR, Johnson BA, Benavides ML, Moore ML, Stratton MT, Harty PS, et al. Agreement of bioelectrical resistance, reactance, and phase angle values from supine and standing bioimpedance analyzers. *Physiol Meas.* (2021) 6:42. doi: 10.1088/1361-6579/abe6fa

Conflict of Interest: FJT, CNM, JF, and RG are currently employees of a biotechnology company (Bettery S.A.) that produces dietary supplements. This company also developed the plant-based protein matrix (BETTPRO®) used in this study. BJS formerly served on the scientific advisory board of Dymatize Nutrition, a manufacturer of sports supplements. HF is employed by José de Mello Saúde. RC, VHT are advisors for Futebol Clube do Porto.

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: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fnut.2022.962773

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Găman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Denisa Margina,
Carol Davila University of Medicine and Pharmacy, Romania
Gail Rees,
University of Plymouth, United Kingdom

*CORRESPONDENCE
Naheed Aryaeian
aryaeian.n@iums.ac.ir

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 06 June 2022
ACCEPTED 27 June 2022
PUBLISHED 22 July 2022

CITATION
Khorshidi M, Sayyari A, Aryaeian N, Olang B, Alaei M, Khalili M, Hosseini A and Salehi M (2022) Effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes mellitus: A randomized clinical trial. *Front. Nutr.* 9:962773. doi: 10.3389/fnut.2022.962773

Effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes mellitus: A randomized clinical trial

Masoud Khorshidi[1], Aliakbar Sayyari[2], Naheed Aryaeian[1]*, Beheshteh Olang[2,3], Mohammadreza Alaei[4], Mitra Khalili[5], Amirhossein Hosseini[2] and Masoud Salehi[6]

[1]Department of Nutrition, School of Public Health, Iran University of Medical Sciences (IUMS), Tehran, Iran, [2]Pediatric Gastroenterology, Hepatology and Nutrition Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3]Children Emergency Department Karolinska University Hospital, Stockholm, Sweden, [4]Department of Pediatric Endocrinology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [5]Department of Radiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [6]Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran

Background: Vascular dysfunction is a major complication of diabetes mellitus that leads to cardiovascular disease (CVD). This study aimed to examine the effects of omega-3 consumption on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes mellitus (T1DM).

Methods: In this randomized, double-blind, placebo-controlled clinical trial, 51 adolescents (10–18 years) with T1DM completed the study. Patients received 600 mg/day [containing 180 mg eicosapentaenoic acid (EPA) and 120 mg docosahexaenoic acid (DHA)] of omega-3 or placebo for 12 weeks. Flow-mediated dilation (FMD), carotid intima-media thickness (CIMT), high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, blood urea nitrogen (BUN), creatinine, fasting blood sugar (FBS), hemoglobin A1C (HbA1c), homeostatic model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), serum insulin (SI), urine albumin-creatinine ratio (uACR), blood pressure, and anthropometric indices were assessed at the baseline and after the intervention.

Results: Following supplementation, omega-3 significantly increased FMD (3.1 ± 4.2 vs. −0.6 ± 4%, $p = 0.006$) and decreased TG (−7.4 ± 10.7 vs. −0.1 ± 13.1 mg/dl, $p = 0.022$) in comparison with the placebo group. However, no significant difference was observed regarding CIMT (-0.005 ± 0.036 vs. 0.003 ± 0.021 mm, $p = 0.33$). Although hs-CRP was significantly decreased within

the omega-3 group ($p = 0.031$); however, no significant change was observed compared to placebo group ($p = 0.221$). Omega-3 supplementation had no significant effect on other variables.

Conclusion: Given the elevation in FMD and reduction in TG, omega-3 supplementation can improve vascular function and may reduce the risk of cardiovascular disease in adolescents with T1DM patients.

KEYWORDS

omega-3, endothelial function, type 1 diabetes mellitus, vascular structure, triglycerides

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder characterized by insulin deficiency caused by the destruction of pancreatic β-cells (1). Based on recent epidemiological evidence, the prevalence of T1DM was 9.5% globally (2). Also, the incidence rate of T1DM is increasing annually by 2–3% worldwide (3). Children younger than 15 years possess the greatest increase in the incidence rate of T1DM (4). Current evidence suggests that genetic, environmental, and immunologic factors have crucial roles in the pathogenesis of T1DM, however, there are still some gaps in current knowledge (5). Impaired blood sugar control in patients with T1DM leads to structural and functional damage to the cardiovascular system, these factors predict an increased risk of cardiovascular disease (CVD) in adulthood, and studies have shown that more than 35% of people with T1DM develop vascular endothelial dysfunction after 5 years (6, 7). Despite the discovery of insulin in 1921 as the main treatment for T1DM, the disease continues to be correlated with substantial medical complications associated with vascular dysfunction including retinopathy, neuropathy, nephropathy, atherosclerosis, and thrombosis in the heart, peripheral arteries, and brain. The lifetime economic burden of T1DM represents a high burden of the disease on health care systems. Thus, developing new therapeutic strategies for the management of T1DM and its related complications is pivotal (8).

Vascular endothelial dysfunction is caused by decreased bioavailability of vasodilators, and one of the factors used to assess endothelial function is flow-mediated dilation (FMD), which has been shown to be impaired in adolescents with type 1 diabetes and predicts vascular disease in adulthood (9). End products of advanced glycation (AGEs), which are formed by constant exposure to high blood sugar, are one of the main factors in increasing vascular wall thickness such as carotid intima-media thickness (CIMT) in patients with diabetes; on the other hand, Ox-low-density lipoprotein (LDL), reduced bioavailability of nitric oxide (NO), and impaired endothelial NO synthase impair endothelial function (10). According to the American Heart Association, children and adolescents with T1DM and type 2 diabetes mellitus (T2DM) are at high risk for CVD in adulthood (11). However, exploring novel adjuvant therapy for the management of T1DM complications is taken into consideration by researchers.

Omega-3 fatty acids are proposed to exert favorable effects on T1DM (12). Omega-3 fatty acids are polyunsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and are found in oily fish and the liver of white fish. It possesses multiple biological properties including antioxidant, anti-inflammatory, immunomodulatory, antitumor, antidepressant, antihypertensive, and lipid-lowering effects (13). Moreover, a growing body of evidence revealed that various kinds of metabolic disorders underlying the development of diabetes were ameliorated by omega-3 administration (14). Omega-3 fatty acids can also be effective in improving vascular endothelial function by reducing inflammatory cytokines and increasing NO and oxylipins production (15, 16). It has been also shown that controlling blood pressure and lipids has greater beneficial impacts on the prevention of cardiovascular complications than controlling blood glucose, while both are necessary to be monitored (17). Some studies have been conducted to date investigating the effects of omega-3 fatty acids on the

Abbreviations: T1DM, Type 1 diabetes mellitus; FMD, flow-mediated dilation; CIMT, carotid intima-media thickness; BUN, blood urea nitrogen; hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; HOMA-IR, hemostatic model assessment of insulin resistance; QUICKI, and quantitative insulin sensitivity check index; HO-1, heme oxygenase 1; GPx, glutathione peroxidase; NF-kB, nuclear factor-kB; PAQ-C, physical activity questionnaire for children; PAQ-A, physical activity questionnaire for adolescents; PAS, physical activity score; ELISA, enzyme-linked immunosorbent assay.

endothelial function and vascular structure in adults with different diseases and the results are contradictory (18, 19). There is no randomized clinical trial assessing the effects of omega-3 supplementation in children and adolescents with T1DM. Hence, the present clinical study aims to examine the effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with T1DM.

Materials and methods

Study design and participants

This was a randomized, double-blind, placebo-controlled, clinical trial. In the protocol, adolescents with T1DM, who were diagnosed by a specialist, were recruited from Mofid Children's Hospital, Tehran, Iran (20). Patients that satisfied the following inclusion and exclusion criteria were eligible to participate in this research. The inclusion criteria were considered as follows: adolescents aged between 10 and 18 years, whose body mass index (BMI) for age Z-score ranged between 5 and 85 percent, diagnosed with T1DM by a specialist, receiving insulin therapy, and at least 5 years have passed since the diagnosis of T1DM. The exclusion criteria include pregnant or breastfeeding subjects, those afflicted by endocrine and metabolic complications other than T1DM, any acute disorders, and patients who had diabetic ketoacidosis or hypoglycemia (blood sugar level lower than 50 mg/dl) in the last 12 and 3 months, respectively. In addition, subjects who consumed antihypertensive, anti-inflammatory, anticoagulant, lipid-lowering, weight-lowering agents, antioxidant, and omega-3 supplements in the last 6 months were excluded. Patients with fish and seafood allergies and smokers were also excluded. The study flow chart of enrolment, allocation, intervention, and assessment was presented in Figure 1. Written informed consent was taken from all participants or their legal guardians before contribution to the research. This investigation was approved by the Medical Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1400.070) and also registered on the Iranian Registry of Clinical Trials website (identifier: IRCT20210419051010N1).

Sample size calculation

Considering a type I error of 5%, a power of 90%, and the changes in FMD values as one of the primary outcomes (21), the sample size was computed to be 25 for each study group based on the two-sided *t*-test. To compensate for an approximate attrition rate of 20% throughout the research, we increased the final sample size to 30 adolescents in each group.

Randomization and intervention

After obtaining the informed consent eligible subjects ($n = 60$) were divided into two equal groups, stratified by sex and the amount of daily insulin intake using stratified block randomization, with a 1:1 allocation ratio. The block randomization was conducted by an assistant and the intervention allocation was blinded for both the investigators and participants. The manufacturer who was responsible for preparing the supplements was assigned a code.

A total of 60 eligible adolescents with T1DM were randomly assigned to the omega-3 ($n = 30$) and placebo groups ($n = 30$). Omega-3 capsules (600 mg) and identical placebo capsules were respectively taken by patients in the intervention and control group once a day, for 12 weeks. Karen Pharma and Food Supplement company provided omega-3 capsules which contain 180 mg EPA and 120 mg DHA. Omega-3 and placebo capsules were similar in weight, size, shape, taste, color, and odor. Placebo capsules were also produced by Karen Pharma & Food Supplement company and contain 600 mg oral glycerin. Study patients were informed how to use their supplements and they were weekly followed by phone calls. Any adverse events were reported.

Clinical assessments

At the onset and end of the trial, body weight and height were measured to the nearest 0.1 kg and 0.1 cm using the Seca scale and stadiometer (Seca), respectively. The individuals were measured when they were barefoot and wearing light clothing. BMI for age Z-score was computed by dividing weight in kilograms by height in meters squared according to age in the curve standard using WHO growth standards. Dietary intake was assessed through 24-h food recall (two weekdays and one weekend) by an expert person. Recalls were completed at the beginning and end of the intervention by face-to-face interview (two weekdays and one weekend). We analyzed the information about dietary consumption by Nutritionist IV software. For estimating the physical activity level of participants, physical activity questionnaire for children (PAQ-C) and physical activity questionnaire for adolescents (PAQ-A), which were validated in Iran, were applied (22). The PAQ-C, appropriate for ages 8–14, provides a summary of physical activity scores (PASs) derived from 9 items, each scored on a 5-point scale. Also, the PAQ-A which is appropriate for ages 14–20, provides a summary of PAS derived from 8 items, each scored on a five-point scale. The levels of physical activity were reported in three categories including low activity, moderate activity, and high activity levels based on the PAQ-A scoring protocol (22, 23). Blood pressure was measured using a mercury sphygmomanometer after at least 5-min of resting. The measurement was performed on two occasions

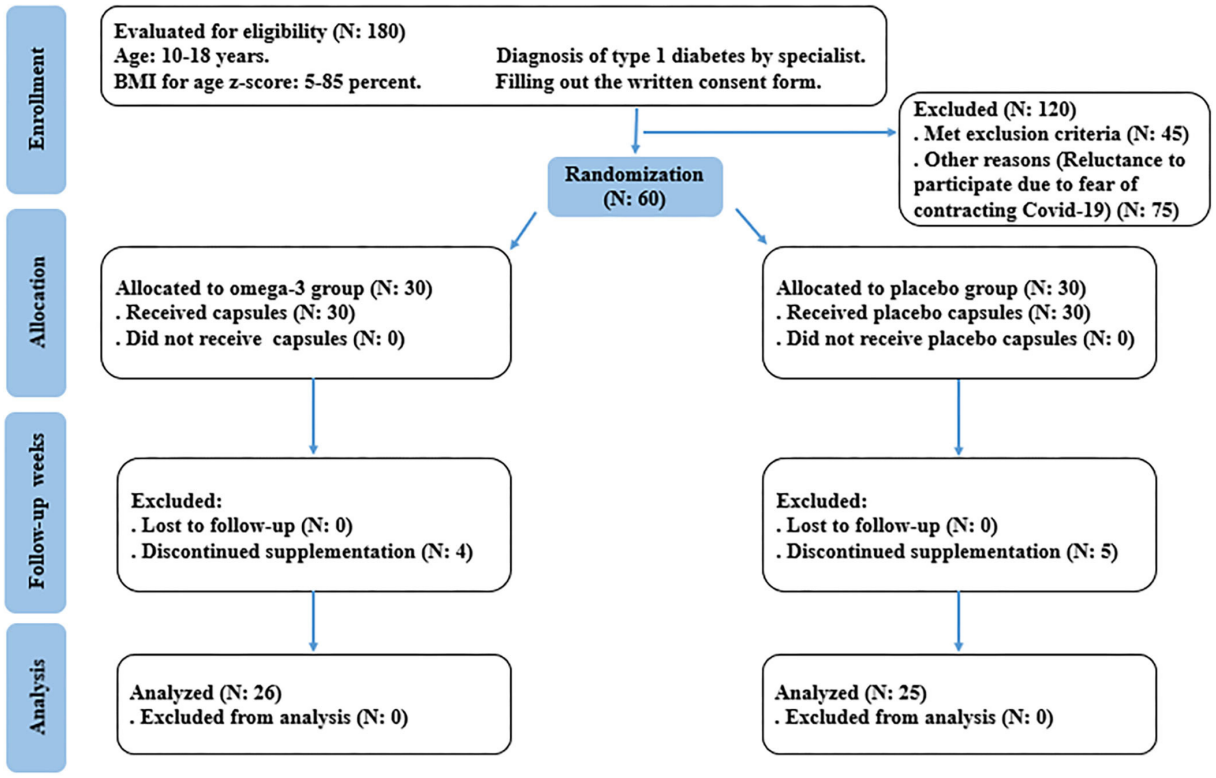


FIGURE 1
Summary of study based on the consolidated standards of reporting trials (CONSORT) flow diagram.

and the mean of the two was considered as the individual's blood pressure.

Biochemical measurements

Blood samples (10 ml) were drawn following 12-h overnight fasting and centrifuged at 3,000 rpm for 5 min to extract serum samples pre- and post-intervention. The concentration of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) was evaluated using commercial kits (DIALAB Inc. kit, Vienna, Austria) before and after the intervention. Serum LDL-C value was calculated using the Friedewald formula. The concentration of fasting serum insulin (SI) and fasting blood sugar (FBS) was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (IBT, USA) and glucose oxidase method, respectively. Hemoglobin A1C (HbA1c) was assessed using a commercial kit (BioRex Inc., Tehran, Iran) by an auto-analyzer (Mindray auto hematology analyzer). Moreover, blood urea nitrogen (BUN) and creatinine were examined by the diacetyl monoxime method and enzymatic method, respectively. Erythrocyte sedimentation rate (ESR) was evaluated by the Westergren method using a special tube. In addition, serum high-sensitivity C-reactive protein (hs-CRP) level was assessed using an ELISA kit (Paadco Inc. kit, Tehran, Iran) pre- and postintervention. Also, the following formulas were used to determine the quantitative insulin sensitivity check index (QUICKI) and homeostatic model assessment for insulin resistance (HOMA-IR). Proteinuria was evaluated by albumin dipstick by collecting urine samples.

QUICKI: 1/[log (fasting insulin μU/ml) + log (fasting glucose mg/dl)].

HOMA-IR: [fasting insulin (μU/ml) × fasting glucose (mg/dl)]/405.

FMD and CIMT assessment

Flow-mediated dilation (FMD) and carotid intima-media thickness (CIMT) were assessed by an expert radiologist. For evaluating FMD, after overnight fasting and 10 min rest, patients lay down in the supine position in a quiet, temperature-controlled room and put their right hand in a comfortable position on a surface for imaging the brachial artery. Ultrasound imaging of the brachial artery was done by Doppler ultrasound (Samsung Medison UGEO H60, Seoul, South Korea) and in a

longitudinal section, 2 cm above of antecubital fossa. An anterior and posterior section were selected between the lumen and the vessel wall for clear imaging and after that, the diameter of the vessel was measured from the anterior part to the posterior part between Media and Adventitia. A baseline rest image was acquired and the arterial diameter was measured. For the second scan, the cuff of the sphygmomanometer was closed around the arm and a 5-min occlusion of at least 50 mmHg above the systolic pressure was applied to the vessel. Finally, 90 s after the cuff was opened, the second measurement was performed. The following formula was used to calculate FMD, where d1 is the baseline brachial artery diameter and d2 is the brachial artery diameter after 90 s of cuff release.

FMD: (d2 – d1) × 100/d1

To assess CIMT, the thickness of two inner layers of the Intima and Media artery were measured by Doppler ultrasound (Samsung Medison UGEO H60, Seoul, South Korea). The distance between the main edge of the first line and the main edge of the second line in the carotid artery was considered CIMT. To increase the accuracy, all measurements were repeated four times.

Statistical analysis

Data entry, coding, security, and storage were checked. Statistical analysis of all data was done by SPSS statistical software (SPSS Inc., Chicago, IL, USA, version 24) and $p < 0.05$ were considered statistically significant. The Kolmogorov–Smirnov test was used for assessing the normality of the data. Quantitative and qualitative variables were presented as mean (standard deviation) or median (25th−75th percentile), and frequency (percentage), respectively. To examine differences in qualitative variables between omega-3 and placebo groups, we applied the chi-square test. We used the independent sample *t*-test or Mann–Whitney U-test to determine differences in numerical variables between the omega-3 and placebo groups. In addition, the aforementioned tests were used to compare between-group alterations in outcome variables. To do a within-group comparison, we used the paired-sample *t*-test or its non-parametric equivalent, the Wilcoxon test. A general linear model was applied to adjust the effects of confounding factors and baseline differences.

Results

Fifty-one patients completed the study ($n = 26$ in the omega-3 and $n = 25$ in the placebo, group). Four patients in the omega-3 group and five patients in the placebo group discontinued the trial due to the COVID-19 pandemic and increased fear of contracting the disease during sampling and low compliance. Also, no side effects were reported during supplementation with omega-3 (Figure 1). The mean age of participants in the intervention group was 13.8 ± 2.3 years and in the placebo group was 12.9 ± 2.4 years ($p = 0.164$). The duration of diabetes in omega-3 and placebo groups were 7.6 ± 2.3 vs. 7.4 ± 2.8 years, respectively ($p = 0.688$). On the other hand, there were 14 boys (54%) and 12 girls (46%) in the omega-3 group and 15 boys (60%) and 10 girls (40%) in the placebo group ($p = 0.657$). Sixteen people in the intervention group and 13 people in the placebo group had low physical activity (PAS between 1 and 2.33) and on the other hand, 10 people in the intervention group and 12 people in the placebo group had moderate physical activity (PAS between 2.34 and 3.66) and none of the participants in the study had intense physical activity (PAS between 3.67 and 5) ($p = 0.492$). Following the analysis of the participants' food recall questionnaire, there was no significant difference in oral intake of vitamins and minerals affecting vascular function and structure, including vitamin A, vitamin C, beta-carotene, vitamin E, zinc, sodium, calcium, selenium, phosphorus, iron, potassium, and fatty acids between the two groups at baseline and the end of supplementation.

Table 1 shows the comparison of post-intervention energy intake, insulin infusion, and anthropometric indices between the 2 groups. After 12-week supplementation, height and weight increased significantly in both groups, and between the two groups this change was not significant; also, BMI Z-score did not change ($p = 0.829$). There were no significant differences in reported insulin infusion ($p = 0.128$) and dietary energy intake ($p = 0.07$) at baseline or the end of the study.

There was a significant reduction about FMD ($p = 0.003$) in omega-3 compared with the placebo group after 12 weeks of supplementation, which remains statistically significant after adjustment for baseline differences between groups, age, sex, and TG changes ($p = 0.006$). However, no significant change in CIMT was observed following omega-3 supplementation ($p = 0.33$) (Table 2).

As shown in Table 3, TG decreased significantly at the end of the intervention, after adjusting for sex, age, and baseline differences between groups ($p = 0.022$). Hs-CRP decrease significantly in omega-3 group ($p = 0.031$); however, this change was not significant compared to the placebo group ($p = 0.192$). There was no significant change in FBS, HgA1c, serum insulin, and other metabolic indices between the two groups at the end of supplementation.

Discussion

We demonstrated that daily supplementation with 600 mg/day omega-3 (containing 180 mg EPA and 120 mg DHA) for 12 weeks in adolescents with T1DM could significantly

10.3389/fnut.2022.962773

TABLE 1 Participant characteristics before and after supplementation with omega-3.

Parameter	Time	Omega-3 ($N = 26$)	Placebo ($N = 25$)	Mean difference (95% CI)	*P*-value
Weight (kg)	Baseline	50.2 ± 14.2	45.3 ± 14.2		0.219[a]
	Endpoint	50.7 ± 13.7	45.9 ± 14	0.1 (−0.5 to 0.6)	0.82[b]
	Change	0.5 ± 1.1	0.6 ± 1.1	0.1 (−0.68 to 0.56)	0.846[a]
	P-within	0.025 [c]	0.016 [c]		
Height (cm)	Baseline	159.8 ± 13.6	153.6 ± 15.8		0.138[a]
	Endpoint	160.8 ± 13.1	155.2 ± 15.7	−0.4 (−1.4 to 0.4)	0.293[b]
	Change	1 ± 1	1.6 ± 2.1	−0.6 (−1.5 to 0.2)	0.175[a]
	P-within	<0.001 [c]	0.001 [c]		
BMI (Z-score)	Baseline	0.01 ± 0.71	0.08 ± 0.75		0.709[a]
	Endpoint	−0.01 ± 0.66	0.05 ± 0.67	0.01 (−0.08 to 0.1)	0.829[b]
	Change	−0.02 ± 0.18	−0.03 ± 0.18	0.02 (−0.08 to 0.12)	0.71[a]
	P-within	0.719 [c]	0.397 [c]		
Calorie intake (kcal)	Baseline	2,348 ± 670	2,328 ± 432		0.899[a]
	Endpoint	2,399 ± 576	2,269 ± 423	114 (−9 to 237)	0.07[b]
	Change	51 ± 265	−58 ± 215	110 (−26 to 246)	0.111[a]
	P-within	0.332 [c]	0.187 [c]		
Insulin infusion (unit)	Baseline	50.3 ± 18.1	52.1 ± 24		0.767[a]
	Endpoint	50.3 ± 18.1	53.1 ± 23.8	−1 (−2.4 to 0.3)	0.128[b]
	Change	0 ± 1.5	1 ± 3.1	−1 (−2.4 to 0.3)	0.134[a]
	P-within	1 [c]	0.1 [c]		

All data presented as mean ± SD. CI: confidence interval; BMI: body mass index.
[a]Independent t-test.
[b]General linear model adjusted for baseline differences between groups.
[c]Paired t-test.

increase FMD. In addition, following supplementation TG was significantly decreased in the intervention group compared to the placebo group. No significant changes were seen between the two groups in terms of other metabolic parameters and CIMT.

It has previously been reported that FMD as a factor of vascular dysfunction assessment, is decreased in patients with diabetes (9). In this study, it has been demonstrated that a daily intake of 600 mg omega-3 could increase FMD in children and adolescents with T1DM. Many studies have reported increased levels of inflammatory factors in people with T1DM compared to people without diabetes which can cause vascular dysfunction (24). Moreover, previous studies have shown that consumption of omega-3 fatty acids increases the ratio of these fatty acids to arachidonic acid in the cell membrane phospholipids and reduces the production of pro-inflammatory eicosanoids (25). Also, mediators derived from omega-3 fatty acids such as resolvins have anti-inflammatory effects (26). On the other hand, omega-3 fatty acids cause phosphorylation of endothelial nitric oxide synthase (eNOS) and consequently increase the production of NO and finally improve vascular function through activating AMP-activated protein kinase (27). Also, Hsu et al. showed that omega-3 fatty acids can increase the bioavailability of nitric oxide and improve vascular response by reducing reactive oxygen species (ROS) (28). To date, only one study has been performed addressing the effect of omega-3 supplementation on FMD in children and adolescents in all populations, and the results were consistent with our study. Engler et al. (29) reported a significant increase in FMD, HDL, and LDL following 6 weeks of supplementation with 1.2 g/day DHA in children and adolescents with familial hypercholesterolemia (high risk for CVD in adulthood). However, several studies in adults have examined FMD and vascular function after omega-3 supplementation, with conflicting results. Mahoney et al. showed that 6 months of omega-3 supplementation with 3.3 g/day did not cause any significant change in FMD in 27 adults with T1DM (18). The results of the study by Stirban et al. (19) showed that supplementation with 2 g/day of omega-3 fatty acids for 6 weeks in adults with T2DM did not improve fasting FMD, but significantly reduced postprandial FMD. On the other hand, Wang et al. conducted a meta-analysis study on sixteen clinical trials demonstrating that omega-3 supplementation could significantly increase FMD, however, limitations such as the heterogeneous population and the different doses and duration of treatment affected these results (30). In addition to BMI and duration of diabetes, another reason for the differences between children and

TABLE 2 Effect of omega-3 supplementation on endothelial function and vascular structure.

Parameter	Time	Omega-3 ($N = 26$)	Placebo ($N = 25$)	Mean difference (95% CI)	*P*-value	*P2*	*P3*
FMD (%)							
	Baseline	6.5 ± 3.6	8.2 ± 5		0.169 [a]		
	Endpoint	9.6 ± 2.8	7.6 ± 3.4	2.5 (0.9 to 4.1)	0.003 [b]	0.006	0.006
	Change	3.1 ± 4.2	−0.6 ± 4	3.7 (1.4 to 6)	0.002 [a]		
	P-within	0.001 [c]	0.452 [c]				
CIMT (mm)							
	Baseline	0.443 ± 0.093	0.462 ± 0.069		0.415 [a]		
	Endpoint	0.438 ± 0.094	0.464 ± 0.07	−0.009 (−0.025 to 0.01)	0.312 [b]	0.229	0.33
	Change	−0.005 ± 0.036	0.003 ± 0.021	−0.007 (−0.024 to 0.01)	0.376 [a]		
	P-within	0.522 [c]	0.502 [c]				

All data presented as mean ± SD. CI, confidence interval; FMD, flow-mediated dilation; CIMT, carotid intima-media thickness; mm, millimeter.
[a]Independent t-test.
[b]General linear model adjusted for baseline differences between groups.
[c]Paired t-test.
P2 General linear model adjusted for baseline differences between groups, age, and sex.
P3 General linear model adjusted for baseline differences between groups, age, sex, and triglycerides (TG) changes.

adults could be progressive vascular dysfunction following aging (31).

There was no significant change in CIMT (as markers for carotid atherosclerosis) following the 3 months omega-3 supplementation. In line with our study, Mahoney et al. reported that omega-3 supplementation for 6 months in patients with type 1 diabetes was not associated with a reduction in CIMT (18). However, Tomoya et al. showed that omega-3 supplementation for a longer period (2.1 years) in T2DM patients significantly reduces CIMT (32). Overall, omega-3 fatty acids appear to be able to improve the CIMT through their anti-inflammatory and antithrombotic effects (33), however, due to the relatively stable structure of the vascular wall, longer interventions are required to observe significant changes (34).

Following supplementation with omega-3, there was a significant reduction in TG levels in the intervention group compared to the placebo group. In line with our trial, Natto et al. revealed that administration of omega-3 polyunsaturated fatty acid (PUFAs) causes a significant reduction in TG in patients with diabetes (35). Similarly, in the study performed by Chauhan et al. omega-3 supplementation was effective in reducing TG levels in diabetic dyslipidemia (36). The exact mechanism by which omega-3 FAs had TG lowering effects returns to the high affinity of omega-3 FAs for peroxisome proliferator-activated receptor (PPAR) followed by enhancement of beta-oxidation and fatty acid metabolism. Moreover, omega-3 FAs may decrease hepatic TG synthesis through inhibition of acyl coA1 and diacylglycerol acyltransferase (37). Also, omega-3 FAs stimulate other nuclear receptors including hepatocyte nuclear factor 4α, liver X receptor, and farnesol X receptor, which modulates TG levels (38). Shinnakasu et al. reported that improving lipid profile and TG levels in patients with diabetes can improve FMD (39). It seems that one of the possible reasons that omega-3 supplementation improved FMD in the present study could be its effect on lowering serum triglyceride levels.

In our study, following omega-3 supplementation, hs-CRP levels were significantly reduced in the intervention group. Many systematic review and meta-analysis studies have also shown the effect of omega-3 PUFAs on lowering hs-CRP levels (40, 41). On the other hand, many studies have proven the effect of inflammation on vascular dysfunction (42). According to the reasons mentioned, another factor that can be effective in improving vascular function in our study is reducing inflammation. Also, other factors such as changes in blood pressure, BMI Z-score, and microalbuminuria could distort the results of the present study, none of which were significantly changed.

Strengths and weaknesses

The strengths of the present study include using a randomized, double-blind, placebo-control design and also examining the effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes for the first time. Also, another strength of this study was the assessment of the effect of a dietary anti-inflammatory and antioxidant element with available food sources with low expected adverse effects, complementary to current medications. However, there are some weaknesses in this research. First, participants' self-reporting on dietary intakes and physical

TABLE 3 Metabolic parameters before and after treatment with omega-3.

Parameter	Time	Omega-3 ($N = 26$)	Placebo ($N = 25$)	Mean difference (95% CI)	*P*-value	*P2*
hs-CRP (mg/l)						
	Baseline	2.6 ± 1.3	2.6 ± 1.3		0.981[a]	
	Endpoint	2.2 ± 1.1	2.5 ± 1.2	−0.2 (−0.6 to 0.1)	0.192[b]	0.221
	Change	−0.4 ± 0.8	−0.1 ± 0.8	−0.2 (−0.7 to 0.2)	0.257[a]	
	P-within	0.031[c]	0.45 [c]			
ESR (mm/hr)						
	Baseline	13.6 ± 6.1	13.6 ± 6.2		0.994 [a]	
	Endpoint	12.3 ± 5.2	13.5 ± 6.2	−1.2 (−3.1 to 0.7)	0.205 [b]	0.337
	Change	−1.3 ± 4.1	−0.1 ± 3.1	−1.2 (−3.3 to 0.8)	0.238 [a]	
	P-within	0.115 [c]	0.901 [c]			
Creatinine (mg/dl)						
	Baseline	0.7 ± 0.1	0.7 ± 0.1		0.203 [a]	
	Endpoint	0.7 ± 0.1	0.7 ± 0.1	0 (−0.1 to 0.1)	0.531 [b]	0.267
	Change	0 ± 0.1	0 ± 0.1	0 (−0.01 to 0.01)	0.744 [a]	
	P-within	0.94 [c]	0.573 [c]			
uACR (mg/g)						
	Baseline	9 ± 3.03	9.4 ± 3.06		0.648 [a]	
	Endpoint	9.47 ± 2.75	8.75 ± 2.32	0.93 (−0.19 to 2)	0.103 [b]	0.11
	Change	0.43 ± 1.96	−0.65 ± 2.87	1.07 (−0.3 to 2.45)	0.124 [a]	
	P-within	0.228 [c]	0.271 [c]			
BUN (mg/dl)						
	Baseline	16.6 ± 5.1	16.6 ± 4.6		0.964 [a]	
	Endpoint	17 ± 5.2	16.2 ± 3.9	0.8 (−0.9 to 2.5)	0.355 [b]	0.26
	Change	0.4 ± 3.5	−0.4 ± 3.1	0.8 (−1.1 to 2.7)	0.406 [a]	
	P-within	0.59 [c]	0.522 [c]			
FBS (mg/dl)						
	Baseline	129 ± 39	126 ± 42		0.809 [a]	
	Endpoint	127 ± 36	122 ± 33	3 (−12 to 18)	0.687 [b]	0.631
	Change	−2 ± 37	−4 ± 29	2 (−17 to 21)	0.846 [a]	
	P-within	0.781 [c]	0.519 [c]			
SI (μIU/ml)						
	Baseline	18.6 ± 14.4	19.7 ± 10.5		0.763 [a]	
	Endpoint	19.2 ± 14.5	18 ± 8.2	1.8 (−3.5 to 7.2)	0.493 [b]	0.741
	Change	0.6 ± 9	1.7 ± 12.8	2.3 (−3.8 to 8.5)	0.453 [a]	
	P-within	0.715 [c]	0.517 [c]			
QUICKI						
	Baseline	0.31 ± 0.04	0.3 ± 0.04		0.476 [a]	
	Endpoint	0.3 ± 0.03	0.3 ± 0.02	0 (−0.01 to 0.01)	0.999 [b]	0.93
	Change	−0.01 ± 0.05	0 ± 0.03	0 (−0.02 to 0.02)	0.606 [a]	
	P-within	0.504 [c]	0.948 [c]			
HOMA-IR						
	Baseline	5.7 ± 4.2	6.4 ± 4.9		0.558 [a]	
	Endpoint	5.6 ± 3.5	5.4 ± 2.9	0.3 (−1.4 to 2.1)	0.679 [b]	0.872
	Change	−0.1 ± 3.6	−1 ± 5.5	0.9 (−1.6 to 3.5)	0.474 [a]	
	P-within	0.936 [c]	0.375 [c]			

(Continued)

TABLE 3 Continued

Parameter	Time	Omega-3 ($N = 26$)	Placebo ($N = 25$)	Mean difference (95% CI)	*P*-value	*P2*
HbA1c (%)						
	Baseline	6.8 ± 0.5	7 ± 0.3		0.252 [a]	
	Endpoint	6.7 ± 0.4	7 ± 0.4	0.2 (−0.4 to 0)	0.111 [b]	0.118
	Change	−0.1 ± 0.6	0 ± 0.5	−0.05 (−0.4 to 0.3)	0.723 [a]	
	P-within	0.618 [c]	0.972 [c]			
SBP (mmHg)						
	Baseline	109 ± 8.1	106.4 ± 6.2		0.2 [a]	
	Endpoint	107.7 ± 6.7	106.6 ± 5.3	−0.3 (−3 to 2.2)	0.772 [b]	0.788
	Change	−1.3 ± 5.7	0.2 ± 5.3	0.5 (−1.9 to 3)	0.324 [a]	
	P-within	0.244 [c]	0.852 [c]			
DBP (mmHg)						
	Baseline	64.4 ± 5.3	64 ± 5.9		0.79 [a]	
	Endpoint	65 ± 6	64 ± 5	0.7 (−1.5 to 3)	0.533 [b]	0.659
	Change	0.6 ± 4.3	0 ± 4.5	0.5 (−1.9 to 3)	0.645 [a]	
	P-within	0.502 [c]	1 [c]			
TG (mg/dl)						
	Baseline	102.2 ± 27.4	117.3 ± 37.6		0.106 [a]	
	Endpoint	94.8 ± 28.1	117.2 ± 36.1	−8.5 (−15.3 to −1.7)	0.015 [b]	0.022
	Change	−7.4 ± 10.7	−0.1 ± 13.1	−7.3 (−14 to 0.5)	0.034 [a]	
	P-within	0.002 [c]	0.952 [c]			
Chol (mg/dl)						
	Baseline	142.4 ± 18.5	156.1 ± 29.3		0.052 [a]	
	Endpoint	146.1 ± 19.3	156.8 ± 29.6	0.2 (−7.8 to 8.1)	0.962 [b]	0.881
	Change	3.7 ± 14	0.7 ± 14.5	2.9 (−5.1 to 11)	0.468 [a]	
	P-within	0.193 [c]	0.796 [c]			
LDL (mg/dl)						
	Baseline	91.2 ± 24.3	100.3 ± 30		0.236 [a]	
	Endpoint	95 ± 21.5	100.7 ± 28	1.8 (−4.3 to 7.8)	0.555 [b]	0.532
	Change	3.8 ± 13.9	0.4 ± 8.4	3.4 (−3.1 to 9.9)	0.298 [a]	
	P-within	0.176 [c]	0.814 [c]			
HDL (mg/dl)						
	Baseline	53.8 ± 12.6	53.1 ± 13.1		0.846 [a]	
	Endpoint	54.1 ± 11.8	52.1 ± 10.8	1.5 (−1.7 to 4.7)	0.353 [b]	0.531
	Change	0.3 ± 6.5	−1 ± 6.2	1.3 (−2.2 to 4.9)	0.461 [a]	
	P-within	0.813 [c]	0.419 [c]			

All data presented as mean ± SD. CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BUN, blood urea nitrogen; uACR; urine albumin-creatinine ratio; FBS, fasting blood sugar; SI, serum insulin; QUICKI, quantitative insulin-sensitivity check index; HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; Chol, cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
[a]Independent t-test.
[b]General linear model adjusted for baseline differences between groups.
[c]Paired t-test.
P2 General linear model adjusted for baseline differences between groups, age, and sex.

activity may influence our results. Second, no biomarker was considered for compliance of participants from omega-3 intake. Third, the small number of included subjects could affect the results. And finally, the duration of intervention could have been longer to influence study biomarkers, especially CIMT.

Conclusion

In conclusion, daily consumption of 600 mg omega-3 fatty acid (containing 180 mg EPA and 120 mg DHA) in adolescents with T1DM for 12 weeks, significantly increased FMD along with a significant decrease in triglyceride. Following

supplementation, the level of hs-CRP in the intervention group decreased but these changes were not significant compared with the placebo group and CIMT did not change. The present findings show that omega-3 intake can improve vascular function and may reduce the risk of CVD in adolescents with T1DM patients.

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/s.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1400.070). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MKho: wrote the original draft, laboratory experiments, and data analysis. AS and NA: review and edit. BO, MA, and AH sampling. MS: data analysis. MKha: laboratory experiments. All authors read and approved the final manuscript.

Funding

This research was supported by the Iran University of Medical Sciences (Grant number: 19738). Also, we would like to thank the Mofid Children's Hospital staff, Shahid Beheshti University of Medical Sciences, Tehran, Iran for their help.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). *Nat Rev Endocrinol.* (2021) 17:150–61. doi: 10.1038/s41574-020-00443-4

2. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect.* (2020) 10:98–115. doi: 10.34172/hpp.2020.18

3. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002-2012. *N Engl J Med.* (2017) 376:1419–29. doi: 10.1056/NEJMoa1610187

4. Chobot A, Polanska J, Brandt A, Deja G, Glowinska-Olszewska B, Pilecki O, et al. Updated 24-year trend of type 1 diabetes incidence in children in Poland reveals a sinusoidal pattern and sustained increase. *Diabet Med J Br Diabet Assoc.* (2017) 34:1252–8. doi: 10.1111/dme.13345

5. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet.* (2018) 391:2449–62. doi: 10.1016/S0140-6736(18)31320-5

6. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet.* (2008) 371:1777–82. doi: 10.1016/S0140-6736(08)60765-5

7. Abd El Dayem SM, Battah AA. Effect of glycemic control on the progress of left ventricular hypertrophy and diastolic dysfunction in children with type I diabetes mellitus. *Anadolu Kardiyol Derg.* (2012) 12:498–507. doi: 10.5152/akd.2012.158

8. Sussman M, Benner J, Haller MJ, Rewers M, Griffiths R. Estimated lifetime economic burden of type 1 diabetes. *Diabet Technol Ther.* (2019) 22:121–30. doi: 10.1089/dia.2019.0398

9. Bradley TJ, Slorach C, Mahmud FH, Dunger DB, Deanfield J, Deda L, et al. Early changes in cardiovascular structure and function in adolescents with type 1 diabetes. *Cardiovasc Diabetol.* (2016) 15:1–12. doi: 10.1186/s12933-016-0351-3

10. Lopes-Virella MF, Hunt KJ, Baker NL, Lachin J, Nathan DM, Virella G, et al. Levels of oxidized LDL and advanced glycation end products–modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes. *Diabetes.* (2011) 60:582–9. doi: 10.2337/db10-0915

11. De Ferranti SD, Steinberger J, Ameduri R, Baker A, Gooding H, Kelly AS, et al. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association. *Circulation.* (2019) 139:e603–e34. doi: 10.1161/CIR.0000000000000618

12. Tiwari P. Recent trends in therapeutic approaches for diabetes management: a comprehensive update. *J Diabetes Res.* (2015) 2015:11. doi: 10.1155/2015/340838

13. Siddiqui RA, Shaikh SR, Sech LA, Yount HR, Stillwell W, Zaloga GP. Omega 3-fatty acids: health benefits and cellular mechanisms of action. *Mini Rev Med Chem.* (2004) 4:859–71. doi: 10.2174/1389557043403431

14. Wu JHY, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O, et al. Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. *Br J Nutr.* (2012) 107(Suppl 2):S214–27. doi: 10.1017/S0007114512001602

15. Abeywardena MY, Head RJ. Longchain n-3 polyunsaturated fatty acids and blood vessel function. *Cardiovasc Res.* (2001) 52:361–71. doi: 10.1016/S0008-6363(01)00406-0

16. Du Y, Taylor CG, Aukema HM, Zahradka P. Role of oxylipins generated from dietary PUFAs in the modulation of endothelial cell function. *Prostaglandins Leukot Essent Fatty Acids.* (2020) 160:102160. doi: 10.1016/j.plefa.2020.102160

17. Farmer JA. Diabetic dyslipidemia and atherosclerosis: evidence from clinical trials. *Curr Diab Rep.* (2008) 8:71–7. doi: 10.1007/s11892-008-0013-2

18. O'Mahoney LL, Dunseath G, Churm R, Holmes M, Boesch C, Stavropoulos-Kalinoglou A, et al. Omega-3 polyunsaturated fatty acid supplementation versus placebo on vascular health, glycaemic control, and metabolic parameters in people with type 1 diabetes: a randomised controlled preliminary trial. *Cardiovasc Diabetol.* (2020) 19:1–10. doi: 10.1186/s12933-020-01094-5

19. Stirban A, Nandrean S, Götting C, Tamler R, Pop A, Negrean M, et al. Effects of n−3 fatty acids on macro-and microvascular function in subjects with type 2 diabetes mellitus. *Am J Clin Nutr.* (2010) 91:808–13. doi: 10.3945/ajcn.2009.28374

20. Khorshidi M, Sayyari A, Olang B, Alaei MR, Shab-Bidar S, Khalili M, et al. Study protocol of a randomized controlled clinical trial investigating the effects of omega-3 supplementation on endothelial function, vascular structure, and metabolic parameters in adolescents with type 1 diabetes. *Trials.* (2021) 22:1–7. doi: 10.1186/s13063-021-05930-1

21. Hamilton SJ, Chew GT, Davis TM, Watts GF. Fenofibrate improves endothelial function in the brachial artery and forearm resistance arterioles of statin-treated Type 2 diabetic patients. *Clin Sci.* (2010) 118:607–15. doi: 10.1042/CS20090568

22. Kowalski K, Crocker P, Donen R, Honours B. *The Physical Activity Questionnaire for Older Children (PAQ-C) and Adolescents (PAQ-A) Manual.* Saskatoon, SK: ACADEMIA (2004).

23. Crocker PR, Bailey DA, Faulkner RA, Kowalski KC, McGrath R. Measuring general levels of physical activity: preliminary evidence for the physical activity questionnaire for older children. *Med Sci Sports Exerc.* (1997) 29:1344–9. doi: 10.1097/00005768-199710000-00011

24. Snell-Bergeon JK, West NA, Mayer-Davis EJ, Liese AD, Marcovina SM, D'Agostino Jr RB, et al. Inflammatory markers are increased in youth with type 1 diabetes: the SEARCH case-control study. *J Clin Endocrinol Metabol.* (2010) 95:2868–76. doi: 10.1210/jc.2009-1993

25. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. *Lancet.* (2010) 376:540–50. doi: 10.1016/S0140-6736(10)60445-X

26. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2–nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med.* (2000) 192:1197–204. doi: 10.1084/jem.192.8.1197

27. Wu Y, Zhang C, Dong Y, Wang S, Song P, Viollet B, et al. Activation of the AMP-activated protein kinase by eicosapentaenoic acid (EPA, 20: 5 n-3) improves endothelial function in vivo. *PLoS ONE.* (2012) 7:e35508. doi: 10.1371/journal.pone.0035508

28. Hsu H-C, Chen C-Y, Chen M-F. N-3 polyunsaturated fatty acids decrease levels of doxorubicin-induced reactive oxygen species in cardiomyocytes—involvement of uncoupling protein UCP2. *J Biomed Sci.* (2014) 21:1–11. doi: 10.1186/s12929-014-0101-3

29. Engler M, Engler M, Malloy M, Chiu E, Besio D, Paul S, et al. function in children with hyperlipidemia: results from the EARLY study. *Int J Clin Pharmacol Ther.* (2004) 42:672–9. doi: 10.5414/CPP42672

30. Wang Q, Liang X, Wang L, Lu X, Huang J, Cao J, et al. Effect of omega-3 fatty acids supplementation on endothelial function: a meta-analysis of randomized controlled trials. *Atherosclerosis.* (2012) 221:536–43. doi: 10.1016/j.atherosclerosis.2012.01.006

31. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol.* (1994) 24:471–6. doi: 10.1016/0735-1097(94)90305-0

32. Mita T, Watada H, Ogihara T, Nomiyama T, Ogawa O, Kinoshita J, et al. Eicosapentaenoic acid reduces the progression of carotid intima-media thickness in patients with type 2 diabetes. *Atherosclerosis.* (2007) 191:162–7. doi: 10.1016/j.atherosclerosis.2006.03.005

33. Woodman RJ, Mori TA, Burke V, Puddey IB, Barden A, Watts GF, et al. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis.* (2003) 166:85–93. doi: 10.1016/S0021-9150(02)00307-6

34. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on coronary restenosis, intima–media thickness, and exercise tolerance: a systematic review. *Atherosclerosis.* (2006) 184:237–46. doi: 10.1016/j.atherosclerosis.2005.06.042

35. Natto ZS, Yaghmoor W, Alshaeri HK, Van Dyke TE. Omega-3 fatty acids effects on inflammatory biomarkers and lipid profiles among diabetic and cardiovascular disease patients: a systematic review and meta-analysis. *Sci Rep.* (2019) 9:1–10. doi: 10.1038/s41598-019-54535-x

36. Chauhan S, Kodali H, Noor J, Ramteke K, Gawai V. Role of omega-3 fatty acids on lipid profile in diabetic dyslipidaemia: single blind, randomised clinical trial. *J Clin Diagn Res JCDR.* (2017) 11:OC13. doi: 10.7860/JCDR/2017/20628.9449

37. Hoy SM, Keating GM. Omega-3 ethylester concentrate. *Drugs.* (2009) 69:1077–105. doi: 10.2165/00003495-200969080-00008

38. Davidson MH. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am J Cardiol.* (2006) 98:27–33. doi: 10.1016/j.amjcard.2005.12.024

39. Shinnakasu A, Yamamoto K, Kurano M, Arimura H, Arimura A, Kikuti A, et al. The combination therapy of fenofibrate and ezetimibe improved lipid profile and vascular function compared with statins in patients with type 2 diabetes. *J Atheroscler Thromb.* (2017) 2017:39446. doi: 10.5551/jat.39446

40. Morvaridzadeh M, Sepidarkish M, Yavari M, Tahvilian N, Heydarian A, Khazdouz M, et al. The effects of omega-3 fatty acid supplementation on inflammatory factors in HIV-infected patients: a systematic review and meta-analysis of randomized clinical trials. *Cytokine.* (2020) 136:155298. doi: 10.1016/j.cyto.2020.155298

41. Dezfouli M, Moeinzadeh F, Taheri S, Feizi A. The effect of omega-3 supplementation on serum levels of inflammatory biomarkers and albumin in hemodialysis patients: a systematic review and meta-analysis. *J Renal Nutr.* (2020) 30:182–8. doi: 10.1053/j.jrn.2019.06.007

42. Sinisalo J, Paronen J, Mattila KJ, Syrjälä M, Alfthan G, Palosuo T, et al. Relation of inflammation to vascular function in patients with coronary heart disease. *Atherosclerosis.* (2000) 149:403–11. doi: 10.1016/S0021-9150(99)00333-0

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 26 July 2022
DOI 10.3389/fnut.2022.948655

OPEN ACCESS

EDITED BY
MoezAllslam Ezzat Faris,
University of Sharjah, United Arab Emirates

REVIEWED BY
Faiza Kalam,
Northwestern University, United States
Nahla Al-Bayyari,
Al-Balqa Applied University, Jordan

*CORRESPONDENCE
Azita Hekmatdoost
a_hekmat2000@yahoo.com

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 20 May 2022
ACCEPTED 04 July 2022
PUBLISHED 26 July 2022

CITATION
Kord Varkaneh H, Salehi sahlabadi A, Găman M-A, Rajabnia M, Sedanur Macit-Çelebi M, Santos HO and Hekmatdoost A (2022) Effects of the 5:2 intermittent fasting diet on non-alcoholic fatty liver disease: A randomized controlled trial. *Front. Nutr.* 9:948655. doi: 10.3389/fnut.2022.948655

Effects of the 5:2 intermittent fasting diet on non-alcoholic fatty liver disease: A randomized controlled trial

Hamed Kord Varkaneh[1], Ammar Salehi sahlabadi[1], Mihnea-Alexandru Găman[2,3], Mohsen Rajabnia[4], Melahat Sedanur Macit-Çelebi[5], Heitor O. Santos[6] and Azita Hekmatdoost[1]*

[1]Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [3]Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest, Romania, [4]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Science, Tehran, Iran, [5]Department of Nutrition and Dietetics, Faculty of Health Sciences, Ondokuz Mayis University, Samsun, Turkey, [6]School of Medicine, Federal University of Uberlandia (UFU), Uberlândia, Brazil

Background and aims: Dietary regimens are crucial in the management of non-alcoholic fatty liver disease (NAFLD). The effects of intermittent fasting (IF) have gained attention in this regard, but further research is warranted. Thus, we aimed to ascertain the overall effects of the 5:2 IF diet (5 days a week of normal food intake and 2 consecutive fasting days) in patients with NAFLD compared to a control group (usual diet).

Methods and results: A 12-week randomized controlled trial was performed to evaluate the effects of the 5:2 IF diet on anthropometric indices, body composition, liver indices, serum lipids, glucose metabolism, and inflammatory markers in patients with NAFLD. The IF group ($n = 21$) decreased body weight (86.65 ± 12.57–82.94 ± 11.60 kg), body mass index (30.42 ± 2.27–29.13 ± 1.95 kg/m^2), waist circumference (103.52 ± 6.42–100.52 ± 5.64 cm), fat mass (26.64 ± 5.43–23.85 ± 5.85 kg), fibrosis (6.97 ± 1.94–5.58 ± 1.07 kPa), steatosis scores/CAP (313.09 ± 25.45–289.95 ± 22.36 dB/m), alanine aminotransferase (41.42 ± 20.98–28.38 ± 15.21 U/L), aspartate aminotransferase (34.19 ± 10.88–25.95 ± 7.26 U/L), triglycerides (171.23 ± 39.88–128.04 ± 34.88 mg/dl), high-sensitivity C-reactive protein (2.95 ± 0.62 –2.40 ± 0.64 mg/L), and cytokeratin-18 (1.32 ± 0.06–1.19 ± 0.05 ng/ml) values compared to the baseline and the end of the control group ($n = 23$)—$p \leq 0.05$ were considered as significant. However, the intervention did not change the levels of high-density lipoprotein cholesterol, total cholesterol, low-density lipoprotein cholesterol, fasting blood sugar, insulin, HOMA-IR, and total antioxidant capacity.

Conclusion: Adhering to the 5:2 IF diet can reduce weight loss and related parameters (fat mass and anthropometric indicators of obesity), as well as hepatic steatosis, liver enzymes, triglycerides, and inflammatory biomarkers in patients with NAFLD.

KEYWORDS

non-alcoholic fatty liver disease, intermittent fasting (5:2), liver enzymes, time-restricted eating, fasting

Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the most prevalent liver disorders worldwide and is believed to affect nearly a quarter of the global population, translating into an increased economic burden on healthcare systems (1). Although genetic factors also play a pivotal role in the development of NAFLD, a major contribution to its pathogenesis is attributed to modifiable risk factors, i.e., obesity, metabolic syndrome, and diabetes (2, 3). As patients diagnosed with NAFLD are prone to developing liver cirrhosis as well as hepatocellular carcinoma, urgent measures are needed to combat the spread of the NAFLD epidemic (4–7), particularly as there are no approved pharmacotherapies for this chronic hepatic disorder.

Dietary interventions remain an attractive strategy in the management of NAFLD and cardiometabolic problems, especially due to the improvement in indicators of adiposity, serum lipids, glycemic control, and blood pressure (8–13). In recent years, IF regimens have gained massive popularity in the field of clinical nutrition as a method of energy restriction, possibly because patients are more likely to accept and adhere to lifestyle changes than taking pills daily (14–17). The 5:2 diet is an emerging type of periodic fasting in which individuals fast 2 days a week and eat freely for 5 days a week (18, 19). Time-restricted feeding and the 5:2 diet are all close to mandatory and voluntary Islamic fasting, with time differences (20, 21). More specifically, regarding the adherence to the 5:2 diet, there is substantial energy restriction on the fasting days, in which it is common to prescribe 0–25% of the estimated total daily energy requirements on 2 non-consecutive days of the week without water restriction, while the habitual intake (*ad libitum*) or 100% of estimated total daily energy requirements are used for the remaining 5 days (19).

Randomized controlled trials (RCTs) have examined the effects of the 5:2 diet in different populations. Fudla et al. reported that participants with obesity on the 5:2 diet for 1 month experienced a reduction in body mass index (BMI) (22). Increasing attention has been paid to the effects of IF regimens on the management of NAFLD, as shown by a systematic review and meta-analysis of RCTs supporting reductions in body weight, BMI, as well as liver enzymes in individuals on IF diets (Ramadan fasting, alternate-day fasting, and time-restricted feeding) for 4–12 weeks (23). More recently, Holmer et al. (7) found that adherence to the 5:2 diet improves indicators of obesity, lipid and glycemic indices, and hepatic steatosis in patients with NAFLD (7). However, Holmer et al. did not assess body composition and inflammatory biomarkers, and did not calculate the basal metabolic rate individually; instead, they used general energy intake recommendations based on sex (7).

Thus, we conducted a 12-week RCT to examine the effects of the 5:2 diet on patients with NAFLD, employing assessment of traditional parameters (imaging and blood tests) of this disease, cardiometabolic and inflammatory biomarkers, body composition, and anthropometric indicators of obesity. Our hypothesis was that the 5:2 diet could significantly improve some cardiometabolic and hepatic markers due to the accompanying weight loss.

Methods and materials

We recruited patients diagnosed with NAFLD and overweight/obesity between October 2019 and May 2020 from the Gastroenterology and Liver Diseases Research Center, Tehran, Iran. The following inclusion criteria were applied: BMI = 25–40 kg/m^2, age between 18 and 50 years (as middle-aged and elderly participants are less available for fasting), grade 2 NAFLD, —i.e., a controlled attenuation parameter (CAP) score $\geq$ 260 dB/m and $\geq$ 34% fatty change, with liver stiffness measurement (LSM) levels $<$ 14 kPa (24)—, willingness to participate in the RCT, and no recent participation in a weight loss diet program.

Based on the BMI range, there was exclusion of participants at underweight and healthy levels, as well as Class 3 obesity, to provide greater uniformity. For instance, the patients at Class 3 obesity generally require more calorie in a traditional dietary model, and, thus, enrollment in an IF regimen is difficult in virtue of the shorter feeding window. Regarding the age range, we aimed to avoid middle-aged and elderly participants due to the lower availability of this population for fasting or adherence to new dietary models that yield habitual changes. In addition,

the patients with NAFLD Grade 3 were not included because IF regimens putatively might be dangerous for this population due to the pathophysiological link between the very high liver accumulation of triglycerides (TG) and its worsening with rapid mobilization and oxidation of TG in this organ during fasting (25, 26).

Regarding medication use, the patients should not have used antibiotics for more than 1 week during the study period or prior to enrollment, and should not have consumed herbal medicines, anti-inflammatory drugs, corticosteroids/any type of hormone, weight-loss drugs, or hepatotoxic drugs, e.g., phenytoin, amoxicillin, and lithium. In addition, the patients should not be alcohol consumers or diagnosed with cardiovascular diseases, stroke, diabetes, acute liver disorders (hepatitis B, C, etc.), kidney diseases, chronic inflammatory disorders, depression, cancer, or autoimmune diseases. The RCT only included the participants who met the aforementioned criteria and who were willing to adhere to a 12-week program of the 5:2 IF diet.

We applied the following exclusion criteria: weight loss $\geq$ 10% or weight gain $\geq$ 5% in the last 6 months, use of pharmacological agents that can affect metabolism or liver function (e.g., lipid-lowering or glucose-lowering drugs, drugs used to control blood pressure) or supplements/substances that may interact with the impact of diet during the intervention.

Before enrollment in the RCT, all the patients had signed informed consent form. We registered the RCT at www.irct.ir (IRCT20100524004010N31). This study was approved by the Ethics Committee of the Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.NNFTRI.REC.1399.019).

Study design and randomization

Participant recruitment

The study was designed as an RCT, with blinding for randomization at the study clinic. Eligible individuals suffering from NAFLD were stratified based on age and BMI and then randomized using a computer-generated random-numbers method to the IF (5:2) group ($n = 24$) or a non-interventional control group ($n = 25$).

Sample size

The number of samples required for this study was calculated based on different dependent variables. The highest number of samples was obtained for the cap score-dependent variable. The sample size for this study was based on how many samples should be selected so that the average cap score difference between the 5:2 diet and the control group is at least 25 units per liter. This difference when reaching a probability of 95% ($\alpha = 0.05$) and power of 80% ($\beta = 20\%$) was considered statistically significant. The number of samples for each group was estimated to be 21 patients. The sample size was calculated based on the deviation from the criteria obtained in a previous study [86] and using the following formulas.

$$
\begin{aligned}
n_1 &= 8S_1 = 34 \\
n_2 &= 8S_2 = 23 \\
\mu_1 &= \mu_2 = 25 \\
Z_{1-\alpha/2} &= = 1/96 \\
Z_{1-\beta} &= 0/84 \\
S_p^2 &= \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} = 842/5 \\
n &= \frac{2S^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2} = 21
\end{aligned}
$$

Dietary interventions

The intervention group was allocated to adhere to the 5:2 diet, i.e., 5 days a week of normal food intake and 2 consecutive fasting days (Monday and Tuesday) a week for 12 weeks. On fasting days, the patients received 25% of the recommended calorie intake over a 2-h period from 12:00 to 2:00 p.m. Such a calorie goal was divided into 30% calories from fat, 15% calories from proteins, and 55% calories from carbohydrates.

The 5:2 diet was selected as it seemed more convenient when compared to other IF regimens, e.g., alternate-day or Ramadan-style fasting. The patients allocated to the control group were asked to follow to their usual diet. Data were evaluated by a registered dietitian before the start of the RCT, whose professionals were responsible for body composition, questionnaires, and dietary prescription.

The caloric intake of the patients was evaluated using the Mifflin-St Jeor Equation (27). For both groups, the intake of macronutrients was: 30% calories from fat, 15% calories from proteins, and 55% calories from carbohydrates. Consumption of non-caloric fluids, e.g., water, coffee, or tea, was allowed for the intervention and control groups, and the patients were advised to drink water without restriction during the RCT. Thus, these non-caloric fluids were allowed for fasting and feeding periods.

A diet plan for 3 months was prescribed for each subject. At the beginning of the RCT, all the patients were instructed on how to adhere to the diet and were monitored during the study by weekly phone calls and monthly interviews based on three 24-h recalls. The patients who did not adhere to the diet were dropped from the RCT.

Measurements

All the patients had the body weight, height, waist circumference (WC), and body composition measured at the beginning and the end of the RCT. Fasting blood samples were collected in the morning after 10–12 h of fasting. Liver steatosis and fibrosis were evaluated using the FibroScan® 502 Touch device at the beginning of the RCT end of 12 weeks. In addition, physical exercise questionnaires (28), 24-h recalls (29, 30), and questionnaires related to patients' demography data were completed. Nutritionist IV software was employed to assess the dietary energy and macronutrient content.

Anthropometric measurements and body composition

We evaluated body weight using a digital scale (Seca 808, Germany; ±0.1 kg accuracy), with patients wearing light clothing and barefooted. WC was assessed at the midpoint between the 12th rib and the iliac crest and during exhalation. The standing height of the patients was evaluated to the nearest 0.5 cm, without shoes, using a wall stadiometer (Seca, Germany) and by standard procedures.

We assessed fat mass, lean body mass (LBM), and total body water (TBW) using bioelectrical impedance analysis (BIA) (Tanita-BC 418 MA, Arlington Heights, USA) at the beginning and the end of the RCT. The evaluation was performed after 12 h of fasting. The patients were asked not to perform physical exercise 12 h before the test to empty the urinary bladder 30 min before the measurements and to remove metallic objects immediately before the test. The assessment was performed outside the menstrual period for women of childbearing age as well.

Blood sample measurements

We collected blood samples (10 ml) from the participants between 7 and 10 A.M., then the probes were centrifuged at 2,000 g (RCF) at room temperature for 20 min. Serum biomarkers were assessed at the beginning and the end of the RCT.

Liver enzymes [alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT)] and the lipid profile [total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, and TG] were measured following standard procedures recommended by Delta-dp diagnostic kits (Roche, Germany).

Inflammatory biomarkers [high-sensibility C-reactive protein (hs-CRP) and cytokeratin-18 (CK-18)] and total antioxidant capacity were evaluated using the ELISA method (ZellBio GmbH, Ulm, Germany).

FBS concentrations were assessed using an auto-analyzer by the glucose oxidase method (Cobas c311, Roche Diagnostics, Risch-Rotkreuz, Switzerland). Serum insulin levels were assessed using ELISA kits (Monobind Inc., Lake Forest, California, USA). In order to assess insulin resistance, we calculated the homeostasis model assessment of insulin resistance (HOMA-IR) by using the following formula: fasting insulin (μU/L) x fasting glucose (nmol/L)/22.5.

Statistical analysis

SPSS software version 22 was employed to compute the statistical analysis. Data were reported as mean ± standardized deviation (SD). The Kolmogorov-Smirnov test was employed to evaluate dara compliance with the normal distribution. We only evaluated data from the patients who completed the RCT (i.e., per-protocol analysis).

One-way ANOVA and independent samples *t*-tests were used to compare quantitative variables between groups at the baseline. The chi-squared test was employed to evaluate qualitative variables. An independent sample *t*-test was employed to compare the quantitative variables between groups before and after the intervention.

The Student's *T*-test/paired samples *t*-test was employed to compare quantitative variables within each of the two groups before and after the intervention. Analysis of covariance (ANCOVA) was performed to compare the mean of variables between groups by adjusting for confounding factors (BMI, body weight; WC, metabolic equivalents; dietary energy intake; and baseline value of the outcomes). The statistical significance was set at $p \leq 0.05$.

Results

Three patients dropped out of the intervention group and three patients dropped out of the control group during the 12-week RCT. A total of 21 and 23 individuals completed the intervention and control group procedures, respectively (Figure 1). The mean age, BMI, WC, and body weight of the patients were 45.25 ± 9.84 years, 30.51 ± 2.7 kg/m^2, 105.59 ± 8.11 cm, and 88.05 ± 11.88 kg, respectively. The baseline characteristics of the patients were similar between the intervention and control groups (Table 1).

Anthropometric indices and body composition

Data regarding the anthropometric indices and body composition of the patients are depicted in Table 1. Following the intervention, the individuals who underwent a 5:2 diet experienced a significant decrease in body weight (−3.7 kg on

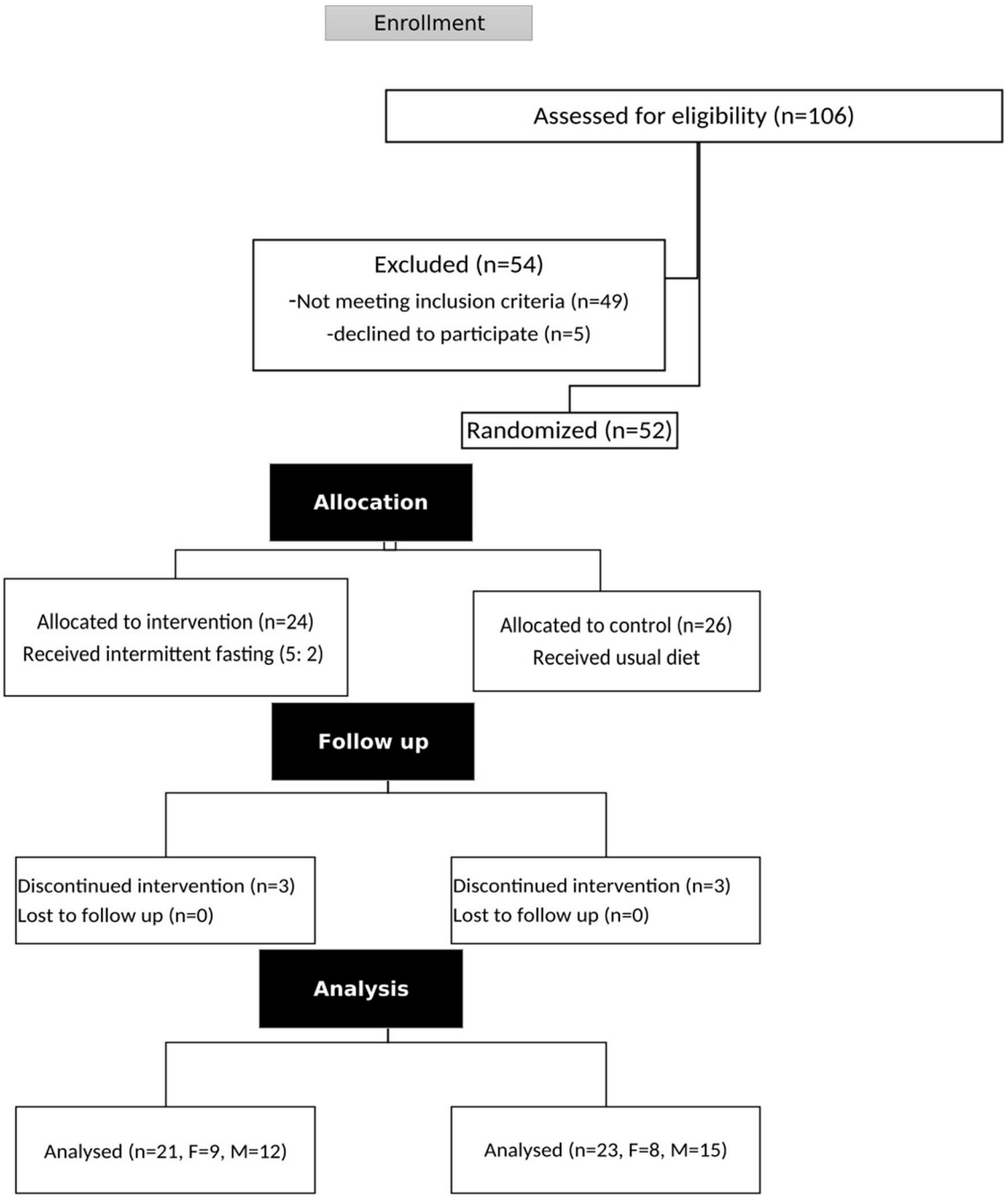


FIGURE 1
Flowchart of the participants evaluated.

average; 86.65 ± 12.57 kg to 82.94 ± 11.60 kg, $p < 0.001$), while there was a non-significant reduction in the control group (−1.02 kg on average, 89.33 ± 18.47 to 88.31 ± 11.01 kg, $p = 0.06$). Moreover, the individuals on the 5:2 diet experienced a significant decrease in BMI (30.42 ± 2.27–29.13 ± 1.95 kg/m^2), WC (103.52 ± 6.42–100.52 ± 5.64 cm), and fat mass (59.97 ± 10.75–59.09 ± 10.37 kg) vs. the control group.

Nutritional intake and physical activity

Information on dietary intake and physical activity (MET.hr/day) of participants is depicted in Table 2, where the dietary data at the end for the intervention group were an average of 5 days of feeding and 2 days of fasting. The intervention and control groups had similar physical activity

TABLE 1 Baseline characteristics, and anthropometric indices of the participants.

Characteristics	Intermittent fasting (5:2) diet ($n = 21$)	Control diet ($n = 23$)	P-value[b]
Age (years)	46.42 ± 13.35	44.17 ± 4.9	0.454
Gender n (%)			
Male	12 (57.1)	15 (65.2)	0.405
Female	9 (42.9)	8 (34.8)	
Smoking n (%)			
Yes	2(9.5)	2(8.7)	0.661
No	19(90.5)	21(91.3)	
Anthropometric indices			
Height (cm)	168.47 ± 10.68	170.91 ± 9.83	0.435
Body weight (kg)			
Before	86.65 ± 12.57	89.33 ± 18.47–88.31 ± 11.01	0.461
After	82.94 ± 11.60	88.31 ± 11.01	0.123
P-value[a]	<0.001	0.064	
BMI (kg/m^2)			
Before	30.42 ± 2.27	30.60 ± 30.09	0.826
After	29.13 ± 1.95	30.26 ± 3.08	0.158
P-value[a]	<0.001	0.063	
Waist circumference (cm)			
Before	103.52 ± 6.42	107.09 ± 9.47	0.107
After	100.52 ± 5.64	107.19 ± 8.93	0.006
P-value[a]	0.001	0.928	
Lean body mass (kg)			
Before	59.97 ± 10.75	62.10 ± 11.29	0.525
After	59.09 ± 10.37	59.33 ± 12.28	0.854
P-value[a]	0.565	<0.0001	
Body fat (kg)			
Before	26.64 ± 5.43	27.21 ± 7.33	0.762
After	23.85 ± 5.85	28.93 ± 7.91	0.025
P-value[a]	0.039	0.041	
Total body water (kg)			
Before	43.76 ± 8.21	45.11 ± 8.57	0.140
After	43.11 ± 7.37	43.19 ± 7.87	0.136
P-value[a]	0.773	0.001	

BMI, body mass index.
Values are expressed as mean ± SD.
[a]Within-group comparison (a paired samples t-test was used for testing two values within group).
[b]Between-group comparison (an independent sample t-test was used for quantitative variables).

levels at the end of intervention vs. the baseline (the intervention group: 29.48 ± 5.60 vs. 29.99 ± 5.15 MET.hr/day, $p > 0.05$; the control group: 30.38 ± 5.13 vs. 30.52 ± 5.19 MET.hr/day, $p > 0.05$). Inter-group differences were not detected. Both intervention and control groups had a significant decrease in total energy intake vs. the baseline (the intervention group: 2,862.95 ± 721.60–2,601.57 ± 726.55 kcal/day, $p < 0.001$; the control group: 3,120.91 ± 733.27–2,967.30 ± 665.80 kcal/day, $p = 0.001$). No significant inter-group differences were detected. The intervention group decreased total protein intake vs. the baseline (93.38 ± 35.32–84.67 ± 25.74 g/day, $p < 0.001$) but did not change for other dietary variables.

Liver parameters

The results of the assessment of liver parameters are reported in Table 3. Fibrosis and steatosis scores decreased after the 5:2 diet (6.97 ± 1.94–5.58 ± 1.07 kPa and 313.09 ± 25.45–289.95 ± 22.36 dB/m, respectively) and reached lower levels than the control group ($p = 0.040$ and $p = 0.042$, respectively). In

TABLE 2 Dietary intakes and physical activity.

Variables	Baseline	End of the study*	*P*-value[a]
Physical activity (MET hr/day)			
Intervention	29.48 ± 5.60	29.99 ± 5.15	0.189
Control	30.38 ± 5.13	30.52 ± 5.19	0.245
P-value[b]	0.531	0.801	
Total energy (kcal/day)			
Intervention	2,862.95 ± 721.60	2,601.57 ± 726.55	<0.001
Control	3,120.91 ± 733.27	2,967.30 ± 665.80	0.001
P-value[b]	0.247	0.089	
Total carbohydrate (g/day)			
Intervention	459.00 ± 127.81	433.29 ± 106.48	0.096
Control	446.17 ± 109.71	437.00 ± 115.66	0.597
P-value[b]	0.722	0.913	
Total protein (g/day)			
Intervention	93.38 ± 35.32	84.67 ± 25.74	0.022
Control	112.96 ± 28.47	107.57 ± 23.34	0.164
P-value[b]	0.048	0.003	
Total fat (g/day)			
Intervention	104.90 ± 34.55	102.67 ± 37.20	0.634
Control	116.39 ± 35.36	111.83 ± 28.07	0.408
P-value[b]	0.283	0.359	
Dietary fiber (g/day)			
Intervention	48.05 ± 11.26	43.38 ± 9.64	0.170
Control	47.96 ± 14.54	48.83 ± 13.07	0.742
P-value[b]	0.982	0.126	

MET, metabolic equivalents.
[a]Within-group comparison (a paired samples t-test was used for testing two values within group).
[b]Between-group comparison (an independent sample t-test was used for quantitative variables).
*Dietary data at the end for the intervention group were an average of 5 days of feeding and 2 days of fasting.

addition, the patients on the 5:2 diet had a significant reduction in ALT (41.42 ± 20.98–28.38 ± 15.21 U/L, $p = 0.043$) and AST (34.19 ± 10.88–25.95 ± 7.26 U/L, $p = 0.01$) levels vs. the control group ($p < 0.05$).

Lipid profile

TG concentrations (171.23 ± 39.88 mg/dL to 128.04 ± 34.88 mg/dL, $p < 0.001$), but not HDL-C, TC, or LDL-C, decreased significantly in the intervention vs. the control group ($p < 0.001$) (Table 3).

Glycemic indices

The decrease in FBS values in the intervention group (106.31 ± 28.11–101.11 ± 20.46 mg/dL) was not statistically significant vs. the control group ($p = 0.52$). Moreover, insulin levels and HOMA-IR values did not change significantly in the intervention or control group (Table 3).

Inflammatory biomarkers

After the intervention, the participants on the 5:2 diet exhibited a decrease in hs-CRP (2.95 ± 0.62–2.40 ±0.64 mg/L, $p < 0.001$) and CK-18 (13.23 ± 0.61–11.92 ±0.53 ng/ml, $p < 0.001$) levels vs. the control group (Table 3). On the other hand, the total antioxidant capacity did not change significantly following the RCT.

Discussion

This RCT shows that the 5:2 diet can be a non-pharmacological tool in the management of NAFLD by improving not only specific markers of NAFLD but also several cardiometabolic parameters. More specifically, we demonstrated significant reductions in body weight, BMI, WC, body fat, ALT, AST, fibrosis and steatosis scores, TG, hs-CRP (mg/L), and CK-18 (ng/ml) after 12 weeks on the 5:2 diet. On the other hand, the 5:2 diet did not alter the levels of HDL-C, TC, LDL-C, glycemic markers (FBS, insulin, and HOMA-IR), and total

TABLE 3 Lipid profile, glycemic indices, liver enzymes, inflammatory biomarkers, and indices of hepatic steatosis and fibrosis.

5:2 Intermittent fasting diet				Control diet			*P*-value[b]
Variables	**Baseline**	**End**	***P*-value[a]**	**Baseline**	**End**	***P*-value[a]**	
Liver parameters							
ALT (U/L)	41.42 ± 20.98	28.38 ± 15.21	0.043	30.34 ± 5.13	28.04 ± 8.12	0.144	0.044
AST (U/L)	34.19 ± 10.88	25.95 ± 7.26	0.013	23.39 ± 8.13	23.77 ± 9.66	0.969	0.015
GGT (U/L)	31.09 ± 26.25	19.52 ± 7.15	0.053	34.77 ± 12.93	39.22 ± 36.95	0.532	0.080
Fibrosis score (kPa)	6.97 ± 1.94	5.58 ± 1.07	0.009	5.82 ± 1.44	5.46 ± 1.32	0.072	0.040
Steatosis score/CAP dB/m	313.09 ± 25.45	289.95 ± 22.36	<0.001	311.52 ± 33.65	306.00 ± 37.35	0.342	0.042
Lipid profile							
TG (mg/dL)	171.23 ± 39.88	128.04 ± 34.88	<0.001	187.6 ± 73.61	199.56 ± 87.43	0.512	<0.001
TC (mg/dL)	165.38 ± 26.06	164.00 ± 22.83	0.865	172.21 ± 37.99	180.72 ± 49.49	0.425	0.285
HDL-C (mg/dL)	41.22 ± 11.64	38.68 ± 11.13	0.079	34.82 ± 7.65	33.73 ± 6.70	0.290	0.167
LDL-C (mg/dL)	90.11 ± 26.50	93.07 ± 28.91	0.551	93.73 ± 31.77	97.45 ± 35.46	0.734	0.015
Glycemic indices							
FBS (mg/dL)	106.31 ± 28.11	101.11 ± 20.46	0.321	102.82 ± 11.71	105.78 ± 14.16	0.230	0.523
Insulin (mU/L)	10.95 ± 3.24	12.18 ± 4.26	0.058	10.78 ± 3.78	12.15 ± 5.25	0.219	0.898
HOMA-IR	3.02 ± 1.34	2.95 ± 1.35	0.528	2.77 ± 1.11	3.15 ± 1.41	0.269	0.249
Inflammatory and antioxidant biomarkers							
hs-CRP (mg/L)	2.95 ± 0.62	2.40 ± 0.64	<0.001	2.72 ± 1.04	2.75 ± 1.13	0.716	<0.001
CK-18 (ng/mL)	13.23 ± 0.61	11.92 ± 0.53	<0.001	13.24 ± 2.76	18.56 ± 3.54	<0.001	<0.001
TAC (mmol/L)	0.35 ± 0.06	0.33 ± 0.09	0.341	0.30 ± 0.8	0.32 ± 0.0.09	0.451	0.296

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CK-18, cytokeratin-18; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitive C-reactive protein; LDL-C, low-density lipoprotein cholesterol; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides.

[a]Within-group comparison (a paired samples t-test was used for testing two values within group).

[b]Analysis based on ANCOVA model for assessing the regression relationship of variables between the groups at the end of the study after adjustments for body mass index, weight, waist circumference, metabolic equivalents, dietary energy intake, and baseline value of the outcome.

antioxidant capacity. Taken together, these results are in line with our hypothesis, given that we expected some improvements in cardiometabolic and hepatic markers with accompanying weight loss and decreased adiposity. So much so that massive research shows beneficial effects of different IF regimens in this regard (31–37).

Different types of IF regimens have been used to improve body composition and anthropometric indices in patients with NAFLD, from the Ramadan fasting to the 5:2 diet. Ramadan is a type of IF regimen that can reduce body weight, body fat, BMI, and WC in patients with NAFLD compared to non-fasting (38), but, overall, our results tend to be better due to the longer length of intervention since Ramadan lasts approximately 4 weeks. Our findings are more similar to the study by Holmer et al. (7), who performed an RCT for patients with NAFLD ($n = 74$) and observed a decrease in body weight by 7.4 kg and in BMI by 2.4 kg/m² over a 12-week period on a 5:2 diet, whose effect was similar to a low-carbohydrate high-fat diet and better than a standard of care (7). We observed a lower decrease in body weight (−3.7 kg) and BMI (−1.3 kg/m^2) lower than in the study by Holmer et al. This was probably because there was a mean reduction of 590 kcal/d at the end of the study by Holmer et al., whereas we observed a mean reduction of 260 kcal. It must be noted that Holmer et al. prescribed the 5:2 diet, guiding a daily intake of 500 kcal for 2 non-consecutive days a week, followed by a general recommendation based on an intake limit of 2,000 kcal/d for women and 2,400 kcal/d for men, while we estimated the patients' basal metabolic rate using the Mifflin-St Jeor Equation and, therefore, providing more reliable data in terms of individual care. Moreover, we observed reductions in WC (−3 cm) and fat mass (−2.8 kg), while, seemingly, Holmer et al. did not assess these parameters.

It is important to note that both the intervention and control groups had a significant decrease in total energy intake compared to the baseline (the intervention group: ∼2,863– ∼ 2,602 kcal/day, $p < 0.001$; the control group: ∼3,121–2,967 kcal/day, $p = 0.001$), but without the inter group differences ($p < 0.05$). In summary, there was a modest reduction in calorie intake in both groups (261 kcal and 154 kcal for intervention and control groups, respectively). Although the control group was instructed to consume the habitual diet, there was a reduction in calorie intake that probably may have occurred because the participants were part of a clinical study. However, reduced calorie intake on the 5:2 diet had a greater clinical magnitude than the control group regardless of the absence of significant intergroup difference, as the 5:2 diet was more effective in improving body composition and laboratory parameters. It is crucial to highlight that we adjusted the intergroup variables

at the end of the study for BMI, body weight, WC, and energy intake.

Reducing energy intake can be considered the main mechanism leading to improvement in adiposity parameters and cardiometabolic markers (38). Decreased energy intake within an IF diet plan may promote mobilization of free fatty acids, increase fat oxidation and production of ketones, as well as can improve circadian rhythm by modulating clock genes and anti-inflammatory molecules with a myriad of metabolic improvements (e.g., melatonin) when the dietary strategy creates a health routine for the subject (39–43). Regarding the molecular basis involving the interplay between liver and lipids, Santos and Macedo suggest that IF reduces the production of apolipoprotein B and thereby increases fatty acid oxidation and decreases hepatic TG content, yielding a reduction in circulating levels of very-low-density lipoprotein cholesterol, LDL-C, and small-dense LDL-C (44). The latter parameter shed light on the anti-atherogenic effects of IF diets, as the small-dense LDL-C represents the LDL particles with highest affinity for arterial damage (45). In contrast, there is a debate about the effects of IF regimens on improving lipid indices, as discussed thoroughly below.

Employing an alternate-day fasting, i.e., alternating between an *ad libitum* feeding day and a 75% energy-restricted fasting day, Cai et al. (46) found improvement in TC and TG within a relatively short length of intervention (4–12 weeks) in patients with NAFLD, but LDL-C and HDL-C levels did not change (46). Holmer et al. found a decrease in TG, TC, and LDL-C levels by 35, 19, and 14 mg/dL, respectively, in patients with NAFLD undergoing a 5:2 diet for 12 weeks, while HDL-C did not alter (7). In our study, we found a decrease in TG (∼43 mg/dL), with a clinical magnitude similar to the reduction by Holmer et al., but we did not observe changes in other lipid parameters.

In patients with NAFLD, liver tests (ALT, AST, alkaline phosphatase, and GGT) are routinely included in the clinical evaluation. We demonstrated a decrease in ALT and AST levels by 13 and 8 U/L, respectively, after the diet 5:2. Johari et al. (47), in turn, found a decrease in ALT and AST levels by 25 and 8 U/L, respectively, in patients with NAFLD after 8 weeks of alternate-day calorie restriction (47). The authors explained this reduction in liver enzymes by the improvement of visceral fat or steatosis in the liver (47). Likewise, we demonstrated improvement in steatosis and fibrosis scores alongside a decrease in GGT levels (∼11 U/L). Collectively, these results can be considered the central tenet of our research, as the primary goal of NAFLD treatment is to improve specific parameters of this ailment, which is secondarily associated with several metabolic benefits.

Increased levels of inflammatory biomarkers in patients with NAFLD might affect the pathogenesis of cardiovascular diseases. CRP is a protein produced by the liver under stimulation of pro-inflammatory cytokines, and the hs-CRP assay is a recommended marker of low-grade inflammation for screening the risk of cardiovascular diseases (48–50). It is no wonder that hs-CRP is a non-invasive complementary marker of NAFLD, and its high levels are common in this population due to the interplay between fatty liver and cardiometabolic problems (51). Aliasghari et al. (38) reported decreased levels of hs-CRP after Ramadan fasting (38). In our research, the group that underwent a 5:2 diet had lower levels of hs-CRP at the end of the study both compared to the baseline (−0.6 mg/L) and end levels of the control group (−0.4 mg/L). CK-18 is another inflammatory marker that circulates as a consequence of oxidative stress, hepatocyte apoptosis, or inflammation in NAFLD (52). In a meta-analysis, CK-18 levels proved to be an important tool for diagnosing NAFLD, especially non-alcoholic steatohepatitis (52), and, in our work, we showed a reduction in CK-18 levels after the 5:2 diet. The reduction in CK-18 levels after the 5:2 diet is not impressive, but, at least, it has a modest clinical magnitude, as we observed a reduction from ∼13.2 to 11.9 ng/ml, and a proposed CK-18 cutoff value to detect steatosis ($S \geq 2$) in NAFLD is 11.7 ng/ml (53).

Recently, in 2022, IF has been highlighted as a safe and effective tool to improve liver histology in NAFLD (54). Behind the scenes of histology, fasting-induced AMP-activated protein kinase (AMPK) is the main biological rationale in this regard, whose enzyme is a master regulator of energy metabolism that activates fatty acid oxidation and breakdown (54), but it is crucial to know that this mechanism is enhanced by carbohydrate restriction and mainly by skeletal muscle contraction during exercise (55). Hepatic steatosis and fibrosis are two of the most studied histological parameters due to their importance in disease diagnosis and staging (56). In patients with NAFLD, excess visceral fat lipids and accompanying inflammation sharply increase the risk of developing complications of chronic liver disease, such as cirrhosis, liver failure, and hepatocellular carcinoma (57). Advanced fibrosis is the most significant predictor of mortality in NAFLD (58). In our study, since 5:2 was associated with improved fibrosis and steatosis scores, we provide evidence that such a dietary regimen is a viable non-pharmacological strategy to improve key clinical parameters that are a result of NAFLD pathology, but RCTs performing liver biopsies are needed to analyze the NAFLD histology.

Type 2 diabetes is a recognized risk factor in the NAFLD severity (59). Patients with NAFLD who underwent Ramadan fasting had reduced FBS, insulin, and HOMA-IR levels but not in the non-fasting group (38). Overall, in a meta-analysis consisting of 12 articles (545 participants), IF diets were associated with a significant decline in FBS (weighted mean difference, −4.16 mg/dL; 95% confidence interval, −6.92– −1.40) when compared with a control diet (60). In our study, we did not find significant changes in FBS, insulin, and HOMA-IR. These null effects may be a result of many patients in our sample being normoglycemic, but mean FBS levels were within the range of pre-diabetes, and this fact cannot be ruled out. At best, there was a non-statistical decrease in FBS by ∼5 mg/dl after the 5:2 diet that

nearly corrected mean FBS levels from the pre-diabetic stage to normoglycemia (from ∼106 to ∼101 mg/dl).

Current guidelines suggest that dietary intervention and exercise are first-line therapy for NAFLD (61, 62). Correspondingly, the European Association for the Study of the Liver (EASL) guidelines focus on lifestyle changes through diet and habitual physical activity and emphasize that diet-induced weight loss is the only treatment to ameliorate liver damage without severe liver fibrosis (63). Given that these guidelines recommend a pragmatic and individualized approach and do not advocate any specific diet for the treatment of NAFLD, our findings are noteworthy as a means of expanding the effect of IF regimens as one of many dietary strategies that can be employed through a personalized perspective, but this does not imply that IF is the best dietary approach. Bearing in mind the importance of personalized dietary plans, the addition of functional foods and supplements could also enhance the effect of IF or other dietary patterns in the management of liver parameters and cardiometabolic markers (64–70).

As for strengths, our data reach different spheres of medicine, as we observed improvement in several laboratory markers with clinical relevance. For instance, (i) ALT and AST are biomarkers for screening liver function; (ii) fibrosis and steatosis are important determinants of NAFLD diagnosis (71); (iii) associated atherogenic dyslipidemia is often observed with increased plasma levels of TG in patients with NAFLD, and cardiovascular diseases are the most common causes of mortality in this population (72); (iv) hs-CRP is recommended for coronary risk assessment in adults and is significantly higher in patients with NAFLD (51); (v) CK-18 (ng/ml) is one of the biomarkers in the circulation as a result of oxidative stress, hepatocyte apoptosis, or inflammation in response to lipid metabolism disorders because of NAFLD (52). In addition, we assessed body composition alongside anthropometric indicators of obesity that is easily replicated in clinical practice and used in nutrition science (73–76).

As for limitations, we did not use gold-standard methods for body composition and energy expenditure nor did we perform liver biopsies. In addition, we used a physical activity questionnaire to assess physical activity. Ultimately, we encourage further research focusing on NAFLD and accompanying diseases, such as dyslipidemia and diabetes, to better expand the clinical magnitude of the 5:2 diet and other types of IF regimens.

Conclusion

The 5:2 diet can reduce weight loss and related parameters (fat mass and anthropometric indicators of obesity), as well as hepatic steatosis, liver enzymes, TG, and inflammatory biomarkers (hs-CRP and CK-18) in patients with NAFLD. However, the intervention did not change the levels of HDL-C, TC, LDL-C, glycemic markers, and total antioxidant capacity. In other words, the 5:2 diet can be a non-pharmacological tool in the management of NAFLD by improving not only specific markers of NAFLD but also several cardiometabolic parameters. Nevertheless, the 5:2 diet cannot be considered the best dietary regimen in this regard, but deserves attention as one of the many strategies that integrate the arsenal of nutrition-based approaches.

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 studies involving human participants were reviewed and approved by Shahid Beheshti University of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HK, AH, MR, and AS conducted the research, analyzed the data, and performed statistical analysis. HS, M-AG, and MS contributed to the writing, design, and revision of the manuscript. AH contributed to the research design. All authors contributed to the article and approved the submitted version.

Funding

This work was supported financially by the National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant Number: 1000328).

Acknowledgments

The authors thank all the volunteers who participated in the study.

Conflict of interest

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

Publisher's note

References

1. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109

2. Godoy-Matos AF, Silva WS Jr, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr.* (2020) 12:60. doi: 10.1186/s13098-020-00570-y

3. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: is insulin resistance the link? *Mol Cell Endocrinol.* (2015) 418 Pt 1:5565. doi: 10.1016/j.mce.2015.02.018

4. Jichitu A, Bungau S, Stanescu AMA, Vesa CM, Toma MM, Bustea C, et al. Non-alcoholic fatty liver disease and cardiovascular comorbidities: pathophysiological links, diagnosis, and therapeutic management. *Diagnostics.* (2021) 11:689. doi: 10.3390/diagnostics11040689

5. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med.* (2018) 24:908–22. doi: 10.1038/s41591-018-0104-9

6. Gheorghe G, Bungău S, Ceobanu G, Ilie M, Bacalbașa N, Bratu OG, et al. The non-invasive assessment of hepatic fibrosis. *J Formos Med Assoc.* (2021) 120:794–803. doi: 10.1016/j.jfma.2020.08.019

7. Holmer M, Lindqvist C, Petersson S, Moshtaghi-Svensson J, Tillander V, Brismar TB, et al. Treatment of NAFLD with intermittent calorie restriction or low-carb high-fat diet - a randomised controlled trial. *JHEP Rep.* (2021) 3:100256. doi: 10.1016/j.jhepr.2021.100256

8. Khodami B, Hatami B, Yari Z, Alavian SM, Sadeghi A, Varkaneh HK, et al. Effects of a low free sugar diet on the management of nonalcoholic fatty liver disease: a randomized clinical trial. *Eur J Clin Nutr.* (2022) 76:987–94. doi: 10.1038/s41430-022-01081-x

9. Sohouli MH, Fatahi S, Sharifi-Zahabi E, Santos HO, Tripathi N, Lari A, et al. The impact of low advanced glycation end products diet on metabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. *Adv Nutr.* (2021) 12:766–76. doi: 10.1093/advances/nmaa150

10. Santos HO, Penha-Silva N. Translating the advanced glycation end products (AGEs) knowledge into real-world nutrition strategies. *Eur J Clin Nutr.* (2021) 76:922–8. doi: 10.1038/s41430-021-01028-8

11. Santos HO, Lavie CJ. Weight loss and its influence on high-density lipoprotein cholesterol (HDL-C) concentrations: a noble clinical hesitation. *Clin Nutr ESPEN.* (2021) 42:90–2. doi: 10.1016/j.clnesp.2021.01.033

12. Lari A, Sohouli MH, Fatahi S, Cerqueira HS, Santos HO, Pourrajab B, et al. The effects of the dietary approaches to stop hypertension (DASH) diet on metabolic risk factors in patients with chronic disease: a systematic review and meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis.* (2021) 31:2766–78. doi: 10.1016/j.numecd.2021.05.030

13. Li J, Jiang B, Santos HO, Santos D, Singh A, Wang L. Effects of walnut intake on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *Phytother Res.* (2020) 34:2921–31. doi: 10.1002/ptr.6740

14. Santos HO. Intermittent fasting and fat mass: what is the clinical magnitude? *Obesities.* (2022) 2:1–7. doi: 10.3390/obesities2010001

15. Varkaneh Kord H, M Tinsley G, O Santos H, Zand H, Nazary A, Fatahi S, et al. The influence of fasting and energy-restricted diets on leptin and adiponectin levels in humans: a systematic review and meta-analysis. *Clin Nutr.* (2021) 40:1811–21. doi: 10.1016/j.clnu.2020.10.034

16. Wang X, Yang Q, Liao Q, Li M, Zhang P, Santos HO, et al. Effects of intermittent fasting diets on plasma concentrations of inflammatory biomarkers: a systematic review and meta-analysis of randomized controlled trials. *Nutrition.* (2020) 79–80:110974. doi: 10.1016/j.nut.2020.110974

17. Meng H, Zhu L, Kord-Varkaneh H, O Santos H, Tinsley GM, Fu P. Effects of intermittent fasting and energy-restricted diets on lipid profile: a systematic review and meta-analysis. *Nutrition.* (2020) 77:110801. doi: 10.1016/j.nut.2020.110801

18. Yang F, Liu C, Liu X, Pan X, Li X, Tian L, et al. Effect of epidemic intermittent fasting on cardiometabolic risk factors: a systematic review and meta-analysis of randomized controlled trials. *Front Nutr.* (2021) 8:669325. doi: 10.3389/fnut.2021.669325

19. Scholtens EL, Krebs JD, Corley BT, Hall RM. Intermittent fasting 5:2 diet: what is the macronutrient and micronutrient intake and composition? *Clin Nutr.* (2020) 39:3354–60. doi: 10.1016/j.clnu.2020.02.022

20. Ismail S, Manaf RA, Mahmud A. Comparison of time-restricted feeding and Islamic fasting: a scoping review. *East Mediterr Health J.* (2019) 25:239–45. doi: 10.26719/emhj.19.011

21. Ismail S, Manaf RA, Mahmud A, Shamsuddin K. Influence of an intervention program promoting voluntary fasting practices and its perceived barriers among overweight or obese Muslim women working in the public sector, Malaysia. *Malay J Med Health Sci.* (2018) 14:1–6. Available online at: https://myjurnal.mohe.gov.my/public/article-view.php?id=122185

22. Fudla H, Mudjihartini N, Khusun H. Effect of four weeks of 5:2 intermittent fasting on energy intake and body mass index among obese male students aged 18-25. *Obesity Med.* (2021) 25:100353. doi: 10.1016/j.obmed.2021.100353

23. Yin C, Li Z, Xiang Y, Peng H, Yang P, Yuan S, et al. Effect of intermittent fasting on non-alcoholic fatty liver disease: systematic review and meta-analysis. *Front Nutr.* (2021) 8:709683. doi: 10.3389/fnut.2021.709683

24. Bonder A, Afdhal N. Utilization of FibroScan in clinical practice. *Curr Gastroenterol Rep.* (2014) 16:1–7. doi: 10.1007/s11894-014-0372-6

25. Tsai J-H, Ferrell LD, Tan V, Yeh MM, Sarkar M, Gill RM. Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition. *Mod Pathol.* (2017) 30:834–42. doi: 10.1038/modpathol.2017.13

26. Jensen MD, Ekberg K, Landau BR. Lipid metabolism during fasting. *Am J Physiol Endocrinol Metab.* (2001) 281:E789–93. doi: 10.1152/ajpendo.2001.281.4.E789

27. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO, et al. New predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr.* (1990) 51:241–7. doi: 10.1093/ajcn/51.2.241

28. Hallal PC, Victora CG. Reliability and validity of the international physical activity questionnaire (IPAQ). *Med Sci Sports Exerc.* (2004) 36:556. doi: 10.1249/01.MSS.0000117161.66394.07

29. Gersovitz M, Madden JP, Smiciklas-Wright H. Validity of the 24-hr dietary recall and seven-day record for group comparisons. *J Am Diet Assoc.* (1978) 73:48–55. doi: 10.1016/S0002-8223(21)05669-8

30. Karvetti R-L. Validity of the 24-hour dietary recall. *J Am Diet Assoc.* (1985) 85:1437–42. doi: 10.1016/S0002-8223(21)03818-9

31. Faris M, Jahrami H, Abdelrahim D, Bragazzi N, BaHammam A. The effects of Ramadan intermittent fasting on liver function in healthy adults: a systematic review, meta-analysis, and meta-regression. *Diabetes Res Clin Pract.* (2021) 178:108951. doi: 10.1016/j.diabres.2021.108951

32. Jahrami HA, Faris ME, Janahi AI, Janahi MI, Abdelrahim DN, Madkour MI, et al. Does four-week consecutive, dawn-to-sunset intermittent fasting during Ramadan affect cardiometabolic risk factors in healthy adults? A systematic review, meta-analysis, and meta-regression. *Nutr Metab Cardiovasc Dis.* (2021) 31:2273–301. doi: 10.1016/j.numecd.2021.05.002

33. Jahrami HA, Alsibai J, Obaideen AA. Impact of Ramadan diurnal intermittent fasting on the metabolic syndrome components in healthy, non-athletic Muslim people aged over 15 years: a systematic review and meta-analysis. *Br J Nutr.* (2020) 123:1–22. doi: 10.1017/S000711451900254X

34. Al-Islam EF, Jahrami HA, Obaideen AA, Madkour MI. Impact of diurnal intermittent fasting during Ramadan on inflammatory and oxidative stress

markers in healthy people: Systematic review and meta-analysis. *J Nutr Intermed Metab*. (2019) 15:18–26. doi: 10.1016/j.jnim.2018.11.005

35. Kacimi S., ef'at A, Fararjeh MA, Bustanji YK, Mohammad MK, Salem ML. Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. *Nutr Res.* (2012) 32:947–55. doi: 10.1016/j.nutres.2012.06.021

36. Jahrami H, BaHammam A, Kalaji Z, Madkour M, Hassanein M. A systematic review, meta-analysis, and meta-regression of the impact of diurnal intermittent fasting during Ramadan on glucometabolic markers in healthy subjects. *Diabetes Res Clin Pract.* (2020) 165:108226. doi: 10.1016/j.diabres.2020.108226

37. Jahrami HA, Alsibai J, Clark CCT, Faris MAE. A systematic review, meta-analysis, and meta-regression of the impact of diurnal intermittent fasting during Ramadan on body weight in healthy subjects aged 16 years and above. *Eur J Nutr.* (2020) 59:2291–316. doi: 10.1007/s00394-020-02216-1

38. Aliasghari F, Izadi A, Gargari BP, Ebrahimi S. The effects of ramadan fasting on body composition, blood pressure, glucose metabolism, and markers of inflammation in NAFLD patients: an observational trial. *J Am Coll Nutr.* (2017) 36:640–5. doi: 10.1080/07315724.2017.1339644

39. Rynders CA, Thomas EA, Zaman A, Pan Z, Catenacci VA, Melanson EL. Effectiveness of intermittent fasting and time-restricted feeding compared to continuous energy restriction for weight loss. *Nutrients*. (2019) 11:2442. doi: 10.3390/nu11102442

40. Santos HO, Genario R, Tinsley GM, Ribeiro P, Carteri RB, Coelho-Ravagnani CF, et al. scoping review of intermittent fasting, chronobiology, and metabolism. *Am J Clin Nutr.* (2022) 115:991–1004. doi: 10.1093/ajcn/nqab433

41. Santos HO, Genario R, Macedo RCO, Pareek M, Tinsley GM. Association of breakfast skipping with cardiovascular outcomes and cardiometabolic risk factors: an updated review of clinical evidence. *Crit Rev Food Sci Nutr.* (2022) 62:466–74. doi: 10.1080/10408398.2020.1819768

42. Genario R, Cipolla-Neto J, Bueno AA, Santos HO. Melatonin supplementation in the management of obesity and obesity-associated disorders: a review of physiological mechanisms and clinical applications. *Pharmacol Res.* (2021) 163:105254. doi: 10.1016/j.phrs.2020.105254

43. Genario R, Morello E, Bueno AA, Santos HO. The usefulness of melatonin in the field of obstetrics and gynecology. *Pharmacol Res.* (2019) 147:104337. doi: 10.1016/j.phrs.2019.104337

44. Santos HO, Macedo RCO. Impact of intermittent fasting on the lipid profile: assessment associated with diet and weight loss. *Clin Nutr ESPE.* (2018) 24:14–21. doi: 10.1016/j.clnesp.2018.01.002

45. Santos HO, Earnest CP, Tinsley GM, Izidoro LFM, Macedo RCO. Small dense low-density lipoprotein-cholesterol (sdLDL-C): analysis, effects on cardiovascular endpoints and dietary strategies. *Prog Cardiovasc Dis.* (2020) 63:503–9. doi: 10.1016/j.pcad.2020.04.009

46. Cai H, Qin YL, Shi ZY, Chen JH, Zeng MJ, Zhou W, et al. Effects of alternate-day fasting on body weight and dyslipidaemia in patients with non-alcoholic fatty liver disease: a randomised controlled trial. *BMC Gastroenterol.* (2019) 19:219. doi: 10.1186/s12876-019-1132-8

47. Johari MI, Yusoff K, Haron J, Nadarajan C, Ibrahim KN, Wong MS, et al. A randomised controlled trial on the effectiveness and adherence of modified alternate-day calorie restriction in improving activity of non-alcoholic fatty liver disease. *Sci Rep.* (2019) 9:11232. doi: 10.1038/s41598-019-47763-8

48. Wang P, Zhang Q, Hou H, Liu Z, Wang L, Rasekhmagham R, et al. The effects of pomegranate supplementation on biomarkers of inflammation and endothelial dysfunction: a meta-analysis and systematic review. *Complement Ther Med.* (2020) 49:102358. doi: 10.1016/j.ctim.2020.102358

49. Chen H, Huang W, Huang X, Liang S, Gecceh E, Santos HO, et al. Effects of green coffee bean extract on C-reactive protein levels: a systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med.* (2020) 52:102498. doi: 10.1016/j.ctim.2020.102498

50. Zhu Y, Huang Y, Santos HO, de Oliveira CVC, Zhou H, Tang N. Effects of purslane supplementation on C-reactive protein levels and biomarkers of oxidative stress as marks for metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. *Phytother Res.* (2021) 35:5477–86. doi: 10.1002/ptr.7182

51. Ajmal MR, Yaccha M, Malik MA, Rabbani MU, Ahmad I, Isalm N, et al. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF-alpha. *Indian Heart J.* (2014) 66:574–9. doi: 10.1016/j.ihj.2014.08.006

52. He L, Deng L, Zhang Q, Guo J, Zhou J, Song W, et al. Diagnostic value of CK-18, FGF-21, and related biomarker panel in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Biomed Res Int.* (2017) 2017:9729107. doi: 10.1155/2017/9729107

53. Darweesh SK, AbdElAziz RA, Abd-ElFatah DS, AbdElazim NA, Fathi SA, Attia D, et al. Serum cytokeratin-18 and its relation to liver fibrosis and steatosis diagnosed by FibroScan and controlled attenuation parameter in nonalcoholic fatty liver disease and hepatitis C virus patients. *Eur J Gastroenterol Hepatol.* (2019) 31:633–41. doi: 10.1097/MEG.0000000000001385

54. Zoe N, Memel JW, Kathleen E. Corey: intermittent fasting as a treatment for nonalcoholic fatty liver disease: what is the evidence? *Clini Cal Liver Dis.* (2022) 19:101–5. doi: 10.1002/cld.1172

55. Macedo RCO, Santos HO, Tinsley GM, Reischak-Oliveira A. Low-carbohydrate diets: effects on metabolism and exercise - a comprehensive literature review. *Clin Nutr ESPEN.* (2020) 40:17–26. doi: 10.1016/j.clnesp.2020.07.022

56. Siddiqui MS, Vuppalanchi R, Van Natta ML, Hallinan E, Kowdley KV, Abdelmalek M, et al. Vibration-controlled transient elastography to assess fibrosis and steatosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol.* (2019) 17:156–63.e2. doi: 10.1016/j.cgh.2018.04.043

57. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol.* (2013) 10:666–75. doi: 10.1038/nrgastro.2013.175

58. Kaswala DH, Lai M, Afdhal NH. Fibrosis assessment in nonalcoholic fatty liver disease (NAFLD). *Dig Dis Sci.* (2016) 61:1356–64. doi: 10.1007/s10620-016-4079-4

59. Marchesini G, Mazzotti A. NAFLD incidence and remission: only a matter of weight gain and weight loss? *J Hepatol.* (2015) 62:15–7. doi: 10.1016/j.jhep.2014.10.023

60. Cho Y, Hong N, Kim KW, Cho SJ, Lee M, Lee YH, et al. The effectiveness of intermittent fasting to reduce body mass index and glucose metabolism: a systematic review and meta-analysis. *J Clin Med.* (2019) 8:1645. doi: 10.3390/jcm8101645

61. Schwimmer JB, Ugalde-Nicalo P, Welsh JA, Angeles JE, Cordero M, Harlow KE, et al. Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: a randomized clinical trial. *JAMA*. (2019) 321:256–65. doi: 10.1001/jama.2018.20579

62. Pugliese N, Plaz Torres MC, Petta S, Valenti L, Giannini EG, Aghemo A. Is there an 'ideal' diet for patients with NAFLD? *Eur J Clin Invest.* (2022) 52:e13659. doi: 10.1111/eci.13659

63. EASL–EASD–EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Clin Pract Guidelines*. (2016) 64:1388–402. doi: 10.1016/j.jhep.2015.11.004

64. Santos HO, Bueno AA, Mota JF. The effect of artichoke on lipid profile: a review of possible mechanisms of action. *Pharmacol Res.* (2018) 137:170–8. doi: 10.1016/j.phrs.2018.10.007

65. Santos HO, Price JC, Bueno AA. Beyond fish oil supplementation: the effects of alternative plant sources of omega-3 polyunsaturated fatty acids upon lipid indexes and cardiometabolic biomarkers-an overview. *Nutrients*. (2020) 12:3159. doi: 10.3390/nu12103159

66. Zuo S, Wang G, Han Q, Xiao H, Santos HO, Avelar Rodriguez D, et al. The effects of tocotrienol supplementation on lipid profile: a meta-analysis of randomized controlled trials. *Complement Ther Med.* (2020) 52:102450. doi: 10.1016/j.ctim.2020.102450

67. Santos HO, da Silva GAR. To what extent does cinnamon administration improve the glycemic and lipid profiles? *Clin Nutr ESPEN.* (2018) 27:1–9. doi: 10.1016/j.clnesp.2018.07.011

68. Zhu C, Yan H, Zheng Y, Santos HO, Macit MS, Zhao K. Impact of cinnamon supplementation on cardiometabolic biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med.* (2020) 53:102517. doi: 10.1016/j.ctim.2020.102517

69. Santos HO, Macedo RCO. Cocoa-induced (*Theobroma cacao*) effects on cardiovascular system: HDL modulation pathways. *Clin Nutr ESPEN.* (2018) 27:10–5. doi: 10.1016/j.clnesp.2018.06.001

70. Santos HO, Kones R, Rumana U, Earnest CP, Izidoro LFM, Macedo RCO. Lipoprotein(a): current evidence for a physiologic role and the effects of nutraceutical strategies. *Clin Ther.* (2019) 41:1780–97. doi: 10.1016/j.clinthera.2019.06.002

71. Permutt T-AL, Peterson MR, Seki E, Brenner D, Sirlin AC, Loomba R. Correlation between liver histology and novel magneticresonance imaging in adult patients with non-alcoholic fattyliver disease–MRI accurately quantifies hepatic steatosis inNAFLDZ. *Aliment Pharmacol Ther.* (2012) 36:22–9. doi: 10.1111/j.1365-2036.2012.05121.x

72. Audrey Deprince JTHS. Dysregulated lipid metabolism links NAFLD to cardiovascular disease. *Mol Metab.* (2020) 42:101092. doi: 10.1016/j.molmet.2020.101092

73. Wang F, He Y, Santos HO, Sathian B, Price JC, Diao J. The effects of dehydroepiandrosterone (DHEA) supplementation on body composition and blood pressure: a meta-analysis of randomized clinical trials. *Steroids*. (2020) 163:108710. doi: 10.1016/j.steroids.2020.108710

74. Fang Z, Dang M, Zhang W, Wang Y, Kord-Varkaneh H, Nazary-Vannani A, et al. Effects of walnut intake on anthropometric characteristics: a systematic review and dose-response meta-analysis of randomized controlled trials. *Complement Ther Med*. (2020) 50:102395. doi: 10.1016/j.ctim.2020.102395

75. de Moraes WMAM, de Almeida FN, Dos Santos LEA, Cavalcante KDG, Santos HO, Navalta JW, et al. Carbohydrate loading practice in bodybuilders: effects on muscle thickness, photo silhouette scores, mood states and gastrointestinal symptoms. *J Sports Sci Med*. (2019) 18:772–9. Available online at: https://pubmed.ncbi.nlm.nih.gov/31827362/

76. Santos HO, Gomes GK, Schoenfeld BJ, de Oliveira EP. The effect of whole egg intake on muscle mass: are the yolk and its nutrients important? *Int J Sport Nutr Exerc Metab*. (2021) 31:514–21. doi: 10.1123/ijsnem.2021-0086

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 16 August 2022
DOI 10.3389/fnut.2022.987921

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Găman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Meysam Zarezadeh,
Tabriz University of Medical Sciences, Iran
Seyed Mohammad Mousavi,
Tehran University of Medical Sciences, Iran

*CORRESPONDENCE
Azita Hekmatdoost
a_hekmat2000@yahoo.com
Ahmed Abu-Zaid
aabuzaid@live.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 06 July 2022
ACCEPTED 25 July 2022
PUBLISHED 16 August 2022

CITATION
Varkaneh HK, Poursoleiman F, Al Masri MK, Alras KA, Shayah Y, Masmoum MD, Alangari FA, Alras AA, Rinaldi G, Day AS, Hekmatdoost A, Abu-Zaid A and Kutbi E (2022) Low fat diet versus low carbohydrate diet for management of non-alcohol fatty liver disease: A systematic review. *Front. Nutr.* 9:987921. doi: 10.3389/fnut.2022.987921

Low fat diet versus low carbohydrate diet for management of non-alcohol fatty liver disease: A systematic review

Hamed Kord Varkaneh[1†], Faezeh Poursoleiman[1†], Mohammad Khaldoun Al Masri[2], Kamar Allayl Alras[2], Yamen Shayah[2], Mohd Diya Masmoum[2], Fulwah Abdulaziz Alangari[2], Abd Alfatah Alras[3], Giulia Rinaldi[4], Andrew S. Day[5], Azita Hekmatdoost[1*], Ahmed Abu-Zaid[2,6*] and Emad Kutbi[7]

[1]Student Research Committee, Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]College of Medicine, Alfaisal University, Riyadh, Saudi Arabia, [3]College of Kinesiology, University of Saskatchewan, Saskatoon, SK, Canada, [4]St. George's Hospital, London, United Kingdom, [5]Department of Paediatrics, University of Otago, Dunedin, New Zealand, [6]Department Pharmacology, College of Graduate Health Sciences, The University of Tennessee Health Science Center, Memphis, TN, United States, [7]Department of Biorepository, Biomedical Research Administration, King Fahad Medical City, Riyadh, Saudi Arabia

Although there is a consensus on beneficial effects of a low calorie diet in management of non-alcoholic fatty liver disease, the optimal composition of diet has not yet been elucidated. The aim of this review is to summarize the results of current randomized controlled trials evaluating the effects of low fat diet (LFD) vs. low carbohydrate diet (LCD) on NAFLD. This is a systematic review of all the available data reported in published clinical trials up to February 2022. The methodological quality of eligible studies was assessed, and data were presented aiming specific standard measurements. A total of 15 clinical trial studies were included in this systematic review. There is an overall lack of consensus on which dietary intervention is the most beneficial for NAFLD patients. There is also an overall lack of consensus on the definition of the different restrictive diets and the percentage of macronutrient restriction recommended. It seems that low calorie diets, regardless of their fat and carbohydrate composition, are efficient for liver enzyme reduction. Both LCD and LFD have similar effects on liver enzymes change; however, this improvement tends to be more marked in LFD. All calorie restrictive dietary interventions are beneficial for reducing weight, liver fat content and liver enzymes in individuals with NAFLD. Low fat diets seem to be markedly successful in reducing transaminase levels. Further research is needed to explore diet intensity, duration and long-term outcome.

KEYWORDS

non-alcoholic fatty liver disease, low fat diet, low carbohydrate diet, NAFLD, liver fat

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent liver diseases worldwide (20–30%) (1). Individuals with NAFLD may develop liver injury with a subset developing progressive fibrosis (non-alcoholic steatohepatitis), cirrhosis and complications including end-stage liver failure and hepatocellular carcinoma (2). Fatty liver is one of the causes of liver transplantation (3). In many aspects, the pathophysiology of NAFLD is similar to that of obesity, dyslipidemia and diabetes (4).

Diet and exercise are first line treatments for NAFLD. Studies have shown that patients with NAFLD consume excessive amounts of total energy, refined carbohydrates (including fructose), fibers and antioxidants (vitamin C and vitamin E), cholesterol and saturated fats (SFA) with an insufficient intake of polyunsaturated fats (PUFA) (5, 6). Since there is no pharmacotherapy currently available for patients with NAFLD, lifestyle changes remain the fundamental management option (7, 8). In patients with NAFLD (overweight and obese), calorie restriction drives the reduction of liver fat, body weight, and histological improvement of non-alcoholic steatohepatitis (NASH) (9, 10). However, there is conflicting data on which hypocaloric dietary plan should be adopted according to the macronutrient composition (11, 12). Nordmann et al. (13) showed that low-fat diets are more effective than low-carbohydrate diets in reducing total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol concentrations. In contrast, low-carbohydrate diets can be more effective than low-fat diets in increasing high-density lipoprotein (HDL) cholesterol concentrations and reducing triglyceride (TG) and transaminase levels with a further decrease in 24-h circulating blood insulin concentrations in both isocaloric and hypocaloric conditions (14–17).

Current evidence suggests a change in diet composition alone can reduce hepatic fat infiltration. Studies in animal models and humans have shown that reducing intake of carbohydrate (CHO) sources such as added sugars, high glycemic grains, and fructose may be an effective approach to reverse fatty liver by significantly reducing insulin resistance and inflammation (18–20). A randomized clinical trial in adults demonstrated that a CHO restricted diet (<20 g/day) compared to a low-fat diet resulted in similar weight loss but greater reduction in hepatic fat (−55 vs. −28%, $p < 0.001$) (21). This suggests a clear metabolic advantage for CHO-restriction, independent of overall weight loss, in adults with NAFLD. A study showed that a low-carbohydrate diet was more effective than a low-fat diet in improving obesity (22, 23). Moreover, another paper explored the impacts of low carbohydrate vs. low-fat/low-calorie diets in achieving weight control and found that the low-carbohydrate diet is more effective than the low-fat/low-calorie diet (24). Furthermore, low-fat diets effectively reduce intrahepatic fat content (24). NAFLD is a significant independent risk factor for cardiovascular disease and type 2 diabetes because of concurrent dyslipidemia and insulin resistance (25), therapeutic diets are believed to be effective for reversal of changes seen with NAFLD (26).

However, despite these data, there is little evidence surrounding an all-inclusive diet for NAFLD. This systematic review focuses on randomized controlled trials (RCTs) of low carbohydrate diets compared to low fat diets, to assess their impact on NAFLD and liver enzymes.

Methods

Conduct of systematic review

A meta-analysis was conducted based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement.

Search strategy

PubMed/Medline, Web of science, Scopus, and Cochrane library databases were searched for relevant articles about the intervention low fat diet or low carbohydrate diet in patients with NAFLD published up to February 2022; without any language restriction. The combination of MESH and non-MESH keywords were used (Supplementary Table 1). Furthermore, all reference lists of related articles, reviews, and meta-analyses were manually checked to avoid missing any studies. The quality of eligible trials was evaluated using the Cochrane quality assessment tool (27), which is comprised of the following: allocation concealment, blinding of participants and personnel, random sequence generation, incomplete outcome data, blinding of outcome assessment, selective reporting and other probable sources of biases (Table 1).

Eligibility criteria

The following criteria were regarded to select eligible studies based on PICO; (1) participants (p): studies that included adult subjects (≥18 years of age) with NAFLD, (2) Intervention (I): examined the effects of low fat diet or low carbohydrate diet in NAFLD patients, (3) comparison: compared with control or baseline value, (4) outcome: those that reported sufficient data related to liver. In addition, studies were excluded if they; (1) were executed on children, pregnant women, other diseases or animals, (2) were not trials, (3) did not provide sufficient information of NAFLD outcomes, or (4) investigated the effects of low fat diet or low carbohydrate diet along with other dietary changes. Unpublished documents and gray literature like

TABLE 1 The risk of bias in studies included in this study.

	Random Sequence Generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias	AHRQ standards
Rodríguez-Hernández et al. (28)	+	+	+	?	?	+	+	Fair
Arefhosseini et al. (29)	+	+	+	+	+	+	+	Good
Haufe et al. (23)	+	+	−	−	+	+	+	Poor
De Luis et al. (30)	+	+	+	+	+	+	+	Good
Kirk et al. (14)	+	+	−	−	+	+	+	Poor
Marina et al. (31)	+	+	+	−	+	+	+	Poor
Kani et al. (26)	+	+	−	−	+	+	+	Poor
Browning et al. (21)	+	+	+	+	−	+	+	Poor
Benjaminov et al. (32)	+	+	+	+	+	+	+	Good
Mardinoglu et al. (25)	+	+	+	+	+	+	+	Good
Yu et al. (33)	+	?	−	?	+	+	+	Poor
Biolato et al. (34)	+	+	+	+	+	+	+	Good
Properzi et al. (35)	+	+	+	+	+	+	+	Good
Ryan et al. (36)	+	+	+	+	+	+	+	Fair
Ravansha et al. (37)	+	+	+	+	+	+	+	Good

10.3389/fnut.2022.987921

conference papers, pilot studies, dissertations, and patents were also excluded.

Data extraction

Two independent investigators (FP and HKV) undertook the study selection whereas a chief investigator (AH) provided further comment if any disagreements. Contact was made with the corresponding authors of any studies where insufficient data was available.

The following data was extracted from eligible studies: first author's name, year of publication and journal, study country, gender of participants, mean age, number of subjects in each group, trial duration, type and energy content of each diet intervention with percent of macronutrient, study design, and the mean and standard deviation of outcome measures at baseline and the end-of-trial.

Definition of low carbohydrate and low fat diet

Low carbohydrate diet definition is very inconsistent, but according to Seidelmann et al. new study (38) optimal proportion of carbohydrate for a healthy diet is 50–55%. Therefore, we considered ≤50% as a low carbohydrate diet (LCD) and according to other studies (13, 39) we defined low fat diet (LFD) as a maximum of 30% of the calorie intake from fat.

Results

Literature search and study characteristics

Out of 1,495 articles identified from PubMed/Medline (n = 375), Scopus (474), Web of Science (465) and Cochrane databases (n = 181), 575 duplicate articles were excluded. A further 906 were excluded based on the title and abstract screening approach. The remaining 14 articles were reviewed with two independent authors by reading the full text. Two additional studied were excluded for the following reasons: no full text available (40) and different definition for low carbohydrate and low fat diet (low fat diet; ≤30% of total calorie from fat and low carbohydrate diet; ≤50% of total calorie from carbohydrate) (4) (Figure 1). After manual search three other articles were also included (14, 29, 32) (Table 2). Finally, 15 articles were included in the current study. Five studies reported LCD vs. other dietary patterns (21, 25, 26, 33), four reported LFD vs. other dietary patterns (34–37), six reported LCD vs. LFD (14, 23, 28–31) and from them nine reported the effects of LCD or LFD on liver fat content (14, 21, 23, 25, 31–33, 35, 36). Fifteen included studies were published between 2005 and 2019. Sample sizes varied from 8 to 140 patients across studies. Mean ages and mean baseline BMIs ranged from 32 to 55 years and 28–45.9 kg.m^2, respectively. One trial was performed exclusively in women (28) and others included both genders. Intervention periods were as wide as 2–24 weeks. According to dietary interventions, carbohydrate percentage in LCD was varied from 8 to 45% and 15 to 30% for fat content in LFD.

Quality assessment

The results of the quality assessment of the eligible studies are presented in Table 1. Most studies had a good quality, two had fair quality (28, 36), and six had a poor quality (14, 21, 23, 26, 31, 33) (an unclear risk of bias for in the allocation concealment and blinding of outcome assessments).

Effects of LCD or LFD on liver enzymes and body weight

LCD vs. other dietary patterns.

Two studies compared LCD (<30% from total calorie intake) with low calorie diet. Kani et al. (26) conducted a randomized parallel trial for 30 patients with NAFLD. After intervention with low calorie (−200 to 500 kcal) and low calorie LCD (−200 to 500 kcal, CHO: 45%, FAT: 35%) diets, both ALT and AST decreased. ALP reduction was only seen in low calorie group. However, the changes in liver enzymes and body weight were not significantly different between these two groups.

Browning et al. (21) compared a very LCD (CHO: 8%, FAT: 59%) with low calorie diet (CHO: 50%, FAT: 34%) in 18 patients with NAFLD. A similar weight loss was observed in both groups. Regardless of the dietary intervention, AST but not ALT changed significantly after 2 weeks weight loss.

In a single arm study, Benjaminov et al. (32) showed that a 4 week intervention with a very LCD (CHO: 14%, FAT: 56%) did not alter ALT and AST. Significant weight reduction was seen. Yu et al. (33) conducted a low calorie, very LFD (CHO <10%, 800 kcal) intervention for patients with obesity and NAFLD. In this single arm study, body mean weight reduction after 8 weeks was 6.8 kg (7% of the pre-intervention weights) ($p = 0.001$).

LFD vs. other dietary patterns

From four studies, three of them compared LFD with Mediterranean diet and one study had no control group. In a crossover study, 20 patients with NAFLD underwent 16 weeks of MD (CHO: 40%, FAT: 40%), 16 weeks of wash-out period, and 16 weeks of LFD (FAT: 18%, CHO: 62%) (34). Both diet interventions were calorie restricted (1,400 kcal). At the end of the MD period, significant weight loss and ALT and AST reduction were observed. But in the LFD group, there were no significant changes in body weight or transaminases. Serum transaminases and body weight at the beginning of LFD period were the same as the end point of MD period: consequently the conditions at the start of the LFD period differed from that of

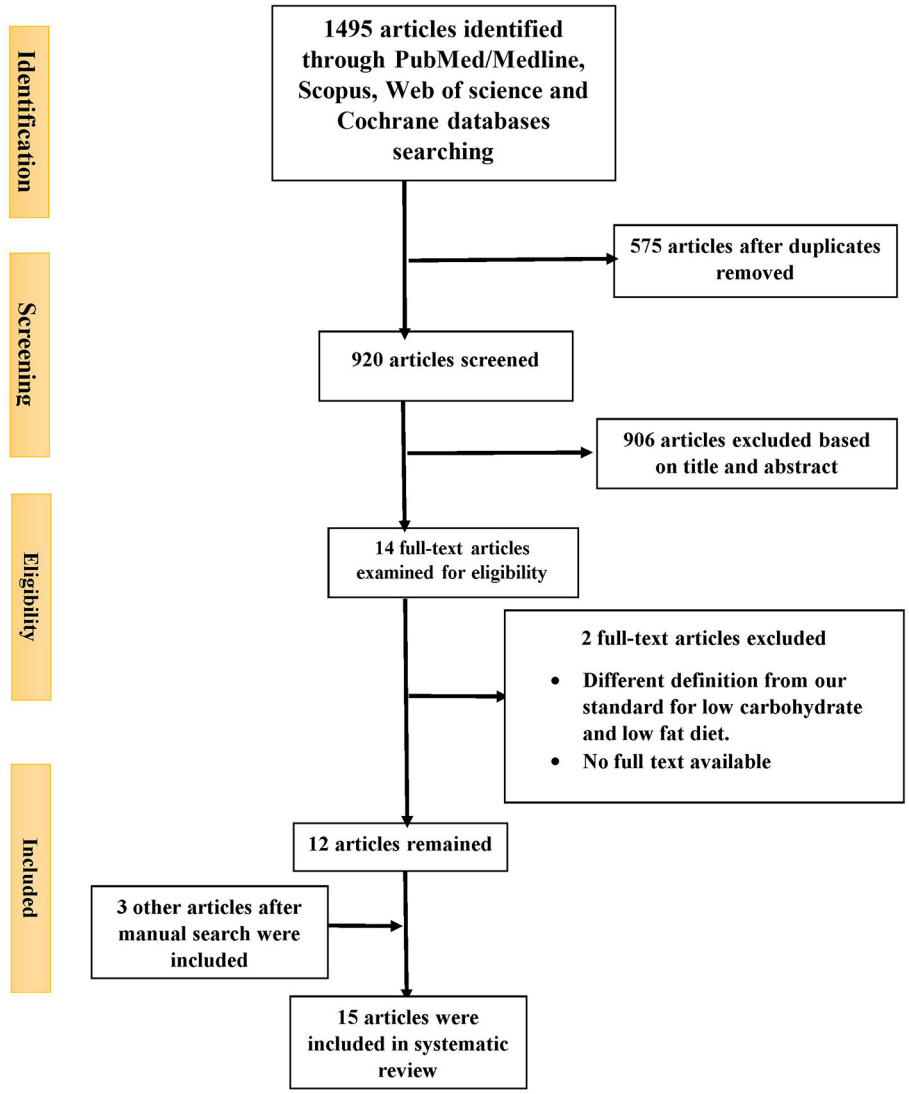


FIGURE 1
PRISMA flowchart for study examined and included into the meta-analysis.

the MD group. These results may reflect the sequence of the dietary intervention.

Properzi et al. (35) compared LFD (FAT: 30%, CHO: 50%) and MD (CHO: 40%, FAT: 35–40%) in a parallel-group RCT. Fifty-six patients with NAFLD were recruited and after 12 weeks intervention data from 49 subjects were available for analysis. At the end of the study, ALT and GGT decreased significantly in both groups. Similar weight loss amounts were seen also, with no no significant differences seen at the final analysis. In a study by Ryan et al. (36) patients with NAFLD randomly received MD (CHO: 40%, FAT: 40%) or LF-HCD (FAT: 30%, CHO: 50%) for 6 weeks. After a 6 week washout period, the subjects swapped to the second diet. ALT and GGT changes were not appreciated in either diet. Similarly, weight loss was not different between two groups ($p = 0.22$).

Ravanshad et al. (37) designed a single arm randomized trial for obese patients with NAFLD.A low calorie LFD (FAT: 25–30%, CHO: 55–60%) for 6 weeks resulted in decreased body weight and mean serum ALP, ALT, AST levels. Although this study appeared to show beneficial effects for LFD with restricted calorie, the differential effects of calorie and fat restriction were able to be demonstrated.

LCD vs. LFD

Six interventional studies evaluated liver enzymes levels by comparing LCD and LFD. Rodríguez-Hernández et al.

TABLE 2 The characteristics of the 15 eligible studies.

References, Country	Subjects	Diagnosis method	Study design	Follow up (weeks)	intervention	Control	Results
Low fat vs. low carb diet							
Rodríguez-Hernández et al. (28), Mexico	Obese & NAFLD	Ultrasonography	Randomized non-controlled crossover trial	24	Low-fat diet (Fat: 21%, Carb: 54%)	Low-carb diet (Carb: 45%, Fat: 28%)	ALT and AST decreased more in the Low-carb diet but not significantly
Arefhosseini et al. (29), Iran	Overweight & NAFLD	Ultrasonography	Randomized controlled crossover trial	6	Low calorie low fat diet (Fat: 25%, Carb: 55%, minus 500 kcal)	Low calorie low carb diet (Carb: 40%, Fat: 40%, minus 500 kcal)	AST decreased in both group but more in LFD weight decreased significantly in both groups
Haufe et al. (23), Germany	Obese & high IHL	MRS	Randomized controlled crossover trial	24	Low calorie low fat diet (Fat ≤ 20%, minus 30% of energy intake before diet)	Low calorie low carb diet (Carb ≤ 90 g/day, Fat ≥ 30%, minus 30% of energy intake before diet)	Both hypocaloric diets decreased liver enzymes, body weight, and liver fat content similarly
De Luis et al. (30)	NAFLD	BMI≥30 ALT≥43	Randomized controlled trial	12	Low calorie low fat diet (Fat: 27%, Carb: 53%, 1,500 kcal)	Low calorie low carb diet (Carb: 38%, Fat: 36%, 1,507 kcal)	ALT and AST decreased more in the low-fat diet weight loss in groups were the same
Kirk et al. (14), USA	Obese	MRS	Randomized controlled trial	11	Low calorie low carb diet (Carb ≤ 50 g/day ∼10%, Fat: 75%, minus 1,000 kcal)	Low calorie high carb diet (Fat: 20%, Carb ≥180 g/day ∼ 65%, minus 1,000 kcal)	ALT and AST did not changed. Liver fat content decreases in low carb at 48 h but not in 11 weeks.
Marina et al. (31), USA	Overweight/obese	BMI > 27MRS	Randomized controlled trial	4	Low fat diet (fat: 20%, carb: 62%)	High fat diet (carb: 27%, fat: 55%)	ALT, AST and Body weight had no changes in groups. Liver fat decreased in LFD but not significantly.
Low carb diet							
Kani et al. (26), Iran	NAFLD	Ultrasonography	Randomized parallel trial	8	Low calorie low carb diet (carb: 45%, fat: 35%, minus 200–500 kcal)	Low calorie diet (carb: 55%, fat: 30%, minus 200–500 kcal)	ALT and ALT reduction and weight loss were the same in both group
Browning et al. (21), Texas	NAFLD	Liver Biopsy	Randomized crossover trial	2	Low carb diet (Carb> 25 kcal/kg of ideal body weight, ∼8%, fat∼ 59%)	Low calorie diet (fat∼34%, car∼50%, 1,200 kcal for women and 1,500 kcal for men)	ALT reduction was not significant in either group but AST reduced significantly in both. Weight were similar loss in both group. Liver fat content reduction in LCD group was more

(Continued)

10.3389/fnut.2022.987921

TABLE 2 Continued

References, Country	Subjects	Diagnosis method	Study design	Follow up (weeks)	intervention	Control	Results
Benjaminov et al. (32), Israel	Obese	CT	Single arm trial	4	Low carb diet (carb: 14%, fat: 56%	–	ALT and AST did not changed. Weight loss was significant Reduction of liver fat content and size.
Mardinoglu et al. (25), Sweden	Obese & NAFLD	Biopsy	Single arm trial	2	Low-carb diet (carb <10%)	–	Liver fat content was dramatically reduced
Yu et al. (33), China	Obese & NAFLD	BMI > 30 liver fat >5.6%	Single arm trial	8	Low calorie low carb diet (carb <10%, minus 800 kcal)	–	Caloric restriction reduced liver fat content and body weight
Low fat diet							
Biolato et al. (34), Itlay	NAFLD	Biopsy-verified NAFLD and increased transaminases	Open-label Crossover trial	16	Low calorie low fat diet (fat: 18%, carb: 62%, 1,400 kcal)	Low calorie Mediterranean diet (carb: 40%, fat: 40%, 1,400 kcal)	Significant weight loss and ALT and AST reduction observed after MD
Properzi et al. (35), Australia	NAFLD	MRS	Randomized parallel trial	12	Low fat diet (fat: 30%, carb: 50%)	Mediterranean diet (carb: 40%, fat: 40%)	ALT and GGT decreased significantly in both groups as well as similar weight loss. No changes were observed in liver fat.
Ryan et al. (36), Australia	NAFLD	Biopsy	Randomized crossover trial	6	Mediterranean diet (carb: 40%, fat: 40%)	Low fat diet (fat: 30%, carb: 50%)	Transaminases (ALT, GGT) level and weight loss changes were not significant and also not different between groups. A significantly greater decrease in liver fat content was seen in MD group.
Ravansha et al. (37), Iran	Obese & NAFLD	BMI > 25 Ultrasonography	Single arm trial	6	Low calorie low fat diet (fat 25–30%, carb: 55–60%, minus 500-1,000 kcal)	–	Compared to the baseline decreased body weight and mean serum ALP, ALT, AST level were seen

NAFLD, non-alcoholic fatty liver disease; IHL, Intrahepatic Lipid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; carb, carbohydrate; MRS, magnetic resonance spectroscopy; carb, carbohydrate; MD, Mediterranean diet; LFD, low fat diet; LCD, low carbohydrate diet.

10.3389/fnut.2022.987921

(28) enrolled 31 obese women with NAFLD in a randomized crossover study. They showed that 24 weeks of LCD (CHO: 45%, FAT: 28%) decreased ALT and AST more than LFD (FAT: 21%, CHO: 54%), but there was no statistical significance. Since both diets were hypocaloric, the authors claimed that weight loss diminished aminotransferase levels regardless of fat or carbohydrate percentage.

Arefhosseini et al. (29) compared LCD (CHO: 40%, FAT: 40%) with LFD (FAT: 25%, CHO: 55%) in 44 overweight patients with NAFLD patients over 6 weeks. The results arising showed that, regardless of the type of diet, calorie deficit (−500 kcal/day) can reduce AST. Unlike the study conducted by Hernández et al. (28), it was only significant for the LFD. Haufe et al. (23) randomized 102 obese patients including 45% with NAFLD into a LCD (CHO: 25%, FAT 45%) and a LFD (FAT: 27%, CHO: 52%). Both diets were hypocaloric and weight loss was observed in both groups. There were no significant differences in liver enzyme reduction between groups. De Luis et al. (30) assessed 12 weeks of hypocaloric diet with LFD (FAT: 27%, CHO: 53%) and LCD (CHO: 38%, FAT: 36%). Diet therapy improved ALT and GGT inboth groups, but AST decreased only with LFD. Compared with LCD, AST and ALT levels were significantly reduced in the LFD group. Weight losses were the same in both groups (−4 kg).

Kirk et al. (14) designed a randomized clinical trial to compare two types of diet: high carbohydrate diet (=LFD) (FAT: 20%, CHO: 65%) and LCD (CHO: 10%, FAT: 75%). Twenty-two obese patients were enrolled and about 50% of them also had NAFLD. Both diets were hypocaloric (−1,000 kcal/day). After 48 h and ∼11 weeks dieting, ALT and AST did not change with either diet. Regardless of the diet group, all subjects lost weight. In a similar study design, Marina et al. (31) evaluated a high fat diet (=LCD) (CHO: 27%, FAT: 55%) and LFD (FAT: 20%, CHO: 62%). About 54% of patients were NAFLD. No changes in liver enzymes or body weight were seen between groups but there was an increasing trend for GGT in LFD.

Overall, high heterogeneity in carbohydrate and fat percent was observed in the designed diets. Low carbohydrate and low fat diets are not well-defined and there is no consensus definition for them and as mentioned in studies percent from total calorie is ranged 8–45% for low carbohydrate diet and 15–30% for low fat diet. Furthermore, most studies combined carbohydrate or fat restriction with calorie restriction preventing clear determination of the effects of the two interventions.

According to the available studies, both LCD and LFD have same effects on liver enzymes in patients with NAFLD. It seems that low caloric diets regardless of their fat and carbohydrate composition are more effective for reduction in liver enzymes. Hypocaloric diets are associated with insulin resistance and metabolic syndrome improvement, so they are effective in reversal of the changes seen with NAFLD. Both LCD and LFD were able to reduce serum transaminase levels but greater improvement appears to be seen with LFD (30).

Effects of LCD or LFD on liver fat content

Haufe et al. (23) in their comparative study showed that the reduction of liver fat following hypocaloric LCD (CHO: 25%, FAT 45%) and hypocaloric LFD (FAT: 27%, CHO: 52%) was similar. Patients with higher baseline intrahepatic fat (IHF) achieved a greater reduction of IHF in this study. Kirk et al. (14) showed that after 48 h, IHF decreased more with the LCD (CHO: 10%, FAT: 75%) than LFD (FAT: 20%, CHO: 65%) but after ∼11 weeks the reduction was similar in both groups.

Mardinoglu et al. (25) performed an isocaloric very LCD (CHO <10) diet for 10 obese patients with NAFLD. From the first day of intervention, liver fat content was dramatically educed and the mean reduction was 43.8% after 2 weeks. Yu et al. (33) conducted a single arm study (CHO < 10%, 800 kcal) and demonstrated a liver fat content reduction of two thirds after 8 weeks ($p = 0.004$).

Marina et al. (31) in a comparative study showed LFD (fat = 20%) could reduce liver triglyceride content but it was not significant in comparison to a high fat diet (LCD).

Browning et al. (21) concluded that after 2 weeks liver fat content decreased significantly with weight loss ($P < 0.001$) but decreased significantly more in LCD ($P = 0.008$). In a study involving XX subjects, managed with a 4 week intervention with very LCD (CHO = 14%), Benjaminov et al. (32) also showed liver fat content reduction.

In comparing LFD and MD, Ryan et al. (36), in a 6-week crossover design incorporating LFD (FAT: 30%, CHO: 50%) or MD (CHO: 40%, FAT: 35–40%), showed a significantly greater reduction in IHF with MD than LFD ($p = 0.03$). However, in a parallel design Properzi et al. (35) observed no difference in liver fat content between groups ($p = 0.32$) with mean (SD) relative reductions in LFD and MD being 25.0 and 32.4%, respectively. Regardless of energy intake, it seems both LFD and LCD can reduce IHF in NAFLD patients.

Discussion

Principal findings

This systematic review evaluating low fat vs. low carbohydrate diets for alcoholic fatty liver disease has demonstrated several major findings. Firstly, considering that NAFLD is one of the commonest causes of liver disease worldwide, there is an overall lack of consensus on which dietary intervention is most beneficial for these patients. Secondly, there is also an overall lack of consensus on the definition of the different restrictive diets and the percentage of macronutrient restriction recommended. Moreover, although most of the included papers relate to trials in middle- or high-income

counties the diagnostic methods for NAFLD varied. With these caveats in mind, it appears that both LCD and LFD have the same effects on liver transaminases in individuals with NAFLD and that low calorie diets, regardless of their fat and carbohydrate composition, are more likely to read to reduced transaminases.

Hypocaloric diets are associated with improvements in insulin resistance and metabolic syndrome, and consequently have beneficial effects in NAFLD. Both LCD and LFD are able to reduce serum transaminase levels, however, this improvement tends to be more marked in LFD (30). Additionally, both LFD and LCD seemed to have report weight loss results in patient groups. Moreover, two studies reported fever impact of low free sugar diet on NAFLD in adolescent boys (41) and adults (42). Lastly, there remains insufficient evidence to deduce what duration, combination or intensity of treatment is most effective in these patients.

Limitations

There are several limitations present in this review. Firstly, the included studies are not homogenous. The authors defined low carbohydrate, low fat and low-calorie diets using different parameters. In the included studies, the percentage restrictions ranged from 8 to 45% for LCD and 15 to 30% for LFD. This can affect the reliability of this systematic review. Moreover, it may be difficult to distinguish results between a low calorie, low carbohydrate and low-fat diet, as most of these diets restricted calories and both macronutrients. Another limitation of this systematic review is that most studies did not explore or categorize patients according to different characteristics. For example, patients with different severities of NAFLD may have responded differently to different types of diet adjustments. Similarly, patients with higher body mass indexes may have more marked reductions in weight or liver fat content than their thinner counterparts. This is an increasingly important limitation now that medicine continues to become more individualized. Lastly, the follow up time period varied amongst included studies, with the shortest being 2 weeks and the longest 24 weeks. Therefore, the long-term clinical outcomes of these diets including weight loss, liver enzymes, liver fat content and patient compliance, remain unexplored.

Comparison with previous literature

The current results agree with previous reviews in that calorie restricted diets are overall successful in reducing, but rarely resolving, NAFLD (43, 44). One previous trial compared hypocaloric LFD and LCD for intrahepatic fat reduction in NAFLD and found no significant difference between the beneficial effects of these two diets over a period of 6 months (23). The current results reflect these outcomes and reiterate the noteworthy impact that dietary modification can have on these markers of liver disease.

Additional large studies have also demonstrated that multidisciplinary lifestyle modification, including exercise and diet, can have an even more pronounced impact on NAFLD with one cohort achieving a 64% remission rate after 12 months (45, 46). Nevertheless, other reviews mention the difficulty of managing NAFLD patients with dietary interventions outside of highly controlled trial settings. These interventions require high-intensity specialist services that may not be available, and, whose cost-effectiveness may need to be explored (43).

This systematic review has contributed to existing literature by summarizing the evidence on LCD vs. LFD and confirming that both of these are demonstrated to be successful interventions to promote weight loss and improve hepatic markers in NAFLD.

Suggestions for further research

Future research is needed to answer the questions elicited by the limitations of this report. For example, the intensity of macronutrient restriction that generates the most beneficial response in patients. Secondly, the duration of treatment needed, and therefore, both the feasibility and effectiveness of long-term dietary interventions for the management of NAFLD. Thirdly, the current review suggests that LFD may be more successful in reducing transaminases in these patients, therefore, further exploration of this effect is warranted. Another important component of lifestyle intervention targeting NAFLD is physical exercise. Future research will need to further explore the ideal combination between physical exercise and dietary interventions to ensure long term positive outcomes for these patients.

Conclusions

All calorie restrictive dietary interventions are beneficial for reducing weight, liver fat content and liver enzymes in individuals with NAFLD. Low fat diets seem to be markedly successful in reducing transaminase levels. Further research is needed to explore diet intensity, duration and long-term outcome.

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/s.

Author contributions

EK, KA, HV, and AH designed the study. MM, MA, FA, AA, and YS contributed to the literature search, screening data, and data extraction. AA-Z and HK carried out the quality assessment. GR and AS analyzed and interpreted data. HV, FP, AS, AH, and AA-Z wrote and edited the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by grants from the Student Research Committee, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran (Grant's ID: 1398/9924).

Acknowledgments

The authors sincerely thank Shahid Beheshti University of Medical Sciences for all moral and material supports.

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.2022.987921/full#supplementary-material

References

1. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis.* (2010) 28:155–61. doi: 10.1159/000282080

2. Adams LA, Lymp JF, Sauver JS, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology.* (2005) 129:113–21. doi: 10.1053/j.gastro.2005.04.014

3. Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. Evolution of indications and results of liver transplantation in Europe. *A report from the European Liver Transplant Registry (ELTR). J Hepatol.* (2012) 57:675–88. doi: 10.1016/j.jhep.2012.04.015

4. Jang EC, Jun DW, Lee SM, Cho YK, Ahn SB. Comparison of efficacy of low-carbohydrate and low-fat diet education programs in non-alcoholic fatty liver disease: a randomized controlled study. *Hepatol Res.* (2018) 48:E22–9. doi: 10.1111/hepr.12918

5. Mouzaki M, Allard JP. The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. *J Clin Gastroenterol.* (2012) 46:457–67. doi: 10.1097/MCG.0b013e31824cf51e

6. Zelber-Sagi S, Ivancovsky-Wajcman D, Isakov NF, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. *J Hepatol.* (2018) 68:1239–46. doi: 10.1016/j.jhep.2018.01.015

7. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association.*Hepatology.* (2012) 55:2005–23. doi: 10.1002/hep.25762

8. George ES, Forsyth A, Itsiopoulos C, Nicoll AJ, Ryan M, Sood S, et al. Practical dietary recommendations for the prevention and management of nonalcoholic fatty liver disease in adults. *Adv Nutr.* (2018) 9:30–40. doi: 10.1093/advances/nmx007

9. Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology.* (2010) 51:121–9. doi: 10.1002/hep.23276

10. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology.* (2015) 149:367–78. e5. doi: 10.1053/j.gastro.2015.04.005

11. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol.* (2012) 56:255–66. doi: 10.1016/j.jhep.2011.06.010

12. Boden G. High-or low-carbohydrate diets: which is better for weight loss, insulin resistance, and fatty livers? *Gastroenterology.* (2009) 136:1490–2. doi: 10.1053/j.gastro.2009.03.019

13. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS, Brehm BJ, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med.* (2006) 166:285–93. doi: 10.1001/archinte.166.3.285

14. Kirk E, Reeds DN, Finck BN, Mayurranjan MS, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology.* (2009) 136:1552–60. doi: 10.1053/j.gastro.2009.01.048

15. Ryan MC, Abbasi F, Lamendola C, Carter S, McLaughlin TL. Serum alanine aminotransferase levels decrease further with carbohydrate than fat restriction in insulin-resistant adults. *Diabetes Care.* (2007) 30:1075–80. doi: 10.2337/dc06-2169

16. McLaughlin T, Carter S, Lamendola C, Abbasi F, Yee G, Schaaf P, et al. Effects of moderate variations in macronutrient composition on weight loss and reduction in cardiovascular disease risk in obese, insulin-resistant adults. *Am J Clin Nutr.* (2006) 84:813–21. doi: 10.1093/ajcn/84.4.813

17. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med.* (2003) 348:2082–90. doi: 10.1056/NEJMoa022207

18. Jensen-Urstad AP, Semenkovich CF. Fatty acid synthase and liver triglyceride metabolism: housekeeper or messenger? *Biochim Biophys Acta Mol Cell Biol Lipids.* (2012) 1821:747–53. doi: 10.1016/j.bbalip.2011.09.017

19. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest.* (2005) 115:1343–51. doi: 10.1172/JCI23621

20. Diraison F, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab.* (2003) 29:478–85. doi: 10.1016/S1262-3636(07)70061-7

21. Browning JD, Baker JA, Rogers T, Davis J, Satapati S, Burgess SC. Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction. *Am J Clin Nutr.* (2011) 93:1048–52. doi: 10.3945/ajcn.110.007674

22. Hession M, Rolland C, Kulkarni U, Wise A, Broom J. Systematic review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities. *Obesity Rev.* (2009) 10:36–50. doi: 10.1111/j.1467-789X.2008.00518.x

23. Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, et al. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology.* (2011) 53:1504–14. doi: 10.1002/hep.24242

24. Kang H, Greenson JK, Omo JT, Chao C, Peterman D, Anderson L, et al. Metabolic syndrome is associated with greater histologic severity, higher carbohydrate, and lower fat diet in patients with NAFLD. *Am Coll Gastroenterol.* (2006) 101:2247–53. doi: 10.1111/j.1572-0241.2006.00719.x

25. Mardinoglu A, Wu H, Bjornson E, Zhang C, Hakkarainen A, Räsänen SM, et al. An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell Metab.* (2018) 27:559–71. e5. doi: 10.1016/j.cmet.2018.01.005

26. Kani AH, Alavian SM, Esmaillzadeh A, Adibi P, Azadbakht L. Effects of a novel therapeutic diet on liver enzymes and coagulating factors in patients with non-alcoholic fatty liver disease: a parallel randomized trial. *Nutrition.* (2014) 30:814–21. doi: 10.1016/j.nut.2013.11.008

27. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928

28. Rodríguez-Hernández H, Cervantes-Huerta M, Rodríguez-Moran M, Guerrero-Romero F. Decrease of aminotransferase levels in obese women is related to body weight reduction, irrespective of type of diet. *Ann Hepatol.* (2016) 10:486–92. doi: 10.1016/S1665-2681(19)31517-0

29. Arefhosseini SR, Ebrahimi-Mameghani M, Naeimi AF, Khoshbaten M, Rashid J. Lifestyle modification through dietary intervention: Health promotion of patients with non-alcoholic fatty liver disease. *Health Promot Perspect.* (2011) 1:147. doi: 10.5681/hpp.2011.016

30. De Luis D, Aller R, Izaola O, Gonzalez Sagrado M, Conde R. Effect of two different hypocaloric diets in transaminases and insulin resistance in nonalcoholic fatty liver disease and obese patients. *Nutr Hosp.* (2010) 25:730–5.

31. Marina A, von Frankenberg A, Suvag S, Callahan H, Kratz M, Richards T, et al. Effects of dietary fat and saturated fat content on liver fat and markers of oxidative stress in overweight/obese men and women under weight-stable conditions. *Nutrients.* (2014) 6:4678–90. doi: 10.3390/nu6114678

32. Benjaminov O, Beglaibter N, Gindy L, Spivak H, Singer P, Wienberg M, et al. The effect of a low-carbohydrate diet on the nonalcoholic fatty liver in morbidly obese patients before bariatric surgery. *Surg Endosc.* (2007) 21:1423–7. doi: 10.1007/s00464-006-9182-8

33. Yu H, Jia W, Guo Z. Reducing liver fat by low carbohydrate caloric restriction targets hepatic glucose production in non-diabetic obese adults with non-alcoholic fatty liver disease. *J Clin Med.* (2014) 3:1050–63. doi: 10.3390/jcm3031050

34. Biolato M, Manca F, Marrone G, Cefalo C, Racco S, Miggiano GA, et al. Intestinal permeability after Mediterranean diet and low-fat diet in non-alcoholic fatty liver disease. *World J Gastroenterol.* (2019) 25:509. doi: 10.3748/wjg.v25.i4.509

35. Properzi C, O'Sullivan TA, Sherriff JL, Ching HL, Jeffrey GP, Buckley RF, et al. *Ad libitum* mediterranean and low-fat diets both significantly reduce hepatic steatosis: a randomized controlled trial. *Hepatology.* (2018) 68:1741–54. doi: 10.1002/hep.30076

36. Ryan MC, Itsiopoulos C, Thodis T, Ward G, Trost N, Hofferberth S, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *J Hepatol.* (2013) 59:138–43. doi: 10.1016/j.jhep.2013.02.012

37. Ravanshad S, Amirkalali B, Saberfirozi M, Zare N, Maram E. Therapeutic effects of restricted diet in obese patients with Non-alcoholic Fatty Liver Disease (NAFLD). *Pak J Med Sci.* (2005) 21:472–5. Available online at: https://www.pjms.com.pk/issues/octdec05/article/article19.html

38. Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, et al. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. *Lancet Public Health.* (2018) 3:e419–28. doi: 10.1016/S2468-2667(18)30135-X

39. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, et al. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med.* (2008) 359:229–41. doi: 10.1056/NEJMoa0708681

40. Xu JH, Ding YM, Wang B, Fu HF, Xu YZ. Impact of low-carbohydrate diet on the clinical indicators of non-alcoholic fatty liver disease. *Chin J Clin Nutr.* (2013) 21:287–91. doi: 10.3760/cma.j.issn.1674-635X.2013.05.005

41. Schwimmer JB, Ugalde-Nicalo P, Welsh JA, Angeles JE, Cordero M, Harlow KE, et al. Effect of a low free sugar diet vs usual diet on nonalcoholic fatty liver disease in adolescent boys: a randomized clinical trial. *JAMA.* (2019) 321:256–65. doi: 10.1001/jama.2018.20579

42. Khodami B, Hatami B, Yari Z, Alavian SM, Sadeghi A, Varkaneh HK, et al. Effects of a low free sugar diet on the management of nonalcoholic fatty liver disease: a randomized clinical trial. *Eur J Clin Nutr.* (2022) 76:987–994. doi: 10.1038/s41430-022-01081-x

43. Kenneally S, Sier JH, Moore JB. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. *BMJ Open Gastroenterol.* (2017) 4:e000139. doi: 10.1136/bmjgast-2017-000139

44. Eslamparast T, Tandon P, Raman M. Dietary composition independent of weight loss in the management of non-alcoholic fatty liver disease. *Nutrients.* (2017) 9:800. doi: 10.3390/nu9080800

45. Wong VW, Chan RS, Wong GL, Cheung BH, Chu WC, Yeung DK, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol.* (2013) 59:536–42. doi: 10.1016/j.jhep.2013.04.013

46. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia.* (2012) 55:885–904. doi: 10.1007/s00125-011-2446-4

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 16 September 2022
DOI 10.3389/fnut.2022.987545

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Gãman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Rajeev K. Singla,
Sichuan University, China
Mohammad Amin Khazeei Tabari,
Mazandaran University of Medical Sciences, Iran

*CORRESPONDENCE
Fengxia Lin
szlinfx@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 06 July 2022
ACCEPTED 29 August 2022
PUBLISHED 16 September 2022

CITATION
Jiang F, Yang R, Xue D, Li R, Tan M, Zeng Z, Xu L, Liu L, Song Y and Lin F (2022) Effects of a natural nutritional supplement on immune cell infiltration and immune gene expression in exercise-induced injury. *Front. Nutr.* 9:987545. doi: 10.3389/fnut.2022.987545

Effects of a natural nutritional supplement on immune cell infiltration and immune gene expression in exercise-induced injury

Feng Jiang[1†], Rongfeng Yang[2†], Diya Xue[1], Rong Li[3], Meiling Tan[4], Zhicong Zeng[1], Luhua Xu[1], Linling Liu[1], Yinzhi Song[1] and Fengxia Lin[1*]

[1]Department of Cardiology, Shenzhen Bao'an Chinese Medicine Hospital, Guangzhou University of Chinese Medicine, Shenzhen, China, [2]Department of Cardiology, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen, China, [3]Department of Obstetrics, Shenzhen Bao'an Chinese Medicine Hospital, Guangzhou University of Chinese Medicine, Shenzhen, China, [4]Wenhua Community Health Service Center, Shenzhen Luohu Hospital Group, Shenzhen, China

Inflammatory immune response plays a key role in exercise-induced injury and healing; however, the relevant regulatory mechanisms of immune infiltration in exercise-induced injuries remain less studied. In the present study, a highly efficient system for screening immunity-related biomarkers and immunomodulatory ability of natural nutritional supplements was developed by integrating intelligent data acquisition, data mining, network pharmacology, and computer-assisted target fishing. The findings demonstrated that resting natural killer cells showed a higher rate of infiltration after exercise, whereas naive B cells and activated dendritic cells showed higher rate of infiltration before exercise. Four key genes, namely *PRF1*, *GZMB*, *CCL4*, and *FASLG*, were associated with exercise-induced injuries and inflammatory immune response. In total, 26 natural compounds including echinacoside, eugenol, tocopherol, and casuariin were predicted by using the HERB databases. Molecular docking analysis showed that GZMB, FASLG, and CCL4 bound to echinacoside. *In vivo* experiments in mice showed that after 30 min swimming, natural killer (NK) cells showed high infiltration rates, and the key genes (*GZMB*, *PRF1*, *FASLG*, and *CCL4*) were highly expressed; however, echinocandin significantly reduced the level of NK cells and decreased the expression of the four key genes post exercise. This natural nutritional supplement may act to protect against inflammatory injury after exercise by suppressing specific immune infiltration.

KEYWORDS

sport injury, nutritional supplement, immune infiltration, inflammation, echinacoside

Introduction

Exercise and physical activity are effective in the prevention and treatment of a wide range of chronic diseases, and there is considerable evidence for a positive association between long-term exercise and health benefits (1). Physical activity lowers diabetic blood sugar levels (2) and blood pressure (3), reduces the risk of acute cardiovascular disease (4, 5) malignancies such as breast and colon cancer (6), prevents memory and cognitive impairment (7), and enhances immunity to pathogens (8). The Physical Activity Guidelines for Americans (9) recommend that children and adolescents should engage in moderate-to-vigorous physical activity for 60 min or more per day, and adults should engage in at least 150 min of moderate-intensity aerobic activity or at least 75 min of vigorous aerobic activity per week. However, injuries are an inevitable part of exercise and participation in sport; they affect continuation of the sport activity, and the inflammatory response caused by the injury is detrimental to health and may affect athletes' ability and performance. Thus, the prevention and treatment of sports-related injuries is particularly important. Inflammation is prevalent in the body after exercise; under normal circumstances, this short-term immune response facilitates the removal of necrotic damaged tissue to achieve renewal and repair (10, 11). Inflammation is also one of the main causes of pain and discomfort from post-exercise injuries, and non-steroidal anti-inflammatory drugs (NSAIDs) are used in certain high-intensity competitive sports (12). Delayed-onset muscle soreness is a common negative manifestation of post-exercise discomfort in the body, which was thought to be the result of a combination of factors such as connective tissue damage, muscle damage, lactic acid accumulation, and inflammatory response (13). Chronic inflammation caused by frequent sports-related injuries may place a greater burden on the body than the benefits of exercise. A survey of college athletes showed that 94% had used NSAIDs and 13.9% had overdosed on NSAIDs (14). However, NSAIDs have side effects such as gastrointestinal bleeding, kidney damage, and even inhibition of cartilage proliferation (15). Nutritional therapy is an important strategy to repair sports injuries; it is more widely available than conventional medical care and results in significant cost savings (16). In addition, dietary supplements that relieve pain and discomfort after sports injuries and promote injury repair have fewer side effects than, for example, NSAIDs, and are more effective in improving physical fitness (17). Natural ingredients, such as curcumin, quercetin, and resveratrol, are a great treasure trove and have been found to be effective for sports injuries (18–20). Our previous studies have found that *Rhodiola rosea* as a natural supplement has a positive effect on exercise capacity and performance, reduces post-exercise pain and skeletal muscle injury, and enhances antioxidant capacity (21). Therefore, we aimed to identify more natural nutritional supplements that are effective in the prevention of and recovery from sports-related injuries. We hope to find some key genes from which to predict some potential natural ingredients that exert anti-inflammatory effects in sports injuries, and use experiments to validate the anti-inflammatory effects of these candidate ingredients, and eventually convert these candidates into natural dietary supplements for use in the anti-inflammatory response to sports injuries.

Materials and methods

Gene expression profiles before and after exercise

Gene expression profiles of individuals before and after exercise was obtained by searching the GEO database; gene IDs were collected and then converted into gene symbols.

Analysis of immune cell infiltration and differentially expressed genes

The CIBERSORT deconvolution method (perm = 1000) was used to analyze immune cell infiltration. The gene expression profiles were screened for differentially expressed genes (DEGs) after normalization using the R limma package based on the cut-off criteria $|\log FC| \geq 1$ and adjP ≤ 0.05 (22).

Immune-related differentially expressed genes

In addition to immune cell infiltration analysis, we also studied the differential expression of immune-related genes before and after exercise. Immune-related genes were downloaded from the ImmPort database; subsequently, they were compared with DEGs to obtain a list of immune-related differentially expressed genes (ImmDEGs) (23).

Protein–protein interaction, hub genes, and enrichment analyses

We used the STRING database for protein–protein interaction (PPI) analysis of DEGs with confidence level ≥ 0.04 and filtered the top five genes, which were considered to be the hub genes according to Cytoscape's Cytohubba plugin (Degree algorithm). DEGs were also subjected to gene ontology (GO) enrichment analyses using the R package clusterProfiler (cutoff: $P \leq 0.05$ and $q \leq 0.05$) (24). Furthermore, to examine the overall gene enrichment more comprehensively, we performed

a gene set enrichment analysis (GSEA) of all genes after sorting by logFC descending order using R soft (pvalueCutoff = 0.05, pAdjustMethod = "BH") (25).

Prediction of key genes and nutrients and molecular docking for validation

Key genes were defined as intersecting genes between ImmDEGs and hub genes. On the basis of the identified key genes, we used the HERB database to back-predict the target nutrients (26). Proteins encoded by key genes were downloaded from the PDB database and subjected to molecular docking analysis using AutoDock software to validate nutrients (**Table 1**).

TABLE 1 All relevant software and websites used in this study.

Name	Entrance
GEO database	https://www.ncbi.nlm.nih.gov/geo/
R soft and main plug-in package	Version: R 4.1.1; Package: limma, clusterprofiler
ImmPort database	https://www.immport.org/home
String database	https://cn.string-db.org/
Cytoscape	Version: Cytoscape_v3.9.0; Plug-in: Degree
HERB database	http://herb.ac.cn/
PubChem database	https://pubchem.ncbi.nlm.nih.gov/
ChemOffice	Chem3D 19.0
Uniprot database	https://www.uniprot.org/
PDB database	https://www.rcsb.org/
Autodock vina	Autodock vina 1.1.2

Materials and experimental animals and treatment

According to the predicted results, we selected echinacoside for *in vitro* validation. Echinacoside was purchased from Chengdu HerbSubstance Co., Ltd. (Chengdu, China) and the percentage purity of echinacoside was 99.85%. Echinacoside was dissolved in 25 mg/mL dimethyl sulfoxide (DMSO) and diluted to 10mg/mL with phosphate-buffered saline. The final concentration of DMSO was less than 10%; the solution was then divided into 1.5 mL aliquots and stored at −80 °C until further use. Specific pathogen-free male C57BL/6 mice (body weight: 20 ± 2 g, age: 56–62 days, n = 12) were purchased from the Guangdong Medical Laboratory Animal Center (Guangdong, China). The mice were maintained under controlled temperature (22 ± 1 °C), humidity (50%), and lighting (12:12 h light/dark) conditions. After 7 days of habituation, the mice were randomly divided into four groups (n = 3 per group; **Figure 3D**). In the normal control group (NC group), mice were gavaged with distilled water (10 mL/kg) once a day for 1 week. In the model group, mice were gavaged with distilled water (10 mL/kg) once a day for 1 week and subjected to passive swimming activity for 30 min on the last day. In the long-term echinacoside group (LE group), mice were gavaged with echinacoside (100 mg/kg) (27) once a day for 1 week and subjected to passive swimming for 30 min on the last day. In the short-term echinacoside group (SE group), mice were gavaged with distilled water (10 mL/kg) once a day for 6 days; on the seventh day, the mice were gavaged with echinacoside (100mg/kg) (27) and, after 30 min, subjected to passive swimming for 30 min. Peripheral blood and muscle tissues were harvested immediately after the swimming session. All animal experiments were approved by the Animal Care and Use Committee of Affiliated Hospital of Guangzhou University of Chinese Medicine (approval number:20200331016), and experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Flow cytometry analysis

Flow cytometry was used to detect the percentage of natural killer (NK) cells and dendritic cell (DC) subsets in the peripheral blood of mice. Peripheral blood was collected into tubes containing ethylenediaminetetraacetic acid. Peripheral blood mononuclear cells (PBMCs) were obtained from whole blood by density gradient centrifugation. PBMCs (1×10^6) were incubated with fluorescein isothiocyanate-conjugated rat anti-mouse antibodies against CD45/PE, NK1.1/APC, CD11C/PE-CY5, and CD3 for 60 min in the dark. The antibodies were purchased from Tonbo (Beijing, China). The cell samples were detected on Novocyte D2060R (Agilent, CA, USA), and the percentage of $CD3^-NK1.1^+$ NK cell subset and $CD11C^+NK1.1^-$ DC subset was analyzed using NovoExpress 1.4.1.

Quantitative reverse transcription PCR

Total RNA was extracted from muscle tissues using TRIzol reagent. cDNA was synthesized using the EvoM-MLV kits. Quantitative reverse transcription-PCR (RT-qPCR) was performed using 2X SYBR Green qPCR Master Mix (K1070-500, APExBIO, US) on a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories) following the manufacturer's protocol and analyzed using the $2^{-\Delta\Delta Ct}$ method. The following optimized thermal conditions were used: 95°C for 30 s, 95°C for 5 s, and 40 cycles at 60°C for 5 s. The levels of mRNA were normalized to endogenous GAPDH, and the expression of target genes was analyzed using the $2^{-\Delta\Delta Ct}$ method.

TABLE 2 Primers used for quantitative reverse transcription-PCR.

Target	Primer	Sequence (5′-3′)
GZMB	FP	CCTGCTACTGCTGACCTTGT
	RP	GGGATGACTTGCTGGGTCTT
FASLG	FP	CAGCCCATGAATTACCCATGT
	RP	ATTTGTGTTGTGGTCCTTCTTCT
CCL4	FP	AAGCCAGCTGTGGTATTCCTGA
	RP	ATCTGAACGTGAGGAGCAAGG
PRF1	FP	CTGCCACTCGGTCAGAATG
	RP	CGGAGGGTAGTCACATCCAT

The experiments were repeated three times independently. The primer sequences used in this study are listed in Table 2.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). GraphPad Prism 8.0 software was used to perform statistical analysis and construct histograms. The differences between groups were evaluated by *t*-tests or one-way analysis of variance (ANOVA) with appropriate *post hoc* tests; and $P < 0.05$ was considered to indicate statistically significant differences.

Results

Gene expression profiles

We downloaded a gene expression matrix for GSE14642 from the GEO database. This data matrix contains data on gene expression levels in peripheral blood of 20 young women before and after 30 min cycle ergometry exercise (28). The work rate was calculated to 50% of the work rate between the anaerobic threshold and the peak oxygen uptake Individually.

Immune cell infiltration and differentially expressed genes

There were marked differences in the immune cell infiltration in these women before and after exercise (Figure 1A). Subsequently, Wilcoxon tests showed that resting NK cells were more highly infiltrated after exercise ($P = 0.001$), whereas naive B cells and activated DCs were more highly infiltrated before exercise ($P = 0.001$ and $P = 0.013$, respectively; Figure 1B). Moreover, 61 DEGs were identified: 8 genes were downregulated, and 53 were upregulated (Figures 1C,D).

Immune-related differentially expressed genes

Analysis of immune-related gene expression levels identified 23 ImmDEGs: all of them (e.g., *PRF1*) were highly expressed after exercise (Figure 1E).

Protein–protein interaction network construction, hub gene selection, and enrichment analysis

Five hub genes (*PRF1*, *GZMB*, *KLRF1*, *CCL4*, and *FASLG*) were identified on the basis of PPI analysis (Figures 2A,B). GO enrichment analysis revealed that DEGs were enriched in 52 biological processes (GO-BP) mainly associated with, for example, NK cell-mediated immunity and cellular defense response. Similarly, DEGs were enriched in eight molecular functions (GO-MF), with the major categories being immune receptor activity and chemokine activity (Figures 2C,D). However, cellular components were not significantly enriched. GSEA enrichment analysis showed that eight pathways were enriched; six pathways including NK cell-mediated cytotoxicity were upregulated after exercise, and two pathways including ECM-receptor interaction were downregulated after exercise (Figure 2E and Table 3).

Prediction of key genes and nutrients and molecular docking

Four common genes (*PRF1*, *GZMB*, *CCL4*, and *FASLG*) were obtained by intersecting the sets of ImmDEGs and hub genes, which were considered as key genes (Figure 2F). The top five natural Chinese herbs with the lowest P value predicted on the basis of key genes in the HERB database were selected (Table 4). The 26 candidate (Table 5) ingredients were identified by the intersection of the related target ingredients directly predicted based on key genes by the HERB database and the active ingredients of above-mentioned Chinese herbs. However, *PRF1* failed to predict natural Chinese medicine by the database. Of these 26 candidates, echinacoside (Figure 2G) was the most interesting to us, as this compound is the active ingredient in our predicted herb, Herba Cistanches, which have been used in China for thousands of years and are still used to this day to enhance physical functions. Based on this fact, we consider echinacoside to be the highest priority research candidate. Molecular docking of selected compound to key genes showed that GZMB, FASLG, and CCL4 bind tightly to echinacoside with their lowest binding free energies of −8.0kcal/mol, −7.2kcal/mol, −6.9kcal/mol, respectively (Figures 3A–C). However, we were unable to analyze the molecular docking

10.3389/fnut.2022.987545

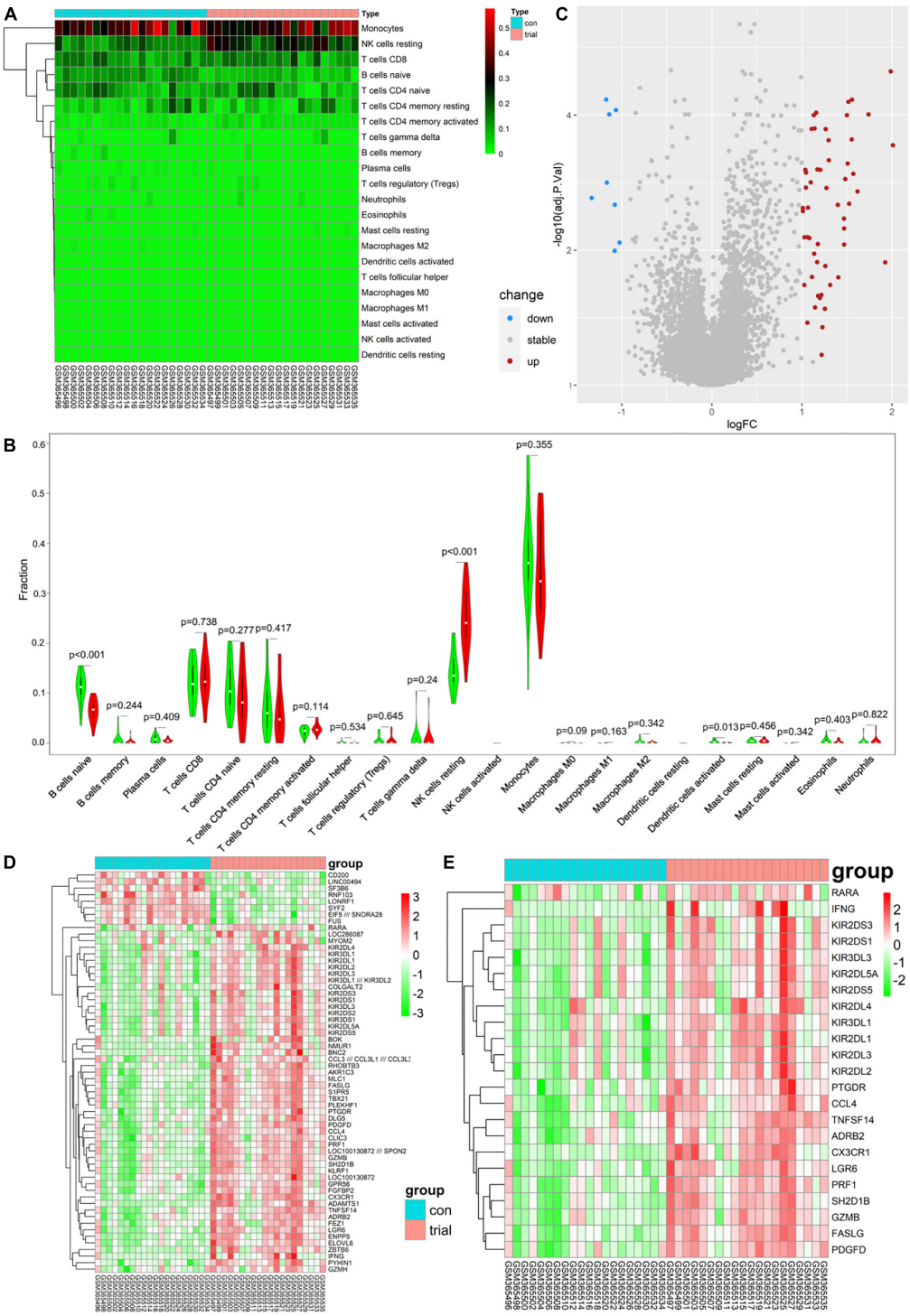


FIGURE 1

(A) Each column represents a sample, and each row represents a type of immune cells. Color transition from green to red represents an increase in immune cell infiltration level. Con group = before exercise; trial group = after exercise. **(B)** Green and red violin columns represent immune cell infiltration levels before and after exercise, respectively. The vertical axis represents the ratio of immune cell infiltration responsible for total immune cell infiltration. *P* value, obtained using the Wilcoxon test, represents the statistical significance of the difference in the immune cell infiltration levels before and after exercise. **(C)** Upregulated and downregulated differentially expressed genes (DEGs) are highlighted in red and blue, respectively. Criteria: $|\log FC| \geq 1$ and $adjP \leq 0.05$. **(D)** Expression levels of 61 DEGs are shown; the darker the red color, the higher the expression level, and the darker the green color, the lower the expression level. Con group = before exercise; trial group = after exercise. **(E)** Expression levels of 23 immune-related differentially expressed genes are shown; the darker the red color, the higher the expression level, and the darker the green color, the lower the expression level. Con group = pre-exercise; trial group = post-exercise.

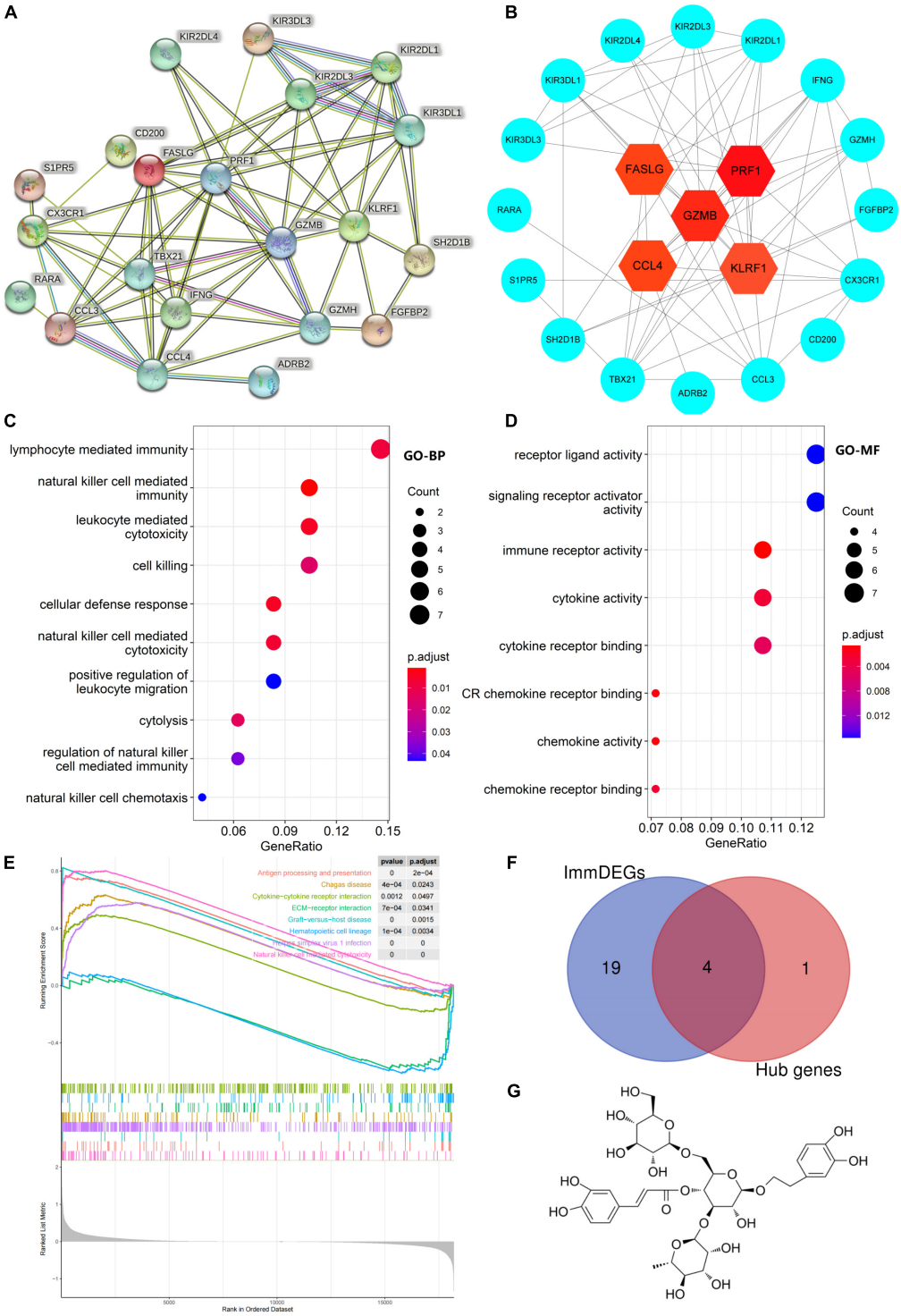


FIGURE 2
(A) Protein–protein interaction network of differentially expressed genes (DEGs). **(B)** Hub gene map, the darker the red color of the node, the stronger the protein interaction. **(C,D)** Top 10 biological processes and all molecular functions observed to be enriched in gene ontology enrichment analysis. The horizontal axis represents the gene ratio, that is, the ratio of the number of DEGs to number of total genes. Dot size is proportional to the gene ratio, and dot color transition from blue to red indicates that the adjusted P value is getting smaller. **(E)** Gene set enrichment analysis results, with each colored line representing an enriched pathway. **(F)** Intersection of immune-related differentially expressed genes (ImmDEGs) and hub genes; the genes common between the two groups were *GZMB*, *FASLG*, *CCL4*, and *PRF1*. **(G)** The two-dimensional structure of echinacoside.

TABLE 3 Gene set enrichment analysis results.

Pathway	Enrichment score	NES	*P*-value	Adjusted *P*	q-value
Natural killer cell mediated cytotoxicity	0.8020	2.1665	1.00E-10	3.33E-08	3.09E-08
Antigen processing and presentation	0.7687	1.9303	1.94E-06	0.0002	0.0002
Graft-versus-host disease	0.8231	1.868	1.79E-05	0.0015	0.0014
Herpes simplex virus 1 infection	0.5788	1.7565	5.57E-10	9.27E-08	8.61E-08
Chagas disease	0.6336	1.6734	0.0004	0.0242	0.0226
Cytokine-cytokine receptor interaction	0.4958	1.4536	0.0012	0.0497	0.0462
ECM-receptor interaction	−0.5803	−1.6812	0.0007	0.0341	0.0317
Hematopoietic cell lineage	−0.6125	−1.7857	5.09E-05	0.0034	0.0031

TABLE 4 Top five herbs with the lowest *P* value predicted on the basis of key genes.

Key gene	GZMB	FASLG	CCL4
Natural herbs	digenea simplex	rhizoma Zingiberis	millettia reticulata
	herba Cistanches	fagopyrum esculentum	rhamnus davurica
	mangifera indica	fructus Choerospondiatis	pericarpium papaveris
	eucalyptus viminalis	fructus Leonuri	cassia mimosoides
	folium camelliae sinensis	radix Platycodi	verbascum thapsus

TABLE 5 Candidate ingredients.

Key gene	GZMB		FASLG	CCL4
Candidate ingredients	Echinacoside	Kainic acid	6-shogaol	Rottlerin
	α-tocopherol	Lysine acid	Acacetin	Morphine
	βetaine	Tea polyphenols	Adeninenucleoside	
	Carotene	TellimagrandinII	Arsenic	
	Casuariin	Tocopherol	Gallic acid	
	Ethylparaben	Vanilloid	Geraniin	
	Vanilloid		Morphine	
	Soleucine		Progesterone	
	Eugenol		Ursolic acid	
	D-Chiro-Inositol		α-tocopherol	
	Hydroxybenzoic acid		Tocopherol	

of PRF1 with echinacoside because its 3D structure was not accessible.

Immune cell infiltration after echinacoside intervention detected by flow cytometry

The frequency of infiltrated immune cells in each group were detected by flow cytometry (**Figures 3E,F**). The percentage of infiltrating NK cells significantly increased after swimming. LE and SE groups both reduced the percentage of infiltrating NK cells with model group. Long-term supplementation with echinacoside restricted the infiltration of NK cells more significantly than short-term supplementation with echinacoside. However, in contrast, the percentage of DCs was significantly increased after swimming. Long-term or short-term supplementation with echinacoside did not significantly decrease the percentage of DCs after swimming.

Expression of key genes after echinacoside intervention detected by quantitative reverse transcription PCR

The mRNA levels of key genes were determined by quantitative reverse transcription PCR, which showed that the mRNA levels of all key genes were

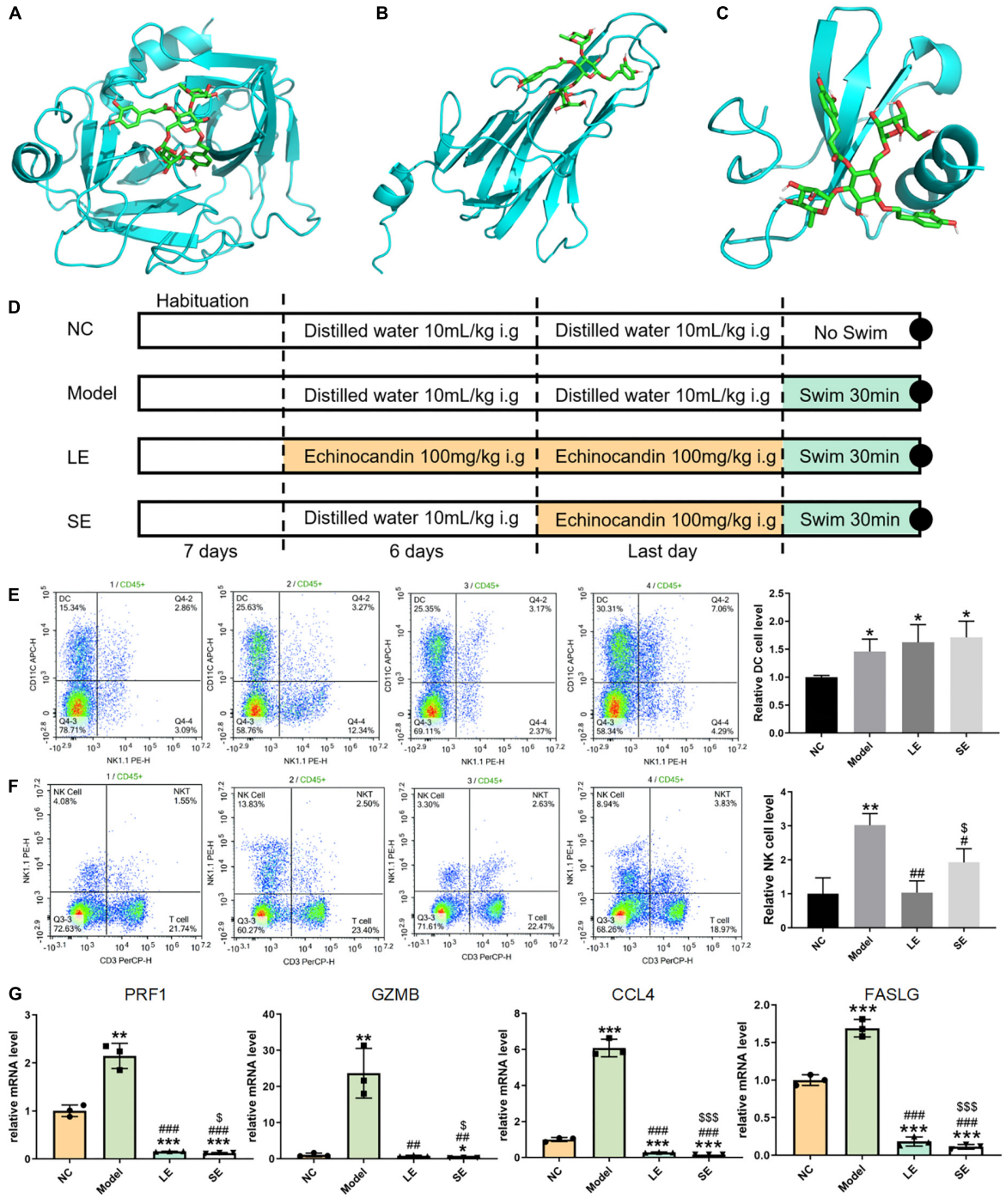


FIGURE 3
(A–C) Lowest-free-energy docking models of echinacoside with GZMB, FASLG, and CCL4, respectively. **(D)** Depiction of the control and experimental groups. **(E,F)** Results of flow cytometry analysis of dendritic cells (DCs) and natural killer (NK) cells, respectively. **(G)** Quantitative reverse transcription-PCR results for key gene expression levels averaged over three independent experiments. Data are shown as mean ± standard error of the mean. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Model; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ vs. LE.

significantly increased after swimming (Figure 3G). Both long-term or short-term supplementation with echinacoside significantly reduced the expression level of key genes. *GZMB*, *PRF1*, and *CCL4* were markedly downregulated in the SE group compared with the LE group.

Discussion

Our analysis of immune cell infiltration in the peripheral blood before and 30 min after exercise showed that NK cells showed a higher rate of infiltration post exercise, whereas naive B cells and activated DCs showed a low rate of infiltration (see **Figure 1B**). DCs, the most effective antigen-presenting cells, play an important role in immune homeostasis, bridging the gap between innate and adaptive immunity (29). They rapidly perform uptake and presentation functions when foreign antigens are present, activating T cells to produce an immune clearance response and inducing immune tolerance in the presence of self-antigens (30). Naive B cells are immature B cells that play an immunomodulatory role in inflammation by secreting cytokines and other pro-inflammatory indicators (31). Both of these cells are adaptive immune cells. NK cells are innate immune cells that determine the inflammatory microenvironment and are important in the removal of foreign infectious agents and own necrotic tissue (32). Therefore, the immune infiltration results do not signify an increase in immunity after 30 min of exercise but a sterile inflammation resulting from exercise-induced injury. We found that 53 genes were upregulated and 8 genes were downregulated post exercise (see **Figures 1C,D**). Notably, a PPI analysis of these differentially expressed genes showed that four of the top five genes (*PRF1*, *GZMB*, *CCL4*, and *FASLG*) were immune-related genes (see **Figure 2F**). GMZB, PRF1, and FASLG are key cytotoxic proteins in the secretory lysosomes in NK cells and typically produce cytotoxic effects in the inflammation zone (33). Thus, the findings suggest that the women developed immune dysregulation after 30 min of exercise, which was primarily associated with abnormally high levels of NK cells.

PRF1 is a potent component of NK cells; *PRF1* expression levels are positively correlated with the inflammatory toxic effects of NK cells, and PRF1 targeting regulates (enhances or decreases) the activity and function of NK cells (34–37). Thus, the high expression of *PRF1* after exercise is consistent with the high rate of NK cell infiltration. GZMB is a component of NK cell endolytic granules and is known for its apoptosis-promoting function (38, 39). It is an important mediator of the inflammatory response, and some studies have found that silencing *GZMB* reduces tissue damage caused by inflammation, for example, in a rat model of rheumatoid arthritis, *GZMB* silencing significantly reduced the degree of swelling in the ankle joint and reduced joint soft tissue damage (40, 41). FASLG, a member of the tumor necrosis factor family, is a transmembrane protein that forms an apoptotic cascade with FAS that is important for cell proliferation homeostasis (42, 43). This factor is widely involved in the inflammatory response and is highly expressed in inflammatory injury responses such as neurological injury, acute lung injury, kidney injury, and traumatic brain injury, and its expression is significantly associated with the extent of acute burns (44–47). Similar to *PRF1*, the high *GZMB* and *FASLG* expression was consistent with a high infiltration rate of NK cells, suggesting an inflammatory response following exercise. CCL4 is also an inflammatory chemokine that mediates the inflammatory immune response by recruiting lymphocytes, NK cells, and eosinophils, among others (48, 49). Several previous studies have found that the expression of this chemokine is significantly elevated in the inflammatory immune response following exercise (50, 51). It has also been found that CCL4 is a key mediator of neuroinflammatory pain following nerve injury (52). Overall, the high expression of these key genes reflects the fact that inflammatory damage does occur after acute exercise.

Notably, we predicted 26 natural immunomodulatory compounds on the basis of these immune infiltration findings. Echinacoside is a key natural component of the traditional Chinese medicinal herb *Herba Cistanches*. This compound remains little studied in the field of exercise, but it is widely used in traditional Chinese medicine because it is believed to play a key role in regulating human body functions. Interestingly, the substance is homologous to food and medicine; therefore, we intended to investigate whether it regulates inflammation after sports-related injury and further examine whether it is a potential sports supplement. Therefore, we selected this natural ingredient, echinacoside, as the optimal candidate compound and as the experimentally verified ingredient. Firstly, we verified the tightness of binding between echinacoside and GZMB using molecular docking and we found a minimum binding free energy of −8.0kcal/mol between them, which indicating a very tight binding. Furthermore, although echinacoside are not among the other key genetically predicted natural ingredients, we wanted to explore whether this component could bind tightly to key genes other than GZMB, such as FASLG. Surprisingly, we found that the binding free energy of echinacoside to FASLG and CCL4 was also very low, at −7.2kcal/mol and −6.9kcal/mol, respectively, suggesting that this natural ingredient also binds tightly to FASLG and CCL4. Finally, Experiments in mice were used to verify the modulatory effect of echinacoside on immune imbalance after sports-related injury. Remarkably, the flow cytometry results showed that mice gavaged with echinacoside had significantly lower levels of NK cells in their peripheral blood; the LE group showed the lowest NK cell levels, equivalent to those in the NC group, followed by the SE group, whereas the Model group, which was not supplemented with echinacoside, showed the highest levels of NK cells in the peripheral blood (see **Figure 3F**). However, for DCs, there was no statistical difference in the DC levels between the Model, LE, and SE groups, which all showed significant higher DC levels than those in the NC group. On the basis of this finding, we analyzed the factors affecting the expression levels of DCs in peripheral blood. DCs maintain overall immune homeostasis through their own active or passive regulation during an inflammatory response occurring after exercise; for example, in tissue damage inflammation, vimentin

prevents autoimmune damage by inhibiting DCs (53). The mode of energy supply is another important factor affecting DC expression. Hypoxia or changes in the nutritional status affect DC metabolism, aerobic glycolysis significantly promotes DC activation, and inhibition of glycolytic efficiency directly prevents DC activation (54, 55). It is difficult to identify which factor most influences the function of DCs, but these factors together lead to the dynamic regulation of DCs.

Furthermore, we examined the expression levels of key genes (*GZMB*, *PRF1*, *FASLG*, and *CCL4*) in each mouse group by RT-qPCR and found that the expression levels of these genes in the LE and SE groups were similar to those in the NC group, significantly lower than those in the Model group, and the lowest in the SE group (see **Figure 3G**). Overall, the flow cytometry results for NK cells were consistent with the expression levels of key genes. However, unexpectedly, the expression of key genes was lower in the SE group than in the LE group. We then reviewed the pharmacokinetics of echinacoside and found that this natural active ingredient is extremely rapidly absorbed in the gastrointestinal tract, with a peak blood concentration time of 15 min and a short half-life of 74 min (56). The changes in the expression levels of these key genes intervened by echinacoside are relatively consistent with the pharmacokinetics of echinacoside. This may therefore explain the lowest expression of key genes in the SE group. Therefore, our preliminary findings suggest that echinacoside administration for a longer period is more effective in regulating peripheral blood NK cell levels after exercise and that this natural supplement is relatively more effective in reducing inflammation when administered 30 min before exercise.

In summary, echinacoside effectively modulates the inflammatory immune response following exercise, primarily by regulating NK cell levels. We report, for the first time to our knowledge, that echinacoside, a promising natural compound, may be used as a dietary supplement to reduce inflammatory damage due to excessive NK cell infiltration following exercise by reducing the elevated NK cell levels and lowering the levels of NK cell-associated cytotoxic proteins such as GZMB, PRF1, FASLG, and CCL4 in the peripheral blood.

In this study, our approach has pioneered a very novel screening model for natural nutritional candidates, a systematic model that allows for rapid screening of a number of potential target natural compounds, greatly improving research efficiency (see **Figure 4**). The results of our study, echinacoside, which can bring preventive and therapeutic effects to patients with sports injuries, are of great clinical application. Particularly

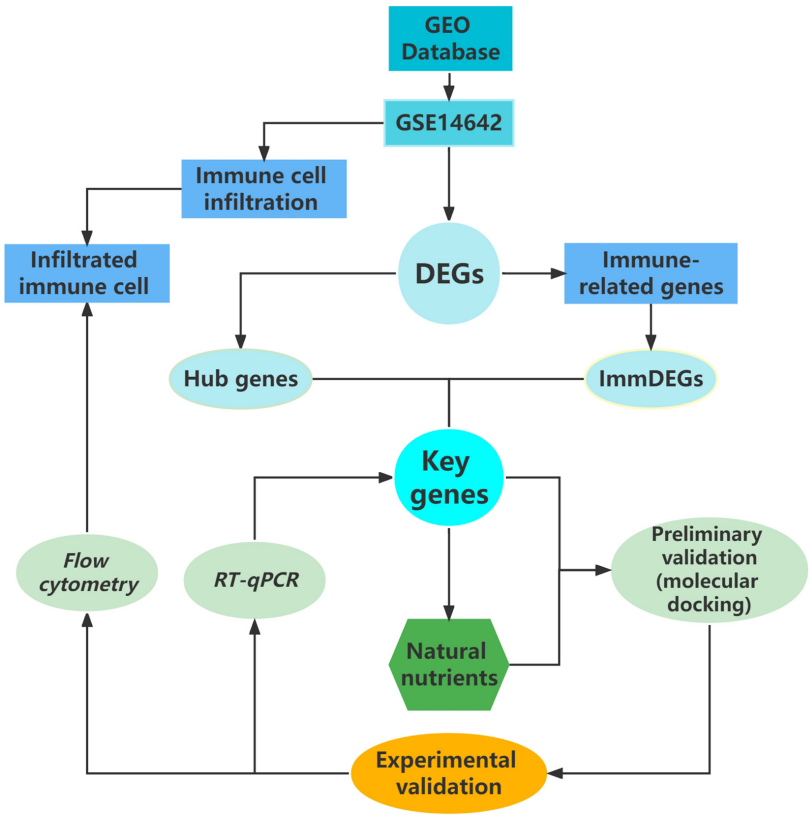


FIGURE 4
Flow chart of research methodology.

in the context of the apparent underdevelopment of anti-inflammatory dietary supplements for the prevention and mitigation of sports injuries, both our research model and echinacoside show strong potential in this area.

Data availability statement

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

Ethics statement

The animal study was reviewed and approved by Animal Care and Use Committee of Affiliated Hospital of Guangzhou University of Chinese Medicine.

Author contributions

FL and RY designed and planned the study and identified, and selected original studies. FJ and DX drafted the manuscript. FL and YS revised the final manuscript. LL and RL performed most experiments with some help from FL and RY. FL performed all the bioinformatics analyses. LX and MT contributed to data analysis. FL and ZZ provided advice during the study and manuscript preparation. All authors read and approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (82004320), the Natural Science Foundation of Guangdong Province of China (2021A1515011095, 2022A1515011710, and 2022A1515010679), the Science and Technology Project of Shenzhen City of China (JCYJ20190807115201653), and Shenzhen Bao'an Chinese Medicine Hospital Research Program (BAZYY20220702).

Acknowledgments

We would like to thank the native English speaking scientists of Elixigen Company (Huntington Beach, California) for editing our manuscript.

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.2022.987545/full#supplementary-material

References

1. Chaput JP, Willumsen J, Bull F, Chou R, Ekelund U, Firth J, et al. 2020 WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5-17 years: summary of the evidence. *Int J Behav Nutr Phys Act.* (2020) 17:141. doi: 10.1186/s12966-020-01037-z

2. Smith AD, Crippa A, Woodcock J, Brage S. Physical activity and incident type 2 diabetes mellitus: a systematic review and dose–response meta-analysis of prospective cohort studies. *Diabetologia.* (2016) 59:2527–45. doi: 10.1007/s00125-016-4079-0

3. Börjesson M, Onerup A, Lundqvist S, Dahlöf B. Physical activity and exercise lower blood pressure in individuals with hypertension: narrative review of 27 RCTs. *Br J Sports Med.* (2016) 50:356–61. doi: 10.1136/bjsports-2015-095786

4. Jeong SW, Kim SH, Kang SH, Kim HJ, Yoon CH, Youn TJ, et al. Mortality reduction with physical activity in patients with and without cardiovascular disease. *Eur Heart J.* (2019) 40:3547–55. doi: 10.1093/eurheartj/ehz564

5. Fiuza-Luces C, Santos-Lozano A, Joyner M, Carrera-Bastos P, Picazo O, Zugaza JL, et al. Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors. *Nat Rev Cardiol.* (2018) 15:731–43. doi: 10.1038/s41569-018-0065-1

6. Schmitz KH, Campbell AM, Stuiver MM, Pinto BM, Schwartz AL, Morris GS, et al. Exercise is medicine in oncology: engaging clinicians to help patients move through cancer. *CA Cancer J Clin.* (2019) 69:468–84. doi: 10.3322/caac.21579

7. Valenzuela PL, Castillo-García A, Morales JS, de la Villa P, Hampel H, Emanuele E, et al. Exercise benefits on Alzheimer's disease: state-of-the-science. *Ageing Res Rev.* (2020) 62:101108. doi: 10.1016/j.arr.2020.101108

8. Chastin SFM, Abaraogu U, Bourgois JG, Dall PM, Darnborough J, Duncan E, et al. Effects of regular physical activity on the immune system, vaccination and

risk of community-acquired infectious disease in the general population: systematic review and meta-analysis. *Sports Med.* (2021) 51:1673–86. doi: 10.1007/s40279-021-01466-1

9. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, et al. The physical activity guidelines for Americans. *JAMA.* (2018) 320:2020–8. doi: 10.1001/jama.2018.14854

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

11. Lee EC, Fragala MS, Kavouras SA, Queen RM, Pryor JL, Casa DJ. Biomarkers in sports and exercise: tracking health, performance, and recovery in athletes. *J Strength Cond Res.* (2017) 31:2920–37. doi: 10.1519/jsc.0000000000002122

12. Matava MJ. Injectable nonsteroidal anti-inflammatory drugs in sport. *Clin J Sport Med.* (2018) 28:443–50. doi: 10.1097/jsm.0000000000000602

13. Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness : treatment strategies and performance factors. *Sports Med.* (2003) 33:145–64. doi: 10.2165/00007256-200333020-00005

14. O'Connor S, McCaffrey N, Whyte E, Moran K, Lacey P. Nonsteroidal anti-inflammatory drug use, knowledge, and behaviors around their use and misuse in Irish collegiate student-athletes. *Phys Sports Med.* (2019) 47:318–22. doi: 10.1080/00913847.2018.1553468

15. Bedi A, Trinh TQ, Olszewski AM, Maerz T, Ramme AJ. Nonbiologic injections in sports medicine. *JBJS Rev.* (2020) 8:e0052. doi: 10.2106/jbjs.Rvw.19.00052

16. Smith-Ryan AE, Hirsch KR, Saylor HE, Gould LM, Blue MN. Nutritional considerations and strategies to facilitate injury recovery and rehabilitation. *J Athl Train.* (2020) 55:918–30. doi: 10.4085/1062-6050-550-19

17. Rawson ES, Miles MP, Larson-Meyer DE. Dietary supplements for health, adaptation, and recovery in athletes. *Int J Sport Nutr Exerc Metab.* (2018) 28:188–99. doi: 10.1123/ijsnem.2017-0340

18. Fernández-Lázaro D, Mielgo-Ayuso J, Seco Calvo J, Córdova Martínez A, Caballero García A, Fernandez-Lazaro CI. Modulation of exercise-induced muscle damage, inflammation, and oxidative markers by curcumin supplementation in a physically active population: a systematic review. *Nutrients.* (2020) 12:501. doi: 10.3390/nu12020501

19. Huang CC, Lee MC, Ho CS, Hsu YJ, Ho CC, Kan NW. Protective and recovery effects of resveratrol supplementation on exercise performance and muscle damage following acute plyometric exercise. *Nutrients.* (2021) 13:3217. doi: 10.3390/nu13093217

20. Borghi SM, Pinho-Ribeiro FA, Fattori V, Bussmann AJ, Vignoli JA, Camilios-Neto D, et al. Quercetin inhibits peripheral and spinal cord nociceptive mechanisms to reduce intense acute swimming-induced muscle pain in mice. *PLoS One.* (2016) 11:e0162267. doi: 10.1371/journal.pone.0162267

21. Lu Y, Deng B, Xu L, Liu H, Song Y, Lin F. Effects of *Rhodiola rosea* supplementation on exercise and sport: a systematic review. *Front Nutr.* (2022) 9:856287. doi: 10.3389/fnut.2022.856287

22. Shen S, Kong J, Qiu Y, Yang X, Wang W, Yan L. Identification of core genes and outcomes in hepatocellular carcinoma by bioinformatics analysis. *J Cell Biochem.* (2019) 120:10069–81. doi: 10.1002/jcb.28290

23. Liu Y, Zhang X, Zhang J, Tan J, Li J, Song Z. Development and validation of a combined ferroptosis and immune prognostic classifier for hepatocellular carcinoma. *Front Cell Dev Biol.* (2020) 8:596679. doi: 10.3389/fcell.2020.596679

24. Sun Q, Li X, Xu M, Zhang L, Zuo H, Xin Y, et al. Differential expression and bioinformatics analysis of circRNA in non-small cell lung cancer. *Front Genet.* (2020) 11:586814. doi: 10.3389/fgene.2020.586814

25. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U.S.A.* (2005) 102:15545–50. doi: 10.1073/pnas.0506580102

26. Fang S, Dong L, Liu L, Guo J, Zhao L, Zhang J, et al. HERB: a high-throughput experiment- and reference-guided database of traditional Chinese medicine. *Nucleic Acids Res.* (2021) 49:D1197–206. doi: 10.1093/nar/gkaa1063

27. Liu J, Yang L, Dong Y, Zhang B, Ma X. Echinacoside, an inestimable natural product in treatment of neurological and other disorders. *Molecules.* (2018) 23:1213. doi: 10.3390/molecules23051213

28. Radom-Aizik S, Zaldivar F Jr, Leu SY, Cooper DM. A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females. *J Appl Physiol.* (2009) 107:168–75. doi: 10.1152/japplphysiol.00121.2009

29. Balan S, Saxena M, Bhardwaj N. Dendritic cell subsets and locations. *Int Rev Cell Mol Biol.* (2019) 348:1–68. doi: 10.1016/bs.ircmb.2019.07.004

30. Waisman A, Lukas D, Clausen BE, Yogev N. Dendritic cells as gatekeepers of tolerance. *Semin Immunopathol.* (2017) 39:153–63. doi: 10.1007/s00281-016-0583-z

31. Frasca D, Diaz A, Romero M, Garcia D, Blomberg B. B cell immunosenescence. *Annu Rev Cell Dev Biol.* (2020) 36:551–74. doi: 10.1146/annurev-cellbio-011620-034148

32. Li Y, Wang F, Imani S, Tao L, Deng Y, Cai Y. Natural killer cells: friend or foe in metabolic diseases? *Front Immunol.* (2021) 12:614429. doi: 10.3389/fimmu.2021.614429

33. Lettau M, Kabelitz D, Janssen O. Lysosome-related effector vesicles in T lymphocytes and NK cells. *Scand J Immunol.* (2015) 82:235–43. doi: 10.1111/sji.12337

34. Romero CA, Sánchez IP, Gutierrez-Hincapié S, Álvarez-Álvarez JA, Pereañez JA, Ochoa R, et al. A novel pathogenic variant in PRF1 associated with hemophagocytic lymphohistiocytosis. *J Clin Immunol.* (2015) 35:501–11. doi: 10.1007/s10875-015-0169-x

35. Huang Q, Ding J, Gong M, Wei M, Zhao Q, Yang J. Effect of miR-30e regulating NK cell activities on immune tolerance of maternal-fetal interface by targeting PRF1. *Biomed Pharmacother.* (2019) 109:1478–87. doi: 10.1016/j.biopha.2018.09.172

36. Lu CC, Wu TS, Hsu YJ, Chang CJ, Lin CS, Chia JH, et al. NK cells kill mycobacteria directly by releasing perforin and granulysin. *J Leukoc Biol.* (2014) 96:1119–29. doi: 10.1189/jlb.4A0713-363RR

37. Chang CJ, Chen YY, Lu CC, Lin CS, Martel J, Tsai SH, et al. *Ganoderma lucidum* stimulates NK cell cytotoxicity by inducing NKG2D/NCR activation and secretion of perforin and granulysin. *Innate Immun.* (2014) 20:301–11. doi: 10.1177/1753425913491789

38. Oboshi W, Watanabe T, Hayashi K, Nakamura T, Yukimasa N. QPY/RAH haplotypes of the GZMB gene are associated with natural killer cell cytotoxicity. *Immunogenetics.* (2018) 70:29–36. doi: 10.1007/s00251-017-1014-6

39. Turner CT, Lim D, Granville DJ. Granzyme B in skin inflammation and disease. *Matrix Biol.* (2019) 75-76:126–40. doi: 10.1016/j.matbio.2017.12.005

40. Velotti F, Barchetta I, Cimini FA, Cavallo MG. Granzyme B in inflammatory diseases: apoptosis, inflammation, extracellular matrix remodeling, epithelial-to-mesenchymal transition and fibrosis. *Front Immunol.* (2020) 11:587581. doi: 10.3389/fimmu.2020.587581

41. Bao CX, Chen HX, Mou XJ, Zhu XK, Zhao Q, Wang XG. GZMB gene silencing confers protection against synovial tissue hyperplasia and articular cartilage tissue injury in rheumatoid arthritis through the MAPK signaling pathway. *Biomed Pharmacother.* (2018) 103:346–54. doi: 10.1016/j.biopha.2018.04.023

42. Ji W, Xin Y, Zhang L, Liu X. ALG2 influences T cell apoptosis by regulating FASLG intracellular transportation. *Biochem J.* (2020) 477:3105–21. doi: 10.1042/bcj20200028

43. Magerus A, Bercher-Brayer C, Rieux-Laucat F. The genetic landscape of the FAS pathway deficiencies. *Biomed J.* (2021) 44:388–99. doi: 10.1016/j.bj.2021.06.005

44. Li Y, Sun Y, Cai M, Zhang H, Gao N, Huang H, et al. FAS Ligand Gene (FASLG) plays an important role in nerve degeneration and regeneration after rat sciatic nerve injury. *Front Mol Neurosci.* (2018) 11:210. doi: 10.3389/fnmol.2018.00210

45. Jiang H, Wang S, Hou L, Huang JA, Su B. Resveratrol inhibits cell apoptosis by suppressing long noncoding RNA (lncRNA) XLOC_014869 during lipopolysaccharide-induced acute lung injury in rats. *J Thorac Dis.* (2021) 13:6409–26. doi: 10.21037/jtd-21-1113

46. Ortiz A, Lorz C, Egido J. The FAS ligand/FAS system in renal injury. *Nephrol Dial Transplant.* (1999) 14:1831–4. doi: 10.1093/ndt/14.8.1831

47. Beer R, Franz G, Schöpf M, Reindl M, Zelger B, Schmutzhard E, et al. Expression of FAS and FAS ligand after experimental traumatic brain injury in the rat. *J Cereb Blood Flow Metab.* (2000) 20:669–77. doi: 10.1097/00004647-200004000-00004

48. Mukaida N, Sasaki SI, Baba T. CCL4 signaling in the tumor microenvironment. *Adv Exp Med Biol.* (2020) 1231:23–32. doi: 10.1007/978-3-030-36667-4_3

49. Kobayashi Y, Konno Y, Kanda A, Yamada Y, Yasuba H, Sakata Y, et al. Critical role of CCL4 in eosinophil recruitment into the airway. *Clin Exp Allergy.* (2019) 49:853–60. doi: 10.1111/cea.13382

50. Muders K, Pilat C, Deuster V, Frech T, Krüger K, Pons-Kühnemann J, et al. Effects of Traumeel (Tr14) on recovery and inflammatory immune response after repeated bouts of exercise: a double-blind RCT. *Eur J Appl Physiol.* (2017) 117:591–605. doi: 10.1007/s00421-017-3554-8

51. Peake JM, Roberts LA, Figueiredo VC, Egner I, Krog S, Aas SN, et al. The effects of cold water immersion and active recovery on inflammation and cell stress responses in human skeletal muscle after resistance exercise. *J Physiol.* (2017) 595:695–711. doi: 10.1113/jp272881

52. Saika F, Kiguchi N, Kobayashi Y, Fukazawa Y, Kishioka S. CC-chemokine ligand 4/macrophage inflammatory protein-1β participates in the induction of neuropathic pain after peripheral nerve injury. *Eur J Pain.* (2012) 16:1271–80. doi: 10.1002/j.1532-2149.2012.00146.x

53. Yu MB, Guerra J, Firek A, Langridge WHR. Extracellular vimentin modulates human dendritic cell activation. *Mol Immunol.* (2018) 104:37–46. doi: 10.1016/j.molimm.2018.09.017

54. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med.* (2016) 213:15–23. doi: 10.1084/jem.20151570

55. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* (2015) 25:771–84. doi: 10.1038/cr.2015.68

56. Jia C, Shi H, Wu X, Li Y, Chen J, Tu P. Determination of echinacoside in rat serum by reversed-phase high-performance liquid chromatography with ultraviolet detection and its application to pharmacokinetics and bioavailability. *J Chromatogr B Analyt Technol Biomed Life Sci.* (2006) 844:308–13. doi: 10.1016/j.jchromb.2006.07.040

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 27 September 2022
DOI 10.3389/fnut.2022.1013310

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Găman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Denisa Margina,
Carol Davila University of Medicine and Pharmacy, Romania
Lolita Matiashova,
L.T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine, Ukraine

*CORRESPONDENCE
Yunpeng Luan
luanyunpeng_sci@outlook.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 06 August 2022
ACCEPTED 06 September 2022
PUBLISHED 27 September 2022

CITATION
Li X, Luan Y, Li Y, Ye S, Wang G, Cai X, Liang Y, Kord Varkaneh H and Luan Y (2022) The effect of high-fructose corn syrup vs. sucrose on anthropometric and metabolic parameters: A systematic review and meta-analysis. *Front. Nutr.* 9:1013310. doi: 10.3389/fnut.2022.1013310

The effect of high-fructose corn syrup vs. sucrose on anthropometric and metabolic parameters: A systematic review and meta-analysis

Xiang Li[1†], Yunqi Luan[2†], Yuejin Li[3], Shili Ye[4], Guihui Wang[5], Xinlun Cai[5], Yucai Liang[6], Hamed Kord Varkaneh[6] and Yunpeng Luan[1,7*]

[1]The First Affiliated Hospital of Yunnan University of Traditional Chinese Medicine, Kunming, China, [2]Beijing Institute for Drug Control (Beijing Center for Vaccine Control), Beijing, China, [3]The General Surgery Department, The First People's Hospital of Yunnan Province, Kunming, China, [4]Faculty of Mathematics and Physics, Southwest Forestry University, Kunming, China, [5]Department of Endocrinology, Kunming Municipal Hospital of Traditional Chinese Medicine, Kunming, China, [6]Lairui Biotechnology (Yunnan) Co., Ltd. Yunnan, China, [7]Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming, China

High-fructose corn syrup (HFCS) has been speculated to have stronger negative metabolic effects than sucrose. However, given the current equivocality in the field, the aim of the present study was to determine the impact of HFCS use compared to sucrose on anthropometric and metabolic parameters. We searched PubMed, Scopus, Cochrane Central and web of sciences, from database inception to May 2022. A random effects model and the generic inverse variance method were applied to assess the overall effect size. Heterogeneity analysis was performed using the Cochran Q test and the I^2 index. Four articles, with 9 arms, containing 767 participants were included in this meta-analysis. Average HFCS and sucrose usage equated to 19% of daily caloric intake. Combined data from three studies indicated that HFCS intake does not significantly change the weight (weighted mean difference (WMD): −0.29 kg, 95% CI: −1.34, 0.77, $I^2 = 0\%$) when compared to the sucrose group. Concordant results were found for waist circumstance, body mass index, fat mass, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), systolic blood pressure (SBP), and diastolic blood pressure (DBP). Moreover, overall results from three studies indicated a significant increase in CRP levels (WMD: 0.27 mg/l, 95% CI: 0.02, 0.52, $I^2 = 23\%$) in the HFCS group compared to sucrose. In conclusion, analysis of data from the literature suggests that HFCS consumption was associated with a higher level of CRP compared to sucrose, whilst no significant changes between the two sweeteners were evident in other anthropometric and metabolic parameters.

KEYWORDS

high-fructose corn syrup, sucrose, fructose, weight, meta-analysis

Introduction

Per capita consumption of sugar in the world increased sharply in the twentieth century. Although reports suggest that this consumption has now leveled off (1), it is of concern that high levels of added sugars have negative effects on human health (2). Sweeteners can be divided into two groups: nutritional and non-nutritional; where nutritional sweeteners contain monosaccharides (fructose and galactose), disaccharides (sucrose, maltose, and maize-based sweeteners) and polyols (sugar alcohol) that produce energy. On the other hand, non-nutritional sweeteners, such as acesulfame K, aspartame, neotame, saccharin and sucralose, do not contain sweet calories (3). Saccharose (GI 61-68) is a sweetener and well-used ingredient in the food industry, however, can cause harmful health problems (4). Glucose, fructose, and sucrose are natural nutrient sweeteners, where sucrose consists of a glucose molecule that is linked to a fructose molecule. High fructose corn syrup is a mixture of glucose and free fructose. The wide distribution of hexokinase enzymes, which are essential for the production of glucose-6-phosphate and glycolytic enzymes, means that glucose is metabolized by all cells of the organism. Fructose, on the other hand, is not so readily phosphorylated by the hexokinases and is metabolized exclusively in the intestine, liver and kidney. Glucose metabolism is regulated by insulin after a meal, whilst after consuming a fructose-only diet, the bulk enters the intestine and the liver, with a markedly longer transit time than glucose. Up to 20% of fructose may be stored as hepatic glycogen, and a large part is converted to LDL/VLDL (5). Furthermore, energy efficiency from fructose metabolism is lower than glucose; where at lower intake, fructose stimulates the metabolic pathway of hepatic de novo lipid production more than glucose does. However, this pathway plays only a minor role in the total excretion of fructose (6–8). Fructose is a natural monosaccharide (glycemic index, GI: 19-23), used as a sweetener in the food industry, whilst high fructose corn syrup (HFCS) is a liquid substitute for sucrose with 42 or 55% (dry) fructose, and is obtained from the hydrolysis of corn starch to glucose using glucoamylase and α-amylase, followed by glucose isomerization to fructose, which results in the production of a mixture of glucose and fructose (9, 10). HFCS can provide flavor, color, texture, stability, and freshness in some food products, such as beverages, processed foods, baking products, ice cream and confectioneries (11). It has been demonstrated in a rat study that the addition of sugars, in general, and HFCS directly or indirectly contribute to obesity, as well as various types of metabolic disorders and diseases (12). Studies have shown that excess consumption of sugar can lead to weight gain, confers a greater risk of developing metabolic heart disease, and an increased risk of early mortality (2, 13–15). HFCS is a nutritional sweetener that is thought to be harmful for human health, partly attributed to preliminary research that shows consumption of large quantities of fructose (i.e. the main constituent of HFCS) can lead to deleterious metabolic consequences in the body (16–18). Sucrose is also comprised of glucose and fructose, which is absorbed in the digestive tract (19). Therefore, there is minimal difference between HFCS and sucrose, due to the ability of the human digestive system to absorb sucrose and fructose. Previous trials have shown that the use of HFCS in comparison with sucrose yields no significant difference in health-related indicators, such as glycemic index, calorie intake, lipid metabolism and inflammation (20–23). However, there is evidence to suggest that fructose consumption in comparison with sucrose has a significantly greater effect on indicators of health (24, 25). Thus, given the current equivocality in the field, the aim of the present study is to determine the impact of HFCS vs. sucrose on anthropometric and metabolic parameters.

Methods

This meta-analysis was performed and reported based on the 'Preferred Reporting Items for Systematic Review and Meta-analysis' (PRISMA) statement (26). The research questions were designed using the population, intervention, comparison, and outcomes (PICO) method.

Search strategy

We searched PubMed, Scopus, Cochrane Central and Clinicaltrials.gov and web of sciences, from database inception to May 2022. The search strategy combined Medical Subject Heading (MeSH) and non-MeSH terms: ("Clinical Trials as Topic"[Mesh] OR "Cross-Over Studies"[Mesh] OR "Double-Blind Method"[Mesh] OR "Single-Blind Method"[Mesh] OR "Random Allocation"[Mesh] OR RCT[Title/Abstract] OR "Intervention Studies"[Title/Abstract] OR "intervention"[Title/Abstract] OR "controlled trial"[Title/Abstract] OR "randomized"[Title/Abstract] OR "randomized"[Title/Abstract] OR "random"[Title/Abstract] OR "randomly"[Title/Abstract] OR "placebo"[Title/Abstract] OR "assignment"[Title/Abstract]) AND (("High Fructose Corn Syrup 55%"[tiab] OR "HFCS-55"[tiab] OR "High Fructose Corn Syrup"[tiab] OR "High Fructose Corn Syrup"[Mesh]) AND ((((Sucrose[Title/Abstract] OR "Sucrose"[Mesh]) OR "Sugar"[tiab]) OR "Sugars"[Mesh]) OR "Sugars"[tiab])) (see Supplementary Table 1). Two of the authors (J.R and H.K) implemented independent searches to avoid missing any relevant articles.

Eligibility criteria

The inclusion criteria were as follows: (1) RTs (2), HFCS use vs. sucrose (3), treatment for at least 48 weeks (4), reported

sufficient information on BMI, WC, and body weight, lipid profile, CRP, blood pressure, and blood glucose both in High Fructose Corn Syrup group and Sucrose administration group (5), inclusion of patients ≥ 18 y old. Publications that did not provide outcome measures at study baseline and end of the intervention (or changes in outcome measures), Observational studies, case reports, case series, non-systematic reviews and trials published as abstracts were excluded.

Data extraction and quality assessment

Titles and abstracts were independently reviewed against eligibility criteria by two investigators (C.C and Y.Z). Any controversies or disagreements were resolved by the third author (H.K) *via* cooperative triangulation. Choice of the final eligible articles was according to the consensus between three investigators (C.C, Y.Z, and H.K). Moreover, with reference to the measures of outcome, the following data were extracted: (1) study's first author, (2) year of publication, (3) type of study population, (4) number of people in each groups, participants' mean age, participants' gender, study design, trial duration and results (means and standard deviations of outcomes at baseline, end of study and/or changes between baseline and end-intervention). To assess the quality of studies, we used the Cochrane Collaboration tool to assess risk of bias (27).

Quantitative data synthesis

The changes in the mean values of body weight, body mass index, waist circumstance, fat mass, TC, HDL, LDL, TG, CRP, SBP, DBP was calculated for all included studies. The combined changes in outcomes were assessed as the difference (High Fructose Corn Syrup minus Sucrose) between its differences (post-trail values minus beginning trail) in the High Fructose Corn Syrup group and the Sucrose group. Standard deviations (SDs) of the mean difference were estimated

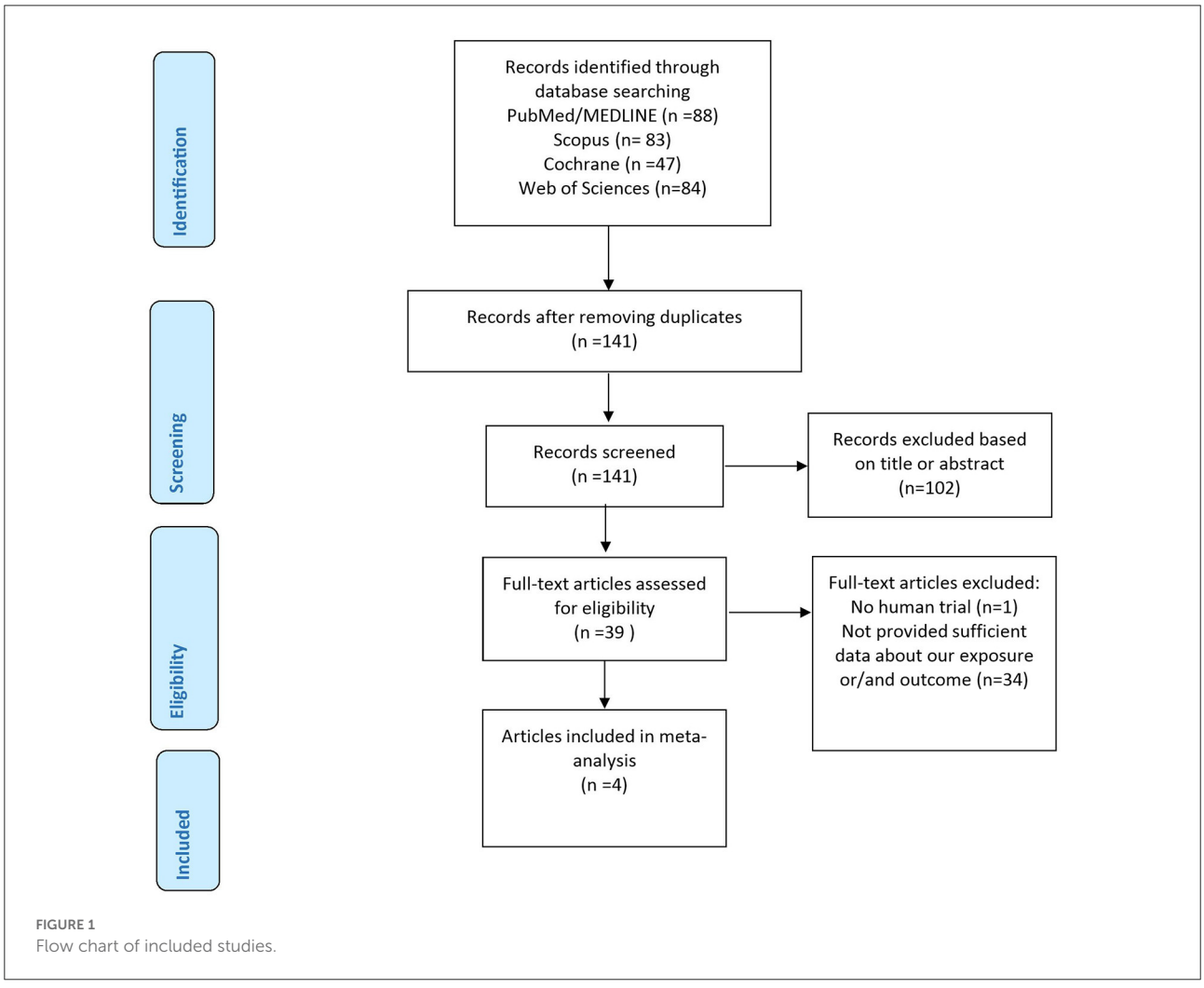


FIGURE 1
Flow chart of included studies.

using the following formula: SD = square root [(SDpre-intervention)2 C (SDpost- intervention)2 – (2R * SDpre-intervention*SDpost- intervention)], supposing a correlation coefficient (R) = 0.5 (28). A random-effects model and the generic inverse variance method were applied to assess overall effect size and the heterogeneity among studies. Heterogeneity analysis was performed using the Cochran Q test and the I^2 index. Moreover, probable publication bias was determined visually by funnel plot and confirmed, statistically, through the Egger's and begg's tests. All statistical analyses were performed using Stata software, version 14 (Stata Corp. College Station, Texas, USA).

Results

Figure 1 details the flow chart of our systematic search. In our comprehensive search of PubMed, Scopus, Web of Science, and Cochrane Library, and after removing duplicates, 141 articles were included in screening, and 102 articles were excluded because they did not meet the aforementioned inclusion criteria. In full-text screening, 35 articles were removed, and four articles, with 9 arms, containing 767 participants were included in this meta-analysis (20, 29–31).

Study characteristics and risk of bias assessment

Characteristics of the included studies are provided in Table 1. Mean age of participants was 41.51 years, ranging from 37 to 52 years and mean length of the interventions was 8.5 weeks, ranging from 2 to 12 weeks. All studies were conducted on both genders in the USA and articles were published between 2012 and 2016. Risk of bias of included studies was evaluated by the Cochrane's Handbook checklist, and most papers were confirmed as having good quality (Figure 2) (20, 29–31).

Meta-analysis results

Three studies, with six arms, including 326 participants in intervention groups and 319 participants in control groups reported weight as an outcome measure (20, 29, 30), and combined results did not show any significant change in HFCS (WMD: −0.29 kg, 95% CI: −1.34, 0.77, I^2 = 0%) compared to the sucrose group (Figure 3). There were comparable results for waist circumstance (WMD: −0.39 cm, 95% CI: −2.04, 1.25, I^2 = 0%) (29, 30), Body mass index (WMD: −0.18 kg/m^2, 95% CI: −0.46, 0.10, I^2 = 0%) (20, 29–31), and fat mass (WMD: −0.24 %, 95% CI: −2.04, 1.55, I^2 = 0%) (30).

Among eligible studies, three trails, with six arms, containing a total of 645 participants (326 participants in HFCS group and 319 participants in sucrose group) reported SBP, DBP, fasting blood glucose (FBG), TG, LDL, HDL, and TC as an outcome measure (20, 29, 30). Pooled results from the random-effects model did not show any significant change between the two groups in SBP (WMD: 0.76 mmHg, 95% CI: −0.86, 2.38, I^2 = 62%), DBP (WMD: −0.29 mmHg, 95% CI: −1.34, 0.77, I^2 = 0%), FBG (WMD: −0.87 mg/dl, 95% CI: −2.56, 0.82, I^2 = 84%), TG (WMD: −4.15 mg/dl, 95% CI: −30.37, 22.07, I^2 = 91%), LDL (WMD: −2.02 mg/dl, 95% CI: −5.69, 1.65, I^2 = 42%), HDL (WMD: −0.61 mg/dl, 95% CI: −1.55, 0.32, I^2 = 0%), and TC (WMD: −2.45 mg/dl, 95% CI: −9.12, 4.22, I^2 = 74%).

Overall results from three studies indicated a significant increase in CRP levels (WMD: 0.27 mg/l, 95% CI: 0.02, 0.52, I^2 = 23%) in the HFCS group compared to the sucrose group (20, 29, 30).

Publication bias

Funnel plot assessment did not show any asymmetry in included studies (Supplemental Figure 1). Furthermore, The Egger's and Begg's tests did not show any significant publication bias for Weight ($p = 0.09$ and $p = 0.57$), WC ($p = 0.25$ and $p = 0.99$), BMI ($p = 0.43$ and $p = 0.32$), Fat mass ($p = 0.62$ and $p = 0.60$), SBP ($p = 0.51$ and $p = 0.57$), DBP ($p = 0.60$ and $p = 0.57$), FBS ($p = 0.39$ and $p = 0.85$), TG ($p = 0.50$ and $p = 0.57$), LDL ($p = 0.84$ and $p = 0.57$), HDL ($p = 0.96$ and $p = 0.57$), TC ($p = 0.69$ and $p = 0.85$), and CRP ($p = 0.23$ and $p = 0.34$), respectively.

Discussion

Numerous empirical evidence has indicated that sugars, particularly HFCS and sucrose, can affect various anthropometric and metabolic parameters (6, 12). However, it remains debatable whether the effects of HFCS and sucrose are of equal magnitude. Some studies have demonstrated that consumption of HFCS and sucrose elicited comparable effects, while some other reports noted a marked difference between the two sugars (20, 22, 25). In this work, we performed a meta-analysis to determine whether the effect of HFCS and sucrose on anthropometric and metabolic parameters were concordant. We found that HFCS was significantly associated with an increased CRP level, compared to sucrose. However, we observed no difference between HFCS and sucrose in terms of their effects on weight, WC, BMI, fat mass, SBP, DBP, FBS, TG, LDL, HDL, and TC.

CRP is a biomarker for inflammation; and several previous investigations have shown that fructose-containing sweeteners, such as HFCS and sucrose, can induce the inflammatory process (32, 33). This is conceivably attributable to the unique metabolic process of fructose, which can cause oxidative stress to cells

TABLE 1 Characteristics of included studies.

Author	Country (year)	Study design (duration)	Gender	Age (year)	Patient features	Sample size HFCS/sucrose	Dose (mg)	Outcome(s)
Theodore J. Angelopoulos	USA (2016)	Parallel (10W)	Both	37.66	Healthy	91/89	18%	Body weight, WC, BMI, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Susan K Raatz	USA (2015)	Crossover (2W)	Both	38.9	Normal glucose tolerance (GT)	28/28	50 g	Body weight, BMI, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Susan K Raatz	USA (2015)	Crossover (2W)	Both	52.1	Impaired glucose tolerance (IGT)	27/27	50 g	Body weight, BMI, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Joshua Lowndes	USA (2014)	Parallel (10W)	Both	40.19	Overweight or obese individuals	51/53	30%	Body weight, WC, BMI, fat mass, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Joshua Lowndes	USA (2014)	Parallel (10W)	Both	40.19	Overweight or obese individuals	60/64	18%	Body weight, WC, fat mass, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Joshua Lowndes	USA (2014)	Parallel (10W)	Both	40.19	Overweight or obese individuals	69/58	8%	Body weight, WC, BMI, fat mass, SBP, DBP, Glucose, TG, LDL, HDL, TC, CRP
Joshua Lowndes	USA (2012)	Parallel (12W)	Both	40	Overweight/obese participants	36/29	10%	BMI
Joshua Lowndes	USA (2012)	Parallel (12W)	Both	42.9	Overweight/obese participants	24/33	30%	BMI

10.3389/fnut.2022.1013310

by elevating the intracellular levels of uric acid and reactive oxygen species (33, 34). To overcome the oxidative stress, cells release molecules such as monocyte chemotactic protein 1 (MCP-1), tumor necrosis factor (TNF) and interleukins, which are pro-inflammatory in nature and thus augment the inflammation process (33). HFCS and sucrose both contain the fructose moiety; however, the content of fructose in HFCS and sucrose is different, which conceivably explains why the two sugars induced inflammation differently. Despite this, in the present study, no significant difference was observed for any other anthropometric or metabolic parameter. The lack of significant difference could be attributed to several reasons. First, in some of the included studies, the duration of the experiment was short. For example, in the study by Raatz et al., the participants consumed the sugars for only 2 weeks (20); this duration is likely insufficient for significant weight changes to be manifest. In addition, compliance with the prescribed sugars was generally obtained through self-reported information from the participants, which could cause validity and bias issues (35, 36). Moreover, the studies generally delivered the sugars through milk or other foods, which contained other nutrients, such as vitamin D and calcium. These nutrients can affect several metabolic parameters, for example, vitamin D has been shown to alter cholesterol levels, especially LDL (37–39). On the other hand, calcium supplementation is known to decrease blood pressure (40). The presence of these nutrients in the foods used to deliver the sugars could act as confounders for our analyses; therefore, the findings should be interpreted with caution. Notwithstanding, the amount/concentration of the sugars consumed were different across the included studies; hence, it is conceivable that the effects elicited by the sugars was masked by studies which examined the lower concentration of the sugars.

There are a number of strengths and limitations of the present study. Firstly, through the amalgamation of studies, we yielded an increased statistical power of analysis compared to the individual studies (41). In addition, most studies included in the present meta-analysis had high methodological quality and employed a randomized trial design. A randomized trial minimizes various types of biases and confounders and produces strong empirical evidence of the effects of an intervention (42). It is also noteworthy that publication bias was not evident in our meta-analysis, which suggests that the results obtained are likely not exaggerated (43). Apart from that, the sugars consumed by the participants were incorporated into their normal diets, which allowed a more accurate evaluation of the real-world effects of the added sugar compared to a totally controlled trial (20). However, one of the limitations of this meta-analysis was that subgroup analyses were not performed due to the lack of incumbent data in the included studies, which was out of the operational control of the study. For instance, the effects of HFCS and sucrose on male and female participants were not evaluated separately in the included studies. Importantly,

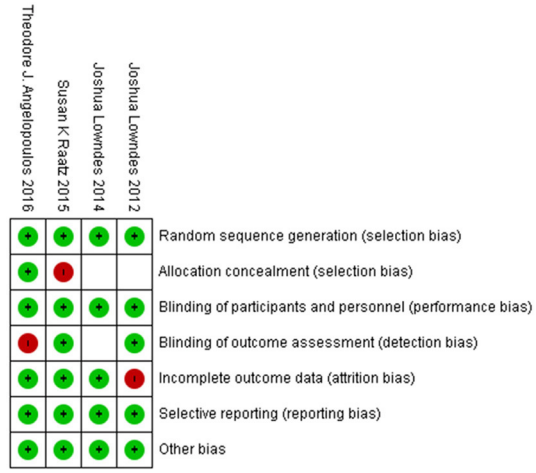


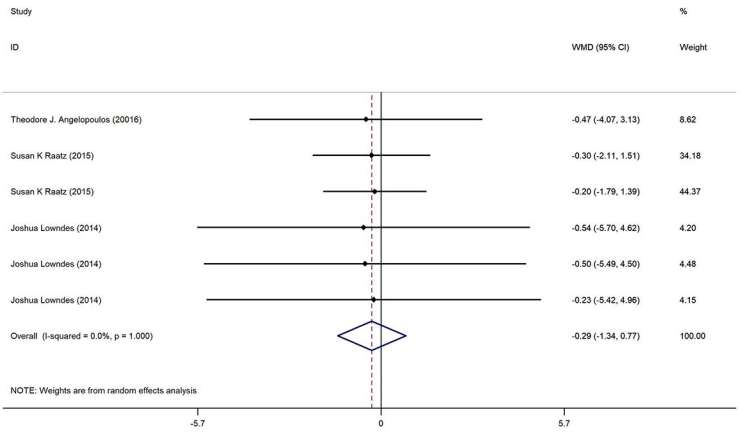
FIGURE 2
Risk of bias assessment of included studies.

many human and animal studies have demonstrated that males and females have different responses to fructose (44–48); for example, triglyceride levels in young women are less sensitive to modifications by fructose compared to men (44, 45, 48), and therefore represents a pragmatic avenue for further research. Besides gender, subgroup analysis by people of different age groups was not performed, although animal studies have indicated that fructose can induce metabolic changes differently in young and adult rats (49). Another limitation of this study was that, as mentioned above, compliance with the assigned sugar intake in the included studies was generally dependent on the self-reported information from the participants. This can cause misleading results if the participants did not adhere to the prescribed diet (35, 36). Finally, in this meta-analysis, we were unable to correct for some potential confounding factors, such as the co-incorporation of added vitamin D and calcium in the food used to deliver the sugars.

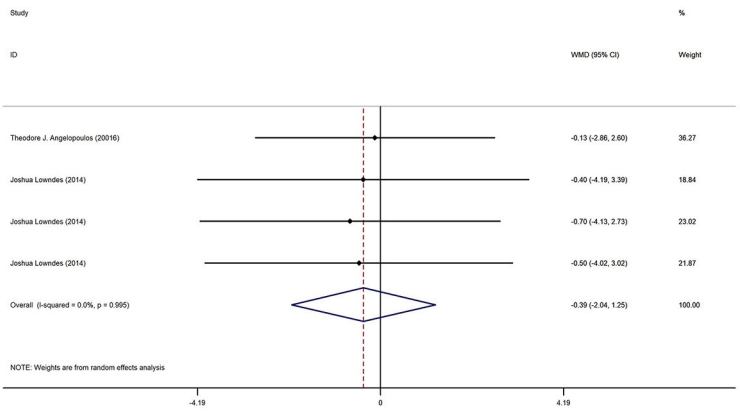
Conclusion

In conclusion, analysis of data from the literature suggests that HFCS is associated with a higher level of CRP compared to sucrose, and that little differences exist in other anthropometric and metabolic parameters. Nonetheless, considering the limitations of the study, careful interpretation of the results is warranted. Future studies are needed to address the limitations above, particularly related to gender-mediated differences. However, the present work successfully provided a reliable estimate of the effect and difference of HFCS and sucrose in a number of anthropometric and metabolic parameters.

A Weight



B Waist circumstance



C Body mass index

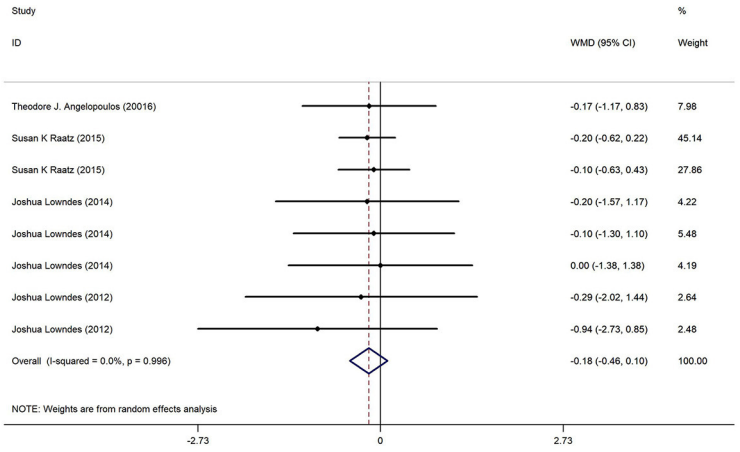
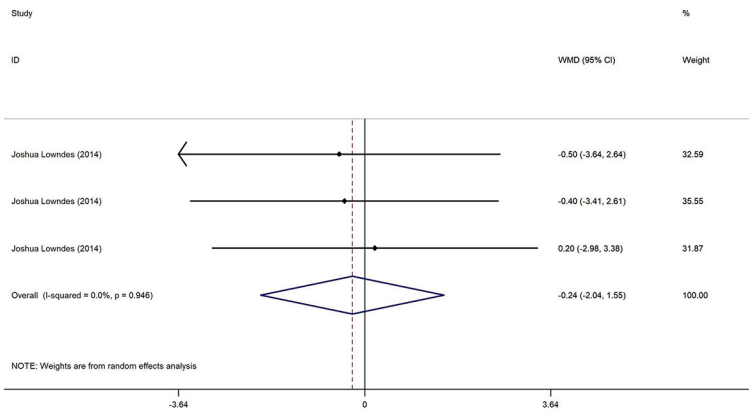
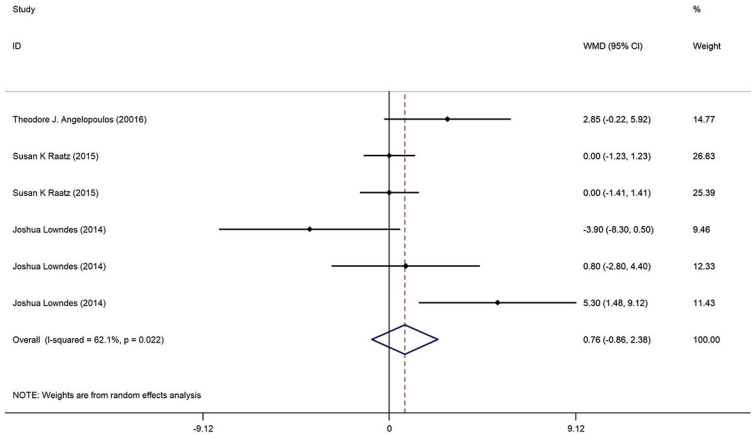


FIGURE 3
(Continued)

D Fat mass



E Systolic blood pressure



F Diastolic blood pressure

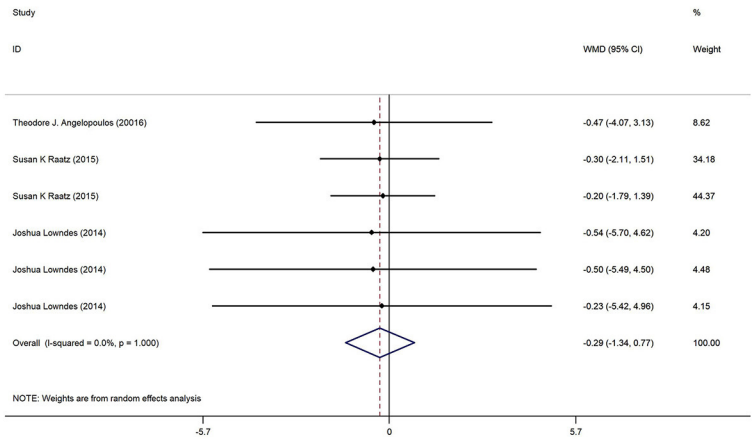
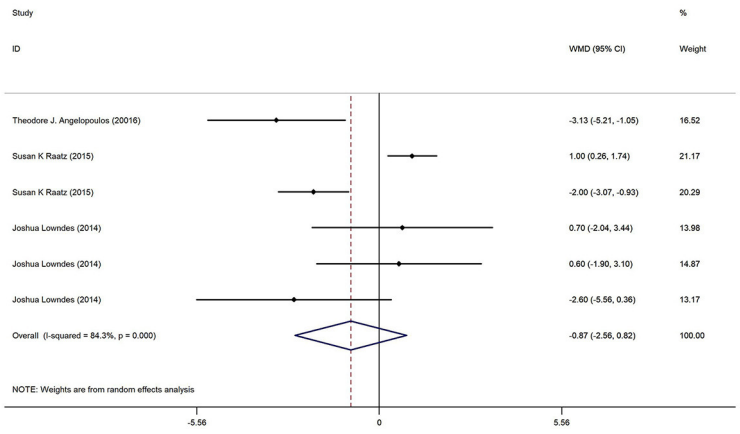
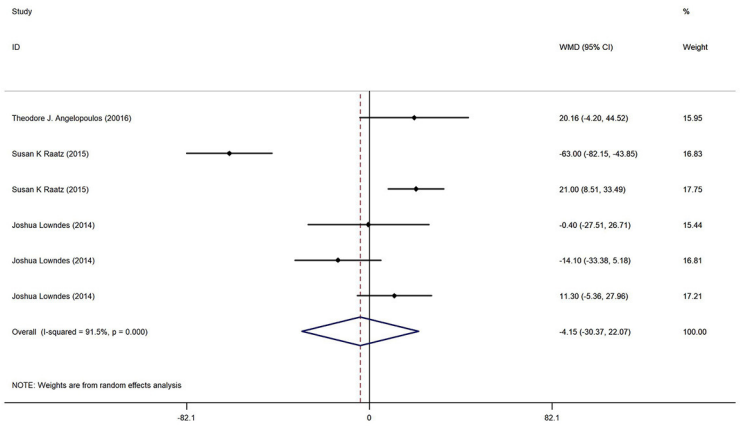


FIGURE 3
(Continued)

G Fasting blood glucose



H Triglyceride



I Low-density lipoprotein

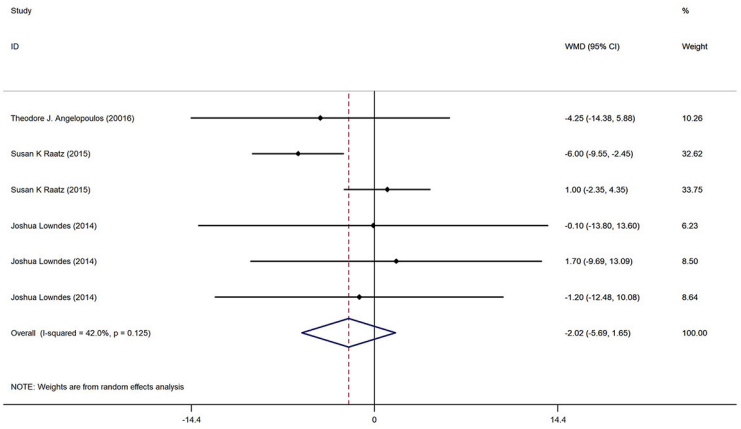


FIGURE 3
(Continued)

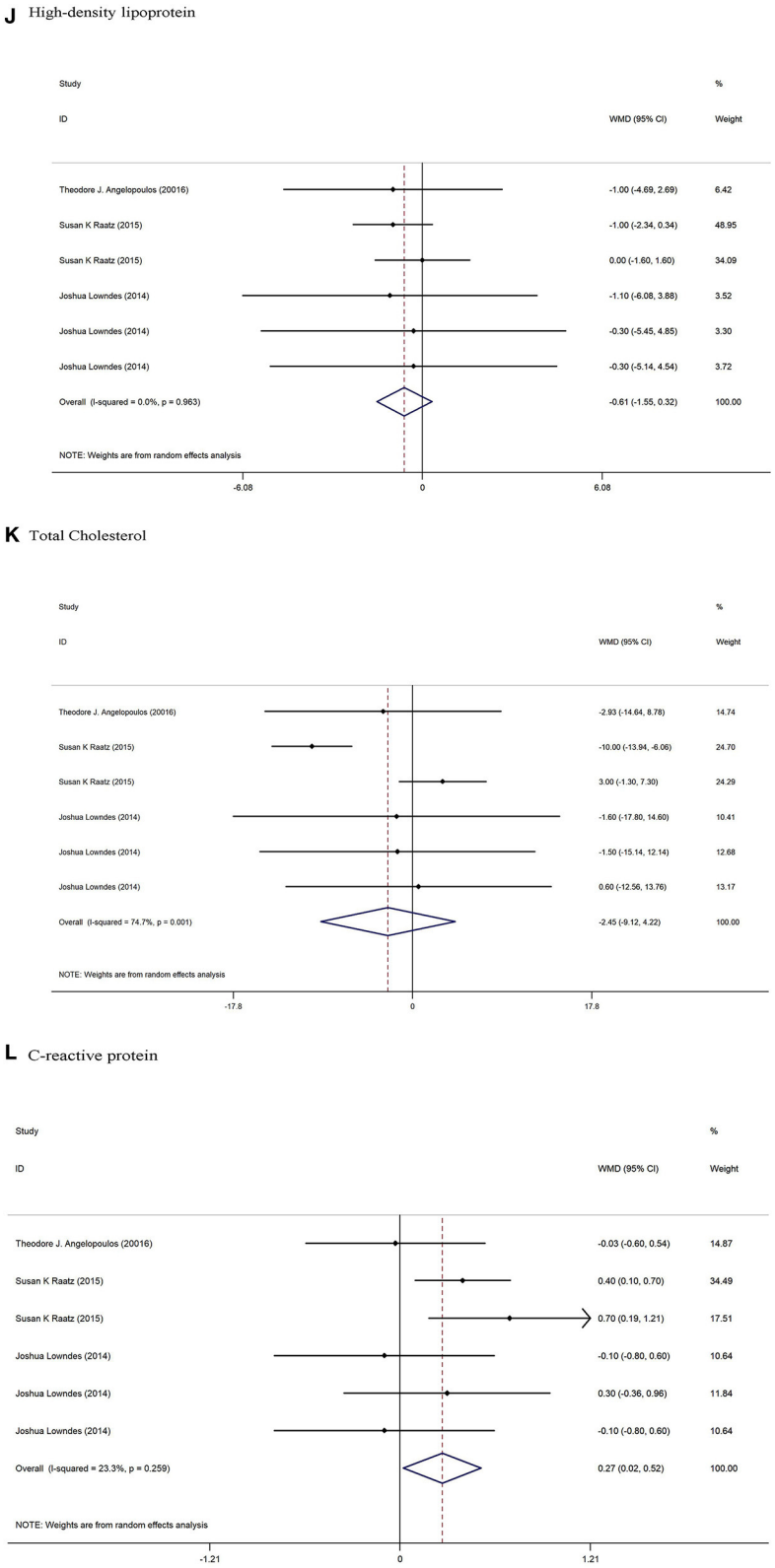


FIGURE 3
Meta-analysis of effect of High-fructose corn syrup on. **(A)** Weight. **(B)** Waist circumstance. **(C)** Body mass index. **(D)** Fat mass. **(E)** Systolic blood pressure. **(F)** Diastolic blood pressure. **(G)** Fasting blood glucose. **(H)** Triglyceride. **(I)** Low-density lipoprotein. **(J)** High-density lipoprotein. **(K)** Total Cholesterol. **(L)** C-reactive protein.

Data availability statement

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

Author contributions

XL, YunqL, YuejL, YunpL, and HK designed the study. The literature search, screening data, and data extraction were done by SY, GW, XC, and YucaL. Quality assessment was carried out by YunpL and HK. XL and YunqL analyzed and interpreted data. XL, YunqL, YuejL, YunpL, and HK wrote and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The present study was funded by National Natural Science Foundation of China (NSFC) (32160223), Young academic and technical leaders of Yunnan Province (202105AC160047), Joint Specific Project of basic Research of TCM Application of Yunnan Province (30370103808), Yunnan Provincial Science and Technology Department-Applied Basic Research Joint Special Funds of Chinese Medicine (202101AZ070001-274), Scientific Research Foundation of the Education Department of Yunnan Province, China (2021J0404), Yunnan Medical Reserve Talents Project (H-2018063), the Opening project of Clinical Medical Center of The First People's Hospital of Yunnan Province (2021LCZXXF-XG02), and doctoral Research Fund of The First People's Hospital of Yunnan Province (20206023).

Conflict of interest

Authors YucaL and HK were employed by Lairui Biotechnology (Yunnan) 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.2022.1013310/full#supplementary-material

References

1. Newens K, Walton J. A review of sugar consumption from nationally representative dietary surveys across the world. *J Hum Nutr Diet.* (2016) 29:225–40. doi: 10.1111/jhn.12338

2. Ruxton C, Gardner E, McNulty H. Is sugar consumption detrimental to health? A review of the evidence 1995-—2006. *Crit Rev Food Sci Nutr.* (2009) 50:1–19. doi: 10.1080/10408390802248569

3. Liauchonak I, Qorri B, Dawoud F, Riat Y, Szewczuk MR. Non-Nutritive Sweeteners and Their Implications on the Development of Metabolic Syndrome. *Nutrients.* (2019) 11:644. doi: 10.3390/nu11030644

4. Stricker S, Rudloff S, Geier A, Steveling A, Roeb E, Zimmer KP. Fructose consumption-free sugars and their health effects. *Dtsch Arztebl Int.* (2021) 118:71–8. doi: 10.3238/arztebl.m2021.0010

5. Sun SZ, Empie MW. Fructose metabolism in humans - what isotopic tracer studies tell us. *Nutr Metab (Lond).* (2012) 9:89. doi: 10.1186/1743-7075-9-89

6. Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming beverages sweetened with fructose, glucose, sucrose, or high-fructose corn syrup. *Am J Clin Nutr.* (2008) 88:1733S—7S. doi: 10.3945/ajcn.2008.25825D

7. Mayes PA. Intermediary metabolism of fructose. *Am J Clin Nutr.* (1993) 58:754S—65S. doi: 10.1093/ajcn/58.5.754S

8. Bar-On H, Stein Y. Effect of glucose and fructose administration on lipid metabolism in the rat. *J Nutr.* (1968) 94:95–105. doi: 10.1093/jn/94.1.95

9. Scaman C. *Corn Sweeteners: Enzyme Use, in Encyclopedia of Biotechnology in Agriculture and Food.* Boka Raton: CRC Press. (2010). p. 178–82.

10. Nabors L, Gelardi R. Alternative sweeteners: an overview. *Altern Sweeteners.* (2001) 2:1–10. doi: 10.1201/b11242-2

11. Moeller S, Fryhofer S, Osbahr AJ 3rd, Robinowitz CB. The effects of high fructose syrup. *Am J Clin Nutr.* (2009) 28:619–26. doi: 10.1080/07315724.2009.10719794

12. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. *Pharmacol Biochem Behav.* (2010) 97:101–6. doi: 10.1016/j.pbb.2010.02.012

13. Bray GA. Potential health risks from beverages containing fructose found in sugar or high-fructose corn syrup. *Diabetes Care.* (2013) 36:11–2. doi: 10.2337/dc12-1631

14. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr.* (2004) 79:537–43. doi: 10.1093/ajcn/79.4.537

15. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr.* (2002) 76:911–22. doi: 10.1093/ajcn/76.5.911

16. Organization WH. *Guideline: Sugars Intake for Adults and Children.* World Health Organization. (2015).

17. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev.* (2005) 63:133–57. doi: 10.1111/j.1753-4887.2005.tb00132.x

18. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohdrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Acad Nutr Diet.* (2002) 102:1621. doi: 10.1016/S0002-8223(02)90346-9

19. Dahlqvist A, Thomson D. The digestion and absorption of sucrose by the intact rat. *J Physiol.* (1963) 167:193–209. doi: 10.1113/jphysiol.1963.sp007141

20. Raatz SK, Johnson LK, Picklo MJ. Consumption of honey, sucrose, and high-fructose corn syrup produces similar metabolic effects in glucose-tolerant and-intolerant individuals. *J Nutr.* (2015) 145:2265–72. doi: 10.3945/jn.115.218016

21. Kuzma JN, Cromer G, Hagman DK, Breymeyer KL, Roth CL, Foster-Schubert KE, et al. No difference in ad libitum energy intake in healthy men and women consuming beverages sweetened with fructose, glucose, or high-fructose corn syrup: a randomized trial. *Am J Clin Nutr.* (2015) 102:1373–80. doi: 10.3945/ajcn.115.116368

22. Yu Z, Lowndes J, Rippe J. High-fructose corn syrup and sucrose have equivalent effects on energy-regulating hormones at normal human consumption levels. *Nutr Res Rev.* (2013) 33:1043–52. doi: 10.1016/j.nutres.2013.07.020

23. Melanson KJ, Zukley L, Lowndes J, Nguyen V, Angelopoulos TJ, Rippe JM. Effects of high-fructose corn syrup and sucrose consumption on circulating glucose, insulin, leptin, and ghrelin and on appetite in normal-weight women. *Nutrition.* (2007) 23:103–12. doi: 10.1016/j.nut.2006.11.001

24. Teff KL, Elliott SS, Tschop M, Kieffer TJ, Rader DM, Heiman, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab.* (2004) 89:2963–72. doi: 10.1210/jc.2003-031855

25. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *Am J Clin Nutr.* (2002) 76:1023–30. doi: 10.1093/ajcn/76.5.1023

26. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* (2015) 4:1. doi: 10.1186/2046-4053-4-1

27. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928. doi: 10.1136/bmj.d5928

28. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135

29. Angelopoulos TJ, Lowndes J, Sinnett S, Rippe JM. Fructose containing sugars at normal levels of consumption do not effect adversely components of the metabolic syndrome and risk factors for cardiovascular disease. *Nutrients.* (2016) 8:179. doi: 10.3390/nu8040179

30. Lowndes J, Sinnett S, Yu Z, Rippe J. The effects of fructose-containing sugars on weight, body composition and cardiometabolic risk factors when consumed at up to the 90th percentile population consumption level for fructose. *Nutrients.* (2014) 6:3153–68. doi: 10.3390/nu6083153

31. Lowndes J, Kawiecki D, Pardo S, Nguyen V, Melanson KJ, Yu Z, et al. The effects of four hypocaloric diets containing different levels of sucrose or high fructose corn syrup on weight loss and related parameters. *Nutr J.* (2012) 11:55. doi: 10.1186/1475-2891-11-55

32. Jameel F, Phang M, Wood LG, Garg ML. Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. *Lipids Health Dis.* (2014) 13:195. doi: 10.1186/1476-511X-13-195

33. Cirillo P, Gersch MS, Mu W, Scherer PM, Kim KM, Gesualdo L, et al. Ketohexokinase-dependent metabolism of fructose induces proinflammatory mediators in proximal tubular cells. *J Am Soc Nephrol.* (2009) 20:545–53. doi: 10.1681/ASN.2008060576

34. Delbosc S, Paizanis E, Magous R, Araiz C, Dimo T, Cristol JP, et al. Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis.* (2005) 179:43–9. doi: 10.1016/j.atherosclerosis.2004.10.018

35. Graziose MM. On the accuracy of self-report instruments for measuring food consumption in the school setting. *Adv Nutr.* (2017) 8:635–6. doi: 10.3945/an.117.015354

36. Rosenman R, Tennekoon V, Hill LG. Measuring bias in self-reported data. *Int J Behav Health Res.* (2011) 2:320. doi: 10.1504/IJBHR.2011.043414

37. Jastrzebski Z, Kortas J, Kaczor K, Antosiewicz J. Vitamin D supplementation causes a decrease in blood cholesterol in professional rowers. *J Nutr Sci Vitaminol.* (2016) 62:88–92. doi: 10.3177/jnsv.62.88

38. Schnatz PF, Jiang X, Vila-Wright S, Aragaki AK, Nudy M. O'sullivan DM, et al. Calcium/Vitamin D (CaD) Supplementation, Serum 25 (OH) Vitamin D Concentrations, and Cholesterol Profiles in the Women's Health Initiative CaD Randomized Trial. *Menopause.* (2014) 21:823. doi: 10.1097/GME.0000000000000188

39. Ponda MP, Dowd K, Finkielstein D, Holt PR, Breslow JL. The short-term effects of vitamin D repletion on cholesterol: a randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol.* (2012) 32:2510–5. doi: 10.1161/ATVBAHA.112.254110

40. van Mierlo LA, Arends LR, Streppel MT, Zeegers M, Kok FJ, Grobbee DE, et al. Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens.* (2006) 20:571. doi: 10.1038/sj.jhh.1002038

41. Lee YH. An overview of meta-analysis for clinicians. *Korean J Intern Med.* (2018) 33:277. doi: 10.3904/kjim.2016.195

42. Bondemark L, Ruf S. Randomized controlled trial: the gold standard or an unobtainable fallacy? *Eur J Orthod.* (2015) 37:457–61. doi: 10.1093/ejo/cjv046

43. Murad MH, Chu H, Lin L, Wang Z. The effect of publication bias magnitude and direction on the certainty in evidence. *BMJ Evid Based Med.* (2018) 23:84–6. doi: 10.1136/bmjebm-2018-110891

44. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest.* (2009) 119:1322–34. doi: 10.1172/JCI37385

45. Swarbrick MM, Stanhope KL, Elliott SS, Graham JL, Krauss RM, Christiansen MP, et al. Consumption of fructose-sweetened beverages for 10 weeks increases postprandial triacylglycerol and apolipoprotein-B concentrations in overweight and obese women. *Br J Nutr.* (2008) 100:947–52. doi: 10.1017/S000711450896825 2

46. Song D, Arikawa E, Galipeau D, Battell M, McNeill JH. Androgens are necessary for the development of fructose-induced hypertension. *Hypertension.* (2004) 43:667–72. doi: 10.1161/01.HYP.0000118018.77344.4e

47. Galipeau D, Verma S, McNeill JH. Female rats are protected against fructose-induced changes in metabolism and blood pressure. *Am J Physiol Heart Circ Physiol.* (2002) 283:H2478–84. doi: 10.1152/ajpheart.00243.2002

48. Hallfrisch J, Reiser S, Prather ES. Blood lipid distribution of hyperinsulinemic men consuming three levels of fructose. *Am J Clin Nutr.* (1983) 37:740–8. doi: 10.1093/ajcn/37.5.740

49. de Castro UGM, Silva ME, de Lima WG, Campagnole-Santos MJ, Alzamora AC. Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. *Lipids Health Dis.* (2013) 12:136. doi: 10.1186/1476-511X-12-136

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 14 October 2022
DOI 10.3389/fnut.2022.959516

OPEN ACCESS

EDITED BY
Heitor O. Santos,
Federal University of Uberlândia, Brazil

REVIEWED BY
Olivier Hue,
Université des Antilles et de la Guyane, France
Toby Mündel,
Massey University, New Zealand

*CORRESPONDENCE
Maria Roriz
maria.roriz@hotmail.com

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 01 June 2022
ACCEPTED 19 September 2022
PUBLISHED 14 October 2022

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

Performance effects of internal pre- and per-cooling across different exercise and environmental conditions: A systematic review

Maria Roriz[1,2]*, Pedro Brito[3], Filipe J. Teixeira[4,5,6], João Brito[7] and Vitor Hugo Teixeira[1,2,8,9]

[1]Faculty of Nutrition and Food Sciences, University of Porto (FCNAUP), Porto, Portugal, [2]Futebol Clube do Porto, Porto, Portugal, [3]Research Center in Sports Sciences, Health Sciences and Human Development, CIDESD, University of Maia, ISMAI, Maia, Portugal, [4]Interdisciplinary Center for the Study of Human Performance (CIPER), Faculdade de Motricidade Humana, Universidade de Lisboa, Cruz-Quebrada, Portugal, [5]Atlântica, Instituto Universitário, Fábrica da Pólvora de Barcarena, Barcarena, Portugal, [6]Bettery Lifelab, Bettery S.A., Lisbon, Portugal, [7]Portugal Football School, Portuguese Football Federation, Oeiras, Portugal, [8]Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sports, University of Porto (FADEUP), Porto, Portugal, [9]Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal

Exercise in a hot and humid environment may endanger athlete's health and affect physical performance. This systematic review aimed to examine whether internal administration of ice, cold beverages or menthol solutions may be beneficial for physical performance when exercising in different environmental conditions and sports backgrounds. A systematic search was performed in PubMed, Web of Science, Scopus and SPORTDiscus databases, from inception to April 2022, to identify studies meeting the following inclusion criteria: healthy male and female physically active individuals or athletes (aged ≥18 years); an intervention consisting in the internal administration (i.e., ingestion or mouth rinse) of ice slush, ice slurry or crushed ice and/or cold beverages and/or menthol solutions before and/or during exercise; a randomized crossover design with a control or placebo condition; the report of at least one physical performance outcome; and to be written in English. Our search retrieved 2,714 articles in total; after selection, 43 studies were considered, including 472 participants, 408 men and 64 women, aged 18-42 years, with a VO_{2max} ranging from 46.2 to 67.2 $mL \cdot kg^{-1} \cdot min^{-1}$. Average ambient temperature and relative humidity during the exercise tasks were 32.4 ± 3.5°C (ranging from 22°C to 38°C) and 50.8 ± 13.4% (varying from 20.0% to 80.0%), respectively. Across the 43 studies, 7 exclusively included a menthol solution mouth rinse, 30 exclusively involved ice slurry/ice slush/crushed ice/cold beverages intake, and 6 examined both the effect of thermal and non-thermal internal techniques in the same protocol. Rinsing a menthol solution (0.01%) improved physical performance during continuous endurance exercise in the heat. Conversely, the ingestion of ice or cold beverages did not seem to consistently increase performance, being more

likely to improve performance in continuous endurance trials, especially when consumed during exercises. Co-administration of menthol with or within ice beverages seems to exert a synergistic effect by improving physical performance. Even in environmental conditions that are not extreme, internal cooling strategies may have an ergogenic effect. Further studies exploring both intermittent and outdoor exercise protocols, involving elite male and female athletes and performed under not extreme environmental conditions are warranted.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021268197], identifier [CRD42021268197].

KEYWORDS

exercise, heat, internal cooling, nutrition, sports performance

Introduction

Prolonged exercise in hot and humid environments is challenging for health and athletic performance, mainly during endurance and team-sport activities (1, 2). Actually, when environmental conditions are extreme, temperature regulation mechanisms may be disrupted and may not compensate the elevation of core body temperature (3). However, the mechanisms underpinning fatigability and reduced performance during exercise in hot environments are likely to be multifactorial.

Overall, body temperature is regulated by both physiologic and behavioral mechanisms (4, 5). Briefly, physiological temperature regulation operates through responses that are independent of conscious voluntary behavior, and include cutaneous vasodilatation (increasing skin blood flow) and increments pertaining sweat rate (3, 5). On the other hand, behavioral temperature regulation occurs through conscious behavior changes that influence heat accumulation, and include modification of the activity levels, clothing changes and seeking of shade or shelter (4). During exercise under heat stress, the rate of heat production is greater than the rate of heat loss and may lead to hyperthermia (6). Also, a greater cardiovascular distress should be expected because the main challenge is to provide sufficient cardiac output to adequately perfuse skeletal muscles to support metabolism, while simultaneously perfusing the skin to support heat loss (7, 8). Thus, hyperthermia is known to alter cardiovascular function, reducing physical and athletic performance (9).

Several cooling strategies have been tested and used with the primary goal of reducing central temperature and thermal sensation, and further delaying the onset of fatigue (10, 11). Concomitantly, cooling methods can be either applied prior to or during exercise. As a pre-cooling strategy, cooling methods should aim to increase the margin for metabolic heat production and heat increase, while as a per-cooling strategy, the aim is to attenuate the rise of exercise-induced central temperature and to cool the body when already under heat stress (12, 13). Though, the combination of pre-cooling and per-cooling techniques seems to be more effective in improving exercise performance in the heat than any method applied individually (10).

Similarly, diverse external and internal thermal cooling techniques are often used to reduce the detrimental effects of heat stress. Notably, external methods (e.g., using a cooling vest, ice pack, cold-water immersion) have proved to be effective in decreasing core, muscle and skin temperatures, and improving physical performance (10), whether internal methods (e.g., ice slurry, crushed ice, cold beverage ingestion) have shown to decrease brain temperature and to improve thermal perception via stimulation of thermoreceptors located within oral and abdominal regions (10, 14). Recently, the research on non-thermal cooling methods, either external or internal, has grown, with particular attention to L-menthol, due to its properties in relieving the thermal strain associated with exercise in the heat (15, 16). L-menthol can be applied topically (creams, gels, or sprays) or internally, via mouth rinse or ingestion of menthol flavored solutions (17).

The effects of external cooling methods on sports performance are well documented (18, 19). However, no systematic review has exclusively focused on the effects of internal thermal and non-thermal cooling strategies on physical performance, specifically in different types of exercise backgrounds. This is of utter importance, because more and more sport events worldwide will occur under adverse environmental conditions. Also, a better understanding of such strategies may provide practical and cost-effective insights on how to improve athletic performance in the heat. Therefore, the purpose of the current systematic review is to summarize the existing scientific literature regarding the role of internal administration of cooling methods in physical performance,

applied either before and/or during exercise, in healthy physically active male and female individuals or athletes, in different environmental and exercise contexts.

Materials and methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (20). The protocol was previously registered at the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42021268197.

Search strategy

All studies were identified through a search on four electronic databases (PubMed, Web of Science, Scopus and SPORTDiscus) from their inception until the 4th of April 2022. Reference lists of the included articles were also searched for additional references to be included in case they would fulfill all inclusion criteria. There was no limit on the status, language, and dates of publication. Twenty-two terms and keywords ("exercise," "sports performance," "athletic performance," "sports," "physical activity," "athletes," "pre-cooling," "cooling," "per-cooling," "mid-cooling," "body temperature," "heat mitigation," "ice-slurry," "ice-slushy," "ice-slush," "beverage," "drinking," "cold fluid," "cold water," "menthol," "mint," "peppermint") were combined by Boolean logic operators (AND) and (OR) (**Supplementary Appendix A**).

Eligibility criteria

The specified eligibility criteria for the systematic review research followed the PICOS model eligibility criteria, which consider the factors of population (P), intervention (I), comparators (C), and outcomes (O), along with study design (S) (21).

Inclusion criteria

To be considered for analysis, the studies must have: (1) involved male or female healthy physically active or athletes (age ≥18 years) subjects; (2) an intervention that includes the internal administration (ingestion or mouth rinse) of ice slush, ice slurry or crushed ice and/or cold beverage and/or menthol solution before and/or during exercise; (3) reported at least one physical performance outcome (i.e., time to exhaustion, time-trials, distance covered, strength and power, exercise capacity, sprint velocity, etc.); (4) a randomized controlled crossover design, with a control or placebo group; and (5) been written in English.

Authors opted to only include trials with a crossover design because such research design has strong advantages over parallel group trials, particularly in this research area (22). Physical performance is highly variable between participants, as it is conditioned by individual factors. So, a crossover design, where the interventions under investigation are evaluated within the same patients, eliminating between-subject variability, best serves the purpose of our review.

Exclusion criteria

Studies were excluded if they: (1) were conducted in participants with injury or illness; (2) were secondary design studies (such as meta-analyses, systematic reviews and narrative reviews), animal studies, articles with no full-text available, opinion pieces, commentaries, editorials, letters, theses, meeting abstracts or "gray literature" in general; (3) included exclusively external cooling or did not report an exclusive effect of the internal cooling; and (4) evaluated only outcome measures based on non-physical performance parameters (e.g., physiological markers).

Study selection

Following the initial search in each database, all references located were imported into EndNote X20 (Clarivate Analytics, London, United Kingdom). Two independent authors (MR and PB) selected the eligible articles, based on the title and abstract, and removed duplicated articles. The full text of references identified from the previous process was reviewed and assessed independently by two reviewers (MR and PB) using the inclusion and exclusion criteria. Disagreements were solved by consensus between the two researchers (MR and PB) after discussion. Unresolved discrepancies were settled by a third reviewer (VHT).

Data extraction

In the set of included studies, data extraction was completed independently by two authors (MR and PB) to an Excel spreadsheet to collect information for descriptive purposes. The last author of this review (VHT) supervised the process. The following data were extracted and presented in **Table 1** as follows: authors and year of publication, ambient conditions (temperature, relative humidity, and wind speed), sample size, characteristics of the participants (sex, age, and training status), study design, exercise protocol, cooling timing, cooling technique, and physical performance outcomes.

Risk of bias assessment

Risk of bias was assessed according to Cochrane Collaboration guidelines (23) (Risk of Bias Tool V.2.) at

TABLE 1 Summary of the included studies involving an intermittent exercise protocol.

Study	Ambient conditions	Participant characteristics	Design	Exercise protocol	Cooling timing	Cooling technique	Performance outcomes
Menthol							
Best et al. (106)	22 ± 1°C	Recreational male athletes (n = 10). Age 24.6 ± 3.9 y. Recreational female athletes (n = 9). Age 20.2 ± 1.0 y.	Crossover, counterbalanced, randomized	3x Isometric Mid-Thigh Pull + 3x Vertical Jump + 3 × 6 s Peak Power (cycle ergometer)	Per-cooling	25 mL menthol solution mouth rinse (0.1%, 10 s) 60 s before each exercise effort	↓1.4% IMTP ↑0.9% VJH ↓1.5% PP (p = 1.000)
Gibson et al. (103)	35 ± 0.2°C 40 ± 0.5% RH	Non-heat-acclimated trained team sports male (n = 11) and female (n = 3). Age 24 ± 3 y. VO_2max 46.2 ± 12.9 mL·kg^{-1}·min^{-1}	Crossover, randomised	CISP protocol	Per-cooling	25 mL L-menthol solution mouth rinse (0.01%, 5 s, ∼40°C) every 10 min	↔ PP, MP, WD (p > 0.05)
Ice/Cold beverages							
Aldous et al. (119)	30.7 ± 0.3°C 50.9 ± 4.2% RH	University-level male football players (n = 8). Age 22 ± 3 y. VO_2max 56 ± 9 mL·kg^{-1}·min^{-1}	Crossover, counterbalanced, randomized	2 × 45 min iSPT	Pre-cooling Per-cooling	7.5 g kg^{-1} ice slurry ingestion (-1°C) 30 min before exercise + 3.75 g kg^{-1} ice slurry ingestion (-1°C) during 15 min half-time	↔ TD, HSD, VRD
Beaven et al. (134)	25°C 60% RH	Professional rugby sevens male athletes (n = 12). Age 21.5 ± 1.3 y	Crossover, counterbalanced, randomised	5 × 40 min maximal running sprint every 30 s	Pre-cooling	500 mL of non-calorific ice slushy ingestion 15 min before exercise	**↓3.2% RT (p = 0.0015)** ↑1.9% ST (p > 0.05)
Brade et al. (132)	35.2 ± 0.3°C 57.8 ± 1.2% RH	Male team sport players (n = 12). Age 21.8 ± 2.3 y	Crossover, counterbalanced, randomized	70 min sprint cycling (2 × 30 min halves + 10 min interval)	Pre-cooling Per-cooling	7.0 g kg^{-1} of ice slushy ingestion (0.6°C) 30 min before exercise + 2.1 g kg^{-1} of ice slushy ingestion (0.6°C) during half-time	↔ PPO ↔ MPO ↔ PP ↔ MP ↔ W (p > 0.05)
Gerret et al. (122)	30.9 ± 0.9°C 41.1 ± 4.0% RH 1.3 m s^{-1} WS	Moderately to well-trained males (n = 12). Age 30.4 ± 3.4 y. VO_2max 58.5 ± 8.1 mL·kg^{-1}·min^{-1}	Crossover, randomized	2 × 31 min self-paced intermittent protocols	Pre-cooling	7.5 g kg^{-1} ice slurry ingestion (0.14 ± 0.1°C) 30 min before exercise	↑1.5% TD (p > 0.05)
Hue et al. (102)	Water temperature 29.5 ± 0.5°C	Ranked long-distance male (n = 5) and female (n = 4) swimmers. Age 23.4 ± 3.3 y	Crossover, randomized	10 × 100 m swimming (5000 m)	Per-cooling	190 mL cold water ingestion (1.3°C) before and after each 1,000 m	↔ Swimming time (p > 0.05)
Lafata et al. (97)	WBGT = 15°C DBT = 24°C	Healthy, physically fit male (n = 52). Age 30.3 ± 5.4 y. VO_2max 49.8 ± 6.3 mL·kg^{-1}·min^{-1}	Crossover, randomized	60% 1RM bench press to fatigue + broad jump, + cycling TTE	Per-cooling	12 mL kg^{-1} cold beverage ingestion (4°C) during the rest period between sets of exercises	↑0.7% TTE (p = 0.7035) ↑0.9% BJ (p = 0.465)

(Continued)

10.3389/fnut.2022.959516

TABLE 1 (Continued)

Study	Ambient conditions	Participant characteristics	Design	Exercise protocol	Cooling timing	Cooling technique	Performance outcomes
Naito et al. (93)	36.5 ± 0.5°C 50 ± 3% RH	Non-heat acclimated physically active males (n = 7). Age 31 ± 4 y	Crossover, randomized	Intermittent cycling protocol	Per-cooling	1.25 g kg^{-1} ice slurry ingestion (−1°C) during each break + 7.5 g kg^{-1} ice slurry ingestion (−1°C) during 10 min half-time	↔ MPO, PPO ($p > 0.05$) ↓ **4.2% WD ($p < 0.05$)**
Thomas et al. (127)	34.4 ± 1.4°C 36.3 ± 4.6% RH	Healthy trained male (n = 10). Age 30.5 ± 5.8 y. VO_2max 56.2 ± 6.6 mL·kg $^{-1}$·min^{-1}	Crossover, counterbalanced, randomized	46-min intermittent protocol	Pre-cooling	7.5 g kg^{-1} ice slurry ingestion (−0.5 ± 0.4°C) 30 min before exercise	↑4% TD ($p > 0.05$)
Zimmermann and Landers (99)	33.1 ± 0.1°C 60.3 ± 1.5% RH	Trained team sports female (n = 9). Age 21.0 ± 1.2 y	Crossover, randomized	72 min (2 × 36 min) intermittent sprint cycling	Pre-cooling	6.8 g kg^{-1} crushed ice ingestion (¯0.5°C) 30 min before exercise	↔ PPO ($p = 0.799$) ↔ MPO ($p = 0.989$)

↔ no change, ↑ increase, ↓ decrease, BJ: broad jump, CISP cycling intermittent-sprint protocol, DBT: dry-bulb temperature, HSD: high-speed distance covered, IMTP: isometric mid-thigh pull, kJ: kilojoules, kg: kilograms, km: kilometers, m: meters, mL: milliliters, min: minutes, MEN: menthol, MP: mean power, MPO: mean power output, P: power, PO: power output, PP: peak power, PPO: peak power output, RH: relative humidity, RT: rate of fatigue, s: seconds, ST: sprint time; T: time, TD: total distance covered, VJH: vertical jump height, VO2max: maximal oxygen uptake, VRD: variable run distance covered, W: work, WBGT: wet-bulb globe temperature, WBT: wet bulb temperature, WD: total work done, WS: wind speed and y: years. p: significance level. Bold represents a significant p-value at a confidence level of 95% ($p < 0.05$).

study level, using Review Manager 5.4, encompassing seven domains: sequence generation, allocation concealment, blinding of participants and outcome assessors, incomplete outcome data, selective outcome reporting and other bias. Each potential source of bias was graded as low, high, or unclear risk. The process was carried out by two of authors independently (MR and PB) that underwent a calibration exercise before performing the assessment of risk of bias. When the details of a study were unclear, the authors were contacted to provide further information/details. Conflicts were settled through discussion amongst the pair of reviewers or through consultation with a third reviewer (VHT).

Results

Literature search

The database search retrieved 2,714 articles. These were reduced to 1,711 after removal of duplicates (n = 1,003). Further screening by title and/or abstract analysis excluded 1,599 articles. The 112 studies left were assessed for eligibility via full text review, and references lists did not reveal any missing papers. The exclusion criteria determined a further removal of 69 articles. Two were review articles (24, 25), three had a non-crossover design (26–28), nineteen reported only physiological, cognitive or perceptual performance measures (29–47), nine had no control or placebo group (48–56), five were conference abstracts/posters (57–61), three were performed in clinical context (62–64), twenty-seven did not report an exclusive effect of the internal cooling (65–91) and one comprised underage participants (92). Forty-three articles fulfilled the eligibility criteria and data were extracted for qualitative analysis. **Figure 1** details the study search, identification, and selection process using the PRISMA flow diagram.

Characteristics of the included studies

Participants

Overall, 472 subjects (408 male and 64 female) from 43 studies were included in the qualitative analysis, with the number of participants ranging from 7 (93–96) to 52 (97). Thirty-four studies assessed only male participants, three only females (98–100), and six included a mixed-sex sample (101–106). The average age of the participants was 27.1 years (ranging between 18 (107) and 42 years (108)). The average VO_2max of the participants was 56.0 mL·kg^{-1}·min^{-1} (ranging from 46.2 (103) to 67.2 mL·kg^{-1}·min^{-1} (109)). Seventeen studies included physically active participants (93, 94, 97, 101, 104, 105, 107, 110–118), twenty-one included recreational athletes (95, 96, 98–100, 106, 108, 119–131), and five involved professional athletes (102, 109, 132–135).

Exercise protocols and environmental conditions

The exercise protocols involved were divided as the following:

(A). Continuous endurance exercise (n = 32).

- Time-trials (n = 14) (94, 96, 98, 100, 104, 108, 109, 123–126, 129, 130, 133).
- Time to exhaustion (n = 17).
  - at fixed first ventilatory threshold (VT1) (n = 2) (113, 114).
  - at fixed rate of perceived exertion (RPE) (n = 3) (101, 110, 121).
  - at fixed VO_2peak or at maximal aerobic power output (W_{max}) or at respiratory compensation point (n = 12) (94, 95, 107, 111, 112, 115–118, 120, 128, 131).
- Maximum power effort (n = 1) (105).

(B). Intermittent effort exercise (n = 11) (93, 97, 99, 102, 103, 106, 119, 122, 127, 132, 134).

Ambient temperature during the exercise tasks ranged from 22°C (106) to 38°C (131) and relative humidity varied between 20% (126) and 80% (123). Forty studies included indoor protocols and three were performed outdoor (102, 104, 130). Wet bulb globe temperature (WBGT) was calculated from each of the included studies (136), with the exception of the studies where WBGT was recorded and reported and other two studies where WBGT could not be calculated (one carried out in water (102) and another study that did not indicate the value of relative humidity (106)). A WBGT ≥28°C was used as a cut-off point to group studies concerning environmental conditions (137). Thirty-four studies were performed under a WBGT ≥28°C (53, 93–96, 98–101, 103, 105, 107–113, 115, 116, 119, 121–123, 125–129, 131–133, 138) and seven studies under a WBGT <28°C (97, 104, 117, 118, 130, 134, 139).

Interventions

All the included studies analyzed the effect of pre-cooling and/or per-cooling with non-thermal (menthol solution) and/or thermal (ice or cold beverages) methods on physical performance, compared with non-cooling control or placebo.

Across all the 43 studies, seven exclusively included a menthol solution mouth rinse (100, 101, 103, 106, 110, 111, 117), thirty exclusively involved ice slurry/ice slush/crushed ice/cold beverages intake (61, 80, 93–99, 102, 104, 107, 109, 112–115, 118–127, 132–134), and six examined both the effect of thermal and non-thermal internal techniques in the same protocol (105, 108, 116, 128–130).

All studies that exclusively applied non-thermal methods had a per-cooling mode (100, 101, 103, 106, 110, 111, 117). Studies that involved thermal strategies encompassed the ingestion of ice or cold beverages both before and during exercise (107, 119, 120), only before exercise (61, 94, 96, 98, 99, 104, 114, 115, 121–125, 127, 134) or only during exercise (93, 95, 97, 102, 107, 109, 112, 113, 118, 126, 133). In the six studies where thermal and non-thermal strategies were both included, the techniques employed were the administration of cold water or menthol solution before exercise (105), ice slurry before exercise or menthol solution during exercise (129), ice slurry or menthol solution during exercise (116), ice or cold menthol flavored beverages before and during exercise (108), and crushed ice before exercise and menthol solution during exercise (128).

Risk of bias assessment

The included studies generally had low or unclear risk of bias (**Figures 2**, **3**). Only five studies had a low risk of sequence generation bias, because they reported information on the randomization procedure conducted to generate groups (101, 105, 110, 116, 117). Attempts to conceal allocation to an intervention or control group were only well reported on four studies (101, 105, 116, 117), with the others having an unclear risk of bias. Three studies (105, 110, 116) were single-blinded, two studies were performed in a double-blinded fashion (101, 117), and four studies (95, 97, 111, 112) reported having blinded the participants to the purpose of the intervention and thence. Four studies clearly reported no blinding of the participants to the purpose of the study (104, 106, 129, 132), which represents a high risk of bias. In each one of the included studies, it was not clear whether the blinding of outcome assessment occurred, so an unclear risk of bias in this category was considered. All studies were considered to have a low risk of bias for incomplete data and an unclear risk for selective reporting. Finally, regarding other bias, twenty-one studies did not report if beverages were distributed in a counterbalanced order, so a bias related to the distribution order was not clear (93–95, 97–99, 101, 102, 108, 110, 111, 114–116, 121–123, 128–131). A study did not set the amount of beverage to be consumed between groups (*ad libitum* fluid ingestion), meaning that it was not clear if the drinking rate may have influenced the outcomes (112).

Results of the included studies

The summary of outcomes measures and main findings of the forty-three included studies are presented in **Table 1** and **Supplementary Table 1**.

Menthol solution *vs.* non-cooling strategy

Intermittent exercise protocols

Mouth rinsing a menthol solution during an intermittent exercise protocol (3 x isometric mid-thigh pull + 3x vertical jump + 3 × 6 s of peak power on a cycle ergometer) showed no significant improvement in none of these performance parameters (106). Similarly, oral menthol administration

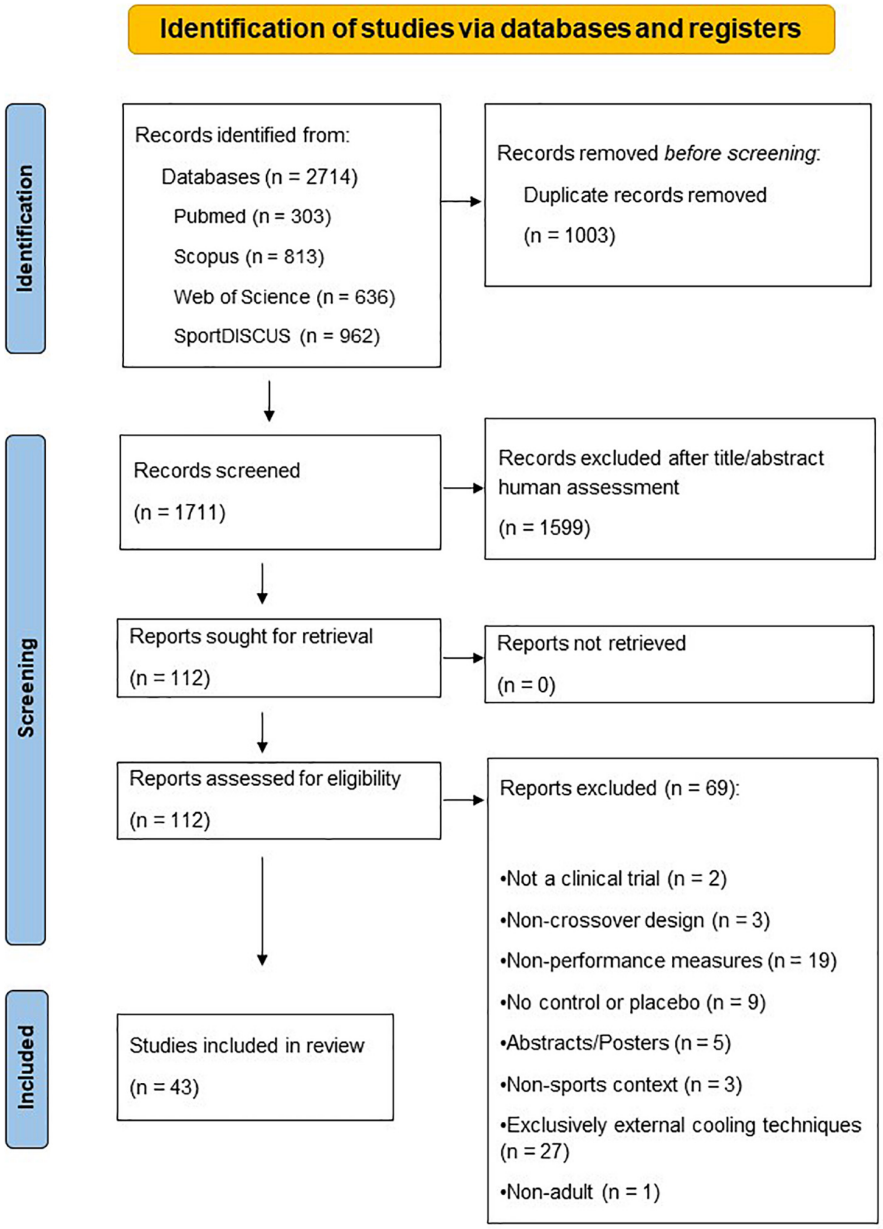


FIGURE 1
PRISMA flow diagram summarizing study selection for inclusion in systematic review.

during a Cycling Intermittent-Sprint Protocol (CISP), which consisted of 10-s rest, 5-s maximal sprint, and 105-s active recovery with the cycles repeated over 10 min (for 20 min), revealed no significant effect on peak power, mean power and total work done (103) (**Table 1** and **Figure 4**).

Continuous endurance exercise protocols

In the only study where menthol was internally applied in a pre-cooling mode, specifically in the minute before a 3-min aerobic test exercise (an exercise protocol much shorter than the other ones), a significant improvement in relative power output was found (13%) (105).

Internal application of menthol solution during continuous exercise significantly enhanced endurance performance in seven out of eight studies. Power output (3.6-6.5%) and completion time (4-7%) were significantly improved at a 16 fixed-RPE cycling protocol (101, 110). Time to exhaustion was significant higher (6-8.6%) with menthol mouth rinsing during a 65 and 70% maximal aerobic power output cycling protocols (111, 116), as well as time to fatigue (34.4%) in a running protocol with an intensity of 100% of VO_2peak (128). Time trial efforts also

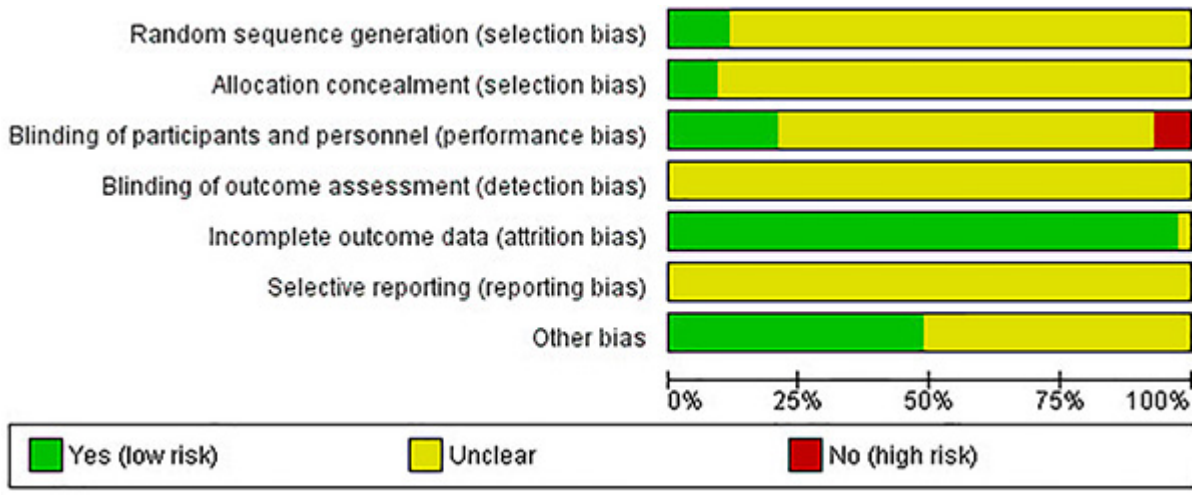


FIGURE 2
Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

showed significant improvements while per-cooling with oral menthol (2.3-2.7%), namely in a 30-km (100) cycling and in a 5-km running protocols (129). However, one study did not find a significant improvement in time to exhaustion when mouth rinsing with a menthol-containing sports drink during a 105% of respiratory compensation point cycling protocol (117).

When oral menthol was administered before and during exercise, which only occurred in one study, completion time was significantly improved (5.3%) in a 20-km time trial cycling protocol (108) (**Supplementary Table 1** and **Figure 4**).

Influence of environmental conditions

Overall, nine of the ten studies that involved the administration of oral menthol and were performed under a WBGT ≥28°C found significant improvements in physical performance (100, 101, 105, 108, 110, 111, 116, 128, 129). When stratifying the analysis by type of exercise, it is possible to observe that the only study that did not report significant improvements in physical performance in more adverse environmental conditions involved an intermittent exercise protocol (103).

On the other hand, the only study that involved menthol mouth rinsing and was completed under a WBGT <28°C did not report significant improvements in physical performance (117), and involved a continuous endurance exercise protocol, with a time to exhaustion at 105% of respiratory compensation point (**Figure 5**).

Ice or cold beverage *vs.* non-cooling strategy

Intermittent exercise protocols

In the four studies where ice or cold beverages were ingested before intermittent exercise, only one found significant improvements in physical performance, specifically on rate of fatigue (3.2%) in a 5 × 30-s of maximal running sprint protocol, even though sprint time was not significantly improved (134). Total distance in a 2 × 31-min (122) and in a 46-min (127) intermittent protocols on non-motorized treadmill was not enhanced by pre-cooling with ice slurry. No significant differences were found in peak power and mean power outputs in a 2 × 36-min intermittent sprint cycling protocol (99).

Across the three studies where ice or cold beverages were ingested during intermittent exercise, none showed a significant ergogenic effect, neither on a 10 × 100-m swimming protocol (101), or in a repeated sprint intermittent cycling protocol (92) or even in a mixed-tasks protocol with bench press to fatigue + broad jump + self-paced cycling time to exhaustion (96).

In the set of the two studies where ice or cold beverages were administered before and during intermittent exercise, none reported significant differences in physical performance. Exercise protocols involved a 2 × 45-min iSPT (consisting of three identical 15-min intermittent exercise blocks) (119) and a 2 × 30-min sprint cycling exercise (132) (**Table 1** and **Figure 6**).

Continuous endurance exercise protocols

Over the thirteen studies that involved the application of ice or cold beverages before a continuous endurance exercise protocol, five observed significant improvements in physical performance. Completion time (0.6-7.2%) and power output (5.4-7.8%) were significantly enhanced in a 40-km (96) and 800-kJ (124) cycling time trials, as well as in a 10-km running time trial (104). Total distance was also improved in a 30-min cycling time trial protocol (2.9%) (94), and time to exhaustion was significant higher in a running to exhaustion at first ventilatory threshold in a treadmill (12.8%) (114). However, time to exhaustion was not significantly improved in a 80%

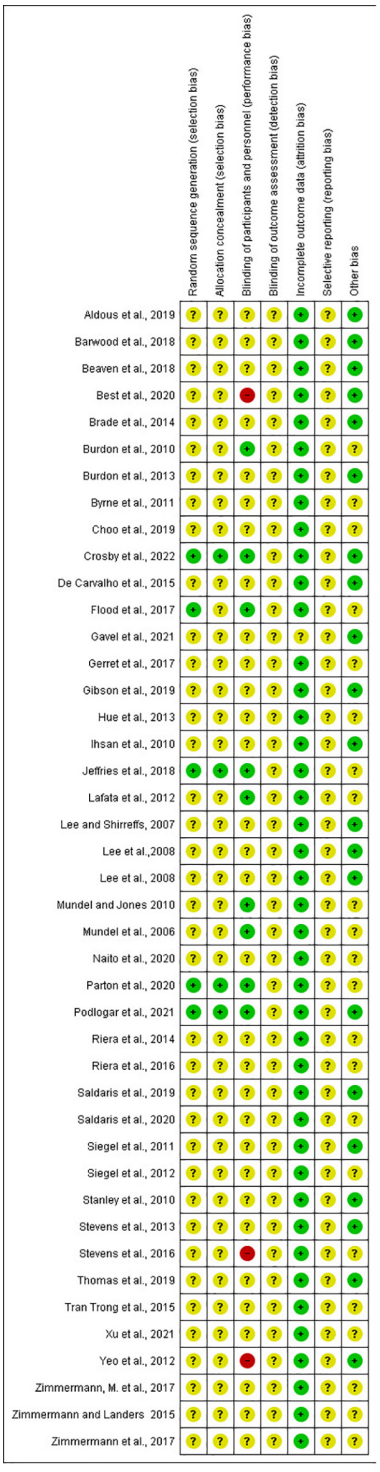


FIGURE 3
Risk of bias summary: review authors' judgments about each risk of bias item for each included study.

VO_2max speed cycling protocol (131), neither mean power output or total work done in a 60-min fixed 15-RPE (121) and 60-min fixed 55%VO_2peak cycling protocols (115). Finally, completion time was not significantly lower while pre-cooling with ice or cold beverages in a 30-km (123), 800-kJ (98) and 30-min fixed 75% of peak power output (125) cycling time trials, not either in a 5-km running time trial protocol (129).

In the nine studies involving per-cooling with ice or cold beverages on continuous endurance exercise protocols, five returned significant enhancements on physical performance. Completion time (10.5%) was significantly improved in a cycling time trial protocol with a resistance of 4 kJ kg^{-1} (133), as well as running time (2.5%) in a triathlon (Olympic distance) protocol (126), and work done (4.4%) in a 15-min maximal intensity cycling protocol (95). Time to exhaustion (7-12.7%) was significantly superior in a 65% (112) and 70% (116) maximal aerobic power output cycling protocols. Thought, time to exhaustion and endurance capacity were not significantly improved in cycling at 50% (139) and 95% (118) VO_2peak intensity, neither in a running to exhaustion at first ventilatory threshold intensity (113). On the other hand, completion time was not significantly lower in a 40-km cycling time trial (109).

In the set of four studies where ice or cold beverages were administered both before and during continuous endurance exercise, all showed significant improvements in physical performance. Significantly higher time to exhaustion (23-118%) was observed in a 80% of maximum power intensity and in a 65%VO_2peak cycling protocols (107, 120), and completion time (6.2-6.8%) was also significantly improved in 20-km cycling (108) and in a 1.5-km running (130) time trials (**Supplementary Table 1** and **Figure 6**).

Influence of environmental conditions

Generally, thirteen out of twenty-nine studies that were performed under a WBGT $\geq$28°C and involved the internal administration of ice or cold beverages revealed significant improvements in physical performance (94–96, 107, 108, 112, 114, 116, 120, 124, 126, 133). When the analysis is differentiated by type of exercise, it is clear that all the thirteen studies involved continuous endurance exercise protocols. In the range of the studies that did not show significant improvements in physical performance while a thermal cooling technique was applied in more adverse environmental conditions, six involved intermittent exercise protocols (93, 99, 119, 122, 127, 132) and ten continuous endurance ones (98, 105, 109, 113, 115, 121, 123, 125, 129, 131).

Moreover, three out of six studies that involved the administration of ice or cold beverages under a WBGT <28°C showed significant improvements in physical performance. Two of them included continuous endurance exercise protocols (104, 130) and one included intermittent exercise (134). In the set of the studies that did not find significant improvements in physical performance, one involved intermittent exercise protocols (97) and two continuous endurance ones (118, 139) (**Figure 7**).

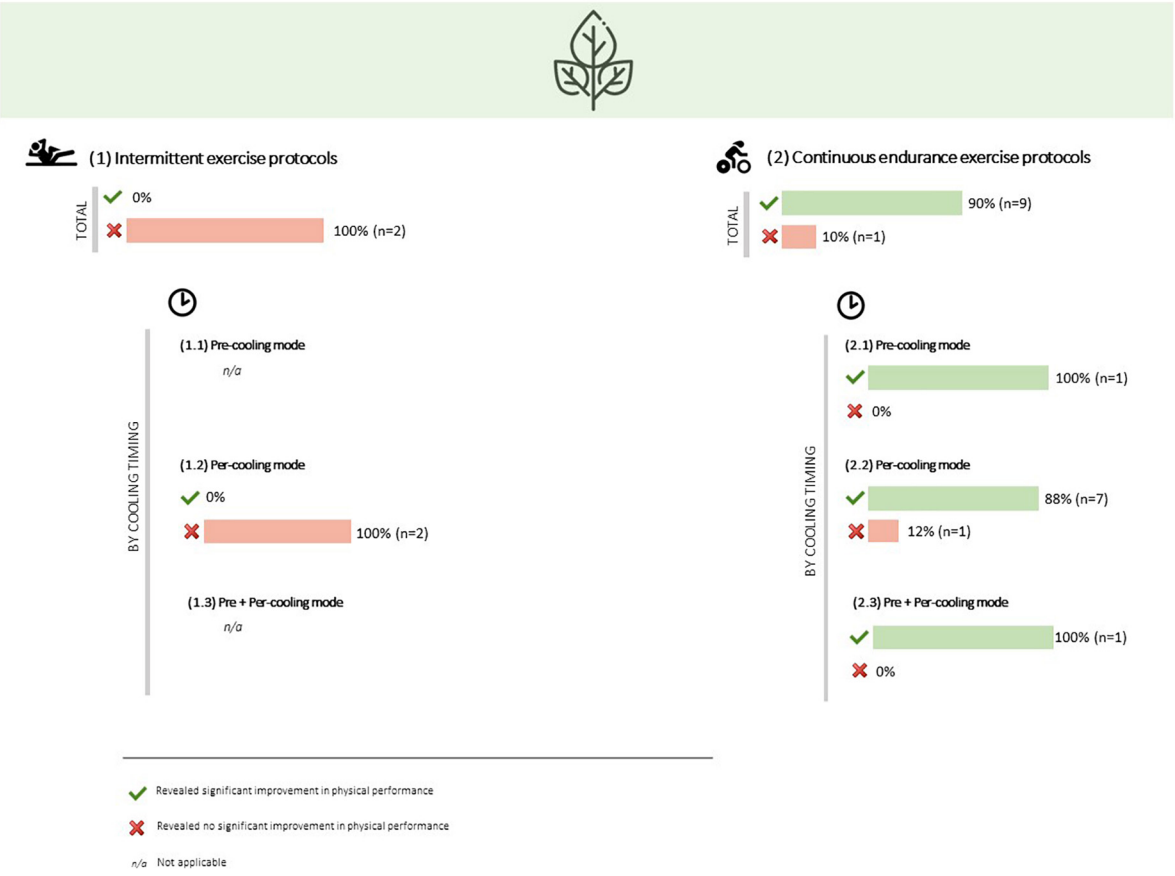


FIGURE 4
Overall results of oral menthol administration effect on physical performance, by type of exercise and cooling timing.

Ice *vs.* cold beverage

Continuous endurance exercise protocols

One study (130) showed that ingesting ice slurry both before and during exercise significantly decreased completion time in a 1.5-km running time trial (3.3%) comparing to drinking a cold beverage at the same timings (**Supplementary Table 1**).

Menthol solution *vs.* ice or cold beverage

Continuous endurance exercise protocols

In the three studies where the ergogenic effect of oral menthol administration was compared to ice or cold beverages, two showed a significant improvement in physical performance with the non-thermal technique, compared to the thermal one. Relative power output was significant higher (6%) while pre-cooling with menthol thermoneutral solution in a 3-min aerobic test compared to pre-cooling with cold water (105). Simultaneously, other study observed that per-cooling with a menthol solution had significant benefits on completion time in a 5-km running time trial (3.8%), compared to pre-cooling with an ice-slurry (129).

On the other hand, per-cooling with a menthol solution at 85% of baseline time to exhaustion showed non-significant differences in physical performance in a 70% maximal aerobic power output cycling protocol, compared to per-cooling at the same time with an ice slurry (116) (**Supplementary Table 1**).

Mixed-method *vs.* non-cooling strategy

Continuous endurance exercise protocols

Mouth rinsing with a menthol solution during exercise and ingesting crushed ice before exercise showed a significant increase in time to fatigue (39.1%) in a running protocol at an intensity of 100% of VO_2peak, comparing to a non-cooling control (128). At the same time, consuming an ice slushy menthol flavored beverage before and during a 20-km cycling time trial revealed a significant decrease in completion time (11%), comparing to a non-cooling strategy (108) (**Supplementary Table 1**).

Discussion

The current systematic review aimed to determine the effects of internal thermal and non-thermal cooling strategies on physical performance in different exercise conditions. The main

10.3389/fnut.2022.959516

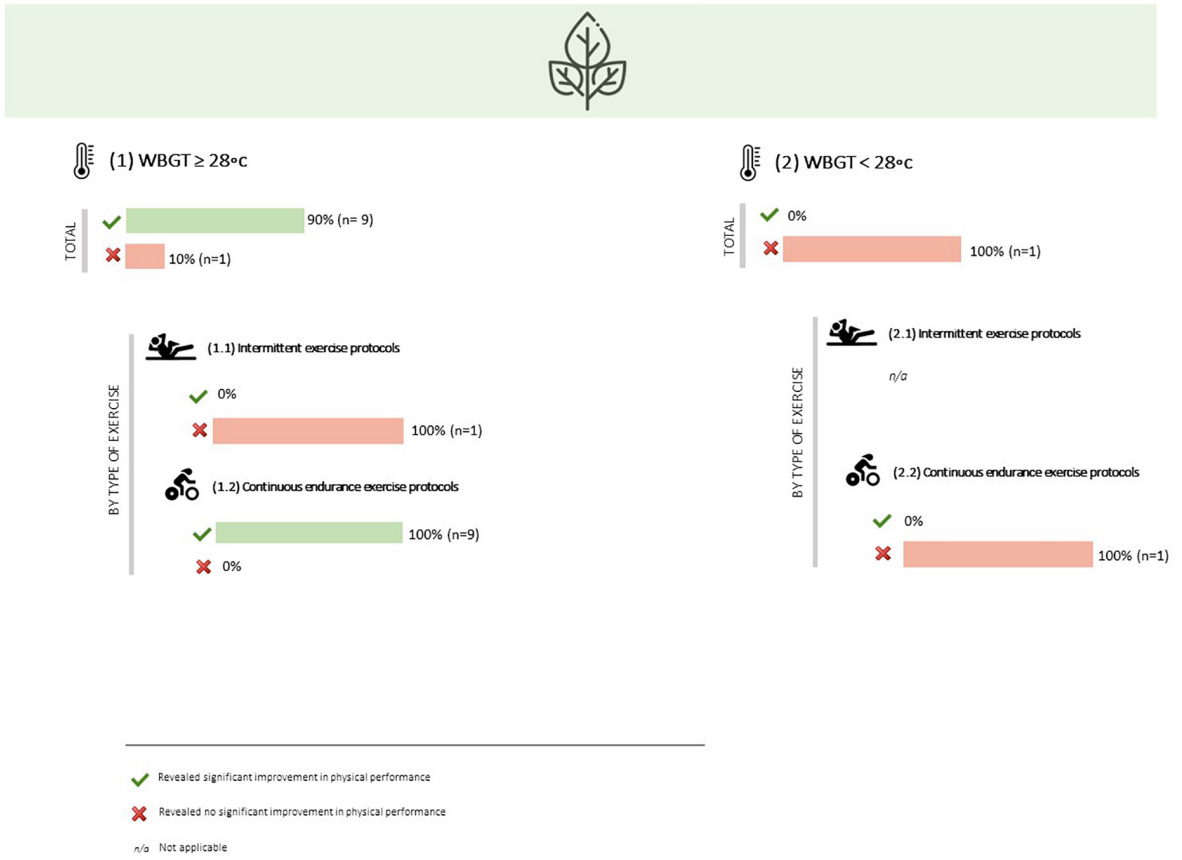


FIGURE 5
Overall results of oral menthol administration effect on physical performance, by environmental conditions and type of exercise.

findings were that mouth rinsing with a menthol solution during continuous endurance exercise in the heat seems to significantly improve physical performance in most of the studies. The impact of thermal methods on physical performance is not consistent, with only less than half of the studies reporting improvements. Ice/cold beverages are more likely to enhance physical performance in continuous endurance exercises, especially when consumed throughout exercises.

Menthol

Ergogenic action and physiological mechanisms

Oral menthol mouth rinsing during exercise in the heat improved continuous exercise, either exhaustive (111, 116, 128), self-modulated to exhaustion over a fixed distance or time (time-trial) (100, 108, 129), or to a fixed RPE (101, 110), which is in line with the recent findings of Barwood and colleagues (138).

Menthol's ergogenic effects seem to be related with an improvement in thermal sensation (101, 108, 110, 116), rate of perceived exertion, ventilatory capacity (111, 129), brain capacity (100), increased glycolytic energy provision or increased bicarbonate buffering (105). Menthol works as a non-thermal cooling stimulus to thermoreceptors, eliciting sensations of coolness when applied to the skin and mucosal surfaces without reductions in temperature, enhancing perceptual cooling effects (140, 141). Concretely, the application of menthol cause a feeling of coolness due to a stimulatory action on "cold" receptors (primarily TRPM8) by inhibiting calcium currents of neuronal membranes (142). The activation of these sensory pathways conducts this information to the brain, lowering the associated thermal strain (143), and may also provide a decrease in subjective airway resistance, a sensation of cool airflow upon inhalation, an increased arousal, and a down-regulation of thirst (142).

Despite menthol did not seem to increase tidal volume, subjects voluntarily increased ventilation following the rinse, perhaps due to the sensation of cooler air (111). A lowered thermal sensation may allow athletes to undergo greater heat stress, through increased blood prolactin concentration (129). Prolactin has been described as a "stress hormone" and a marker of dopaminergic activity in the brainstem (144). It is released in response to increased heat load (145), more specifically

10.3389/fnut.2022.959516

FIGURE 6
Overall results of ice or cold beverages administration effect on physical performance, by type of exercise and cooling timing.

regarding the temperature of the facial surface during exercise (146). A higher prolactin concentration suggests that following the menthol solution rinse the body can tolerate greater stress associated with a higher exercise intensity. For this reason, it is not clear if a decrease in thermal sensation can lead to a greater fatigue or even to a higher risk of hyperthermia, especially in the latter stages of prolonged exercise in elite athletes (110, 129).

Two of the three studies included in the present systematic review in which menthol did not improve performance applied intermittent exercise protocols (103, 106). It is important to mention that the conditions were less demanding in these studies, either by being held in a normothermic environment (22 ± 1°C) (106) or due to a shorter exercise protocol (20 min) and a greater recovery period between sets (103). Also, even if a cycling intermittent-sprint protocol is a reliable and valid tool to determine the physiological responses to intermittent sprinting (147), the specific task being closed in nature could create an experimental artifact, whereby participants are not able to sprint freely in frequency or duration. This raises the need to assess the effect of oral menthol under an intermittent exercise protocol that more closely mimics real conditions. In another study, in which menthol did not significantly enhance the time to exhaustion in a continuous endurance exercise protocol (117), the trial was not performed under heat stress conditions (20.4 ± 0.5°C, 29.5 ± 4.6% RH, WBGT = 18°C). Additionally, this was the only protocol to incorporate menthol into a carbohydrate sports drink, while the control beverage being the same beverage without menthol. Although speculative, the lack of significant benefits on physical performance in this study could be due to the fact that the sensing of carbohydrates and menthol in the mouth affects similar nervous signaling pathways and, hence, the erogenicity of both substances is not additive. In fact, carbohydrate mouth rinsing is known to be ergogenic on its own, without supplying additional energy to the body (148). Therefore, more significant results are found with the administration of menthol, in comparison with no-beverage or beverages that do not exert an ergogenic role.

Timing and administration mode

Overall, the collective evidence presented in the current systematic review suggests that menthol does not need to be swallowed to elicit a positive effect on performance, being

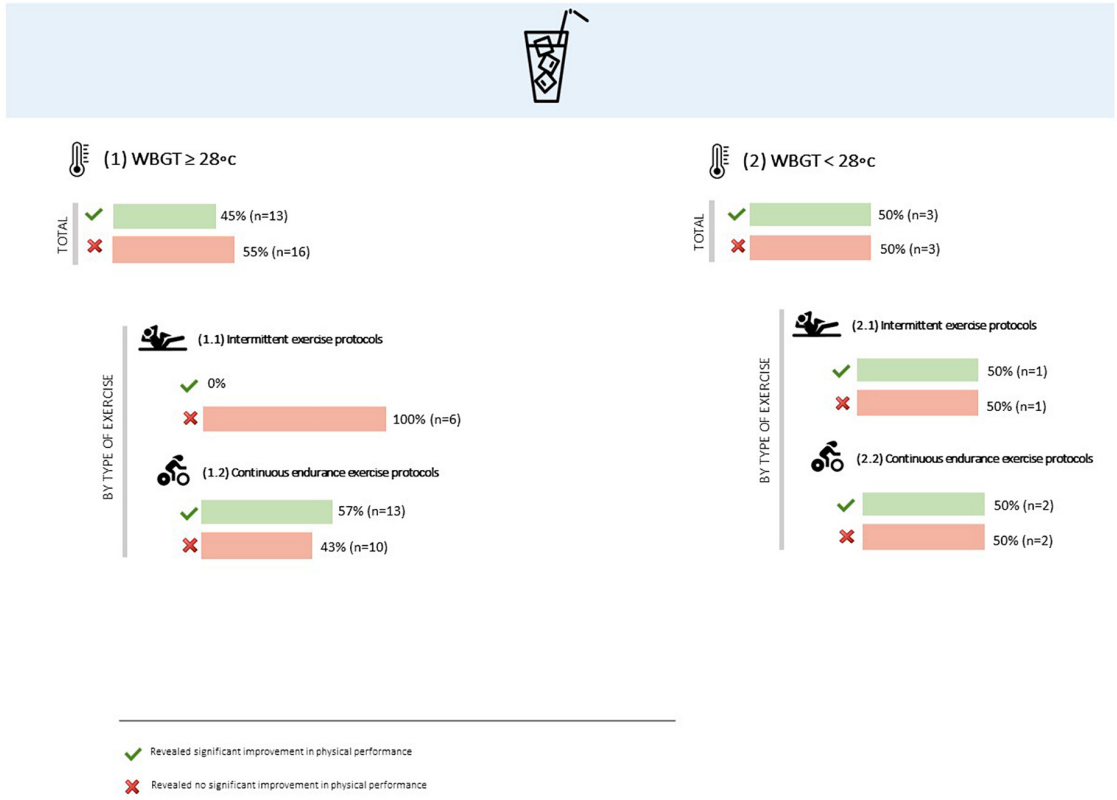


FIGURE 7
Overall results of ice or cold beverages administration effect on physical performance, by environmental conditions and type of exercise.

sufficient to orally rinse and expectorate (100, 101, 105, 110, 111, 116, 128, 129). Menthol activates thermoreceptors located in the oral cavity, one of the most densely innervated parts of the body with several peripheral receptors (149), that my trigger an ergogenic effect (108).

Almost all studies conducted with oral menthol have opted for a per-cooling strategy, which seems more suited to its mode of action. As the proposed mechanism of action of internally applied menthol targets thermal sensation, improved thermal comfort, and rate of perceived exertion (15, 150), the ergogenic effect is more likely to occur with the higher thermal stress obtained in more advanced stages of exercise. Accordingly, menthol seems to benefit performance when given in several successive internal administrations throughout exercise (100, 101, 108, 110, 111, 128, 129) or even as a single dose in the latter stages of exercise (116).

Most studies (100, 105, 110, 116, 128, 129) have demonstrated a beneficial effect on physical performance with a mouth rinsing for 5 s, without no greater effect observed with longer rinse durations (up to 10 s) (116, 117).

Sex differences

Five studies have evaluated the role of oral menthol on physical performance in female participants (100, 101, 103, 105, 106), and two of them reported significant improvement in exercise (100, 105). However, three of the five studies did not present the results separated by sex, which makes impossible to differentiate the effect of oral menthol mouth rinse on physical performance in men and women, no matter it is significant (105) or not (103, 106). In the other two studies, one found significant results with cooling only for men (101) and the other observed a significant improve in completion time and power output in the female subjects (100). Therefore, is not clear if the lack of efficacy on women depends on sex-related physiological differences, or if is due to methodological differences between studies.

Interestingly, oral application of L-menthol reduced the perceptual measures of thermal sensation in men, while in females it was only effective in the early stages of exercise in the heat. Women exhibited a smaller reduction in thermal sensation following L-menthol mouth rinsing, suggesting a sex-specific response to L-menthol efficacy during exercise (101). Mouth rinsing with 25 mL (0.01%) every 10 min of exercise improved non-significantly the time (6%) and power output (2.2%) in women (101). Therefore, these facts highlight the importance of further research to understand sex differences in behavioral thermoregulation and performance response to oral menthol during exercise in the heat.

Influence of environmental conditions

Only one study involving oral menthol administration was carried out under WBGT <28°C and this included continuous endurance exercise protocol (117), revealing no significant improvements in physical performance. In fact, as previously mentioned, in this study menthol was incorporated into a carbohydrate sports drink, possibly not allowing to isolate the ergogenic effect of menthol from that of carbohydrates. Also, the same study was carried out under a WBGT = 18°C, which represents a low risk of overheating environment (137). Therefore, the fact that the only study that included menthol and was carried out under a WBGT <28°C had methodological characteristics so different from the others, makes it difficult to conclude about the role of the environmental conditions. More studies developed under a WBGT <28°C with non-thermal internal cooling techniques are needed to better conclude if these techniques are also efficient in physical performance, under these conditions.

It is important to consider that, in the case of menthol, although more studies have been completed under a WBGT ≥ 28°C, the type of exercise seems to be more important than the adversity of the environmental conditions, since the only study that did not revealed significant improvements in physical performance in these conditions involved an intermittent exercise protocol (103).

As already stated, performance responses to internal cooling seem to be better in continuous endurance exercise than in intermittent exercise. In fact, in continuous exercise, the heat stored is higher and T_{core} and thermal sensation rise faster, compared to the same amount of exercise performed in a variable intensity mode that includes short high intensity bouts followed by rest periods (151–153). Also, sweat loss and dehydration level are lower in intermittent compared to continuous type sports (154). Therefore, regardless of the external environmental conditions, it is expected that in activities where the heat storage and dehydration turns out to be superior, the response to internal cooling will be more effective concerning physical performance, as improvements in T_{core} or thermal sensation are more urgent. Since menthol's main mechanism of action is an improvement in the thermal sensation in more advanced phases of the exercise, if this is higher it is more likely that the ergogenic effects of this compound, previously explained, are felt.

Ice or cold beverage

Ergogenic action and physiological mechanisms

The application of thermal methods before and/or during exercise improved performance in 15 of 35 studies that evaluated exhaustive (time to exhaustion at a fixed intensity) (107, 112, 114, 116, 120, 131), self-modulated to exhaustion over a fixed distance or time (time trial) (94, 96, 104, 108, 124, 126) or to a fixed point (power output) (95, 133), and intermittent protocols (93, 134). An improvement in performance was more prevalent in studies evaluating continuous endurance exercise (54%), than in studies that applied intermittent exercise protocols (11%).

Overall, the improvement in physical performance with the internal administration of ice or cold beverages may have resulted from an improve in behavioral thermoregulation ((thermal sensation (104, 130), thermal comfort (120)), thermal autonomic responses ((T_{core} (94–96, 107, 112, 134)), or from a combination of all these (114, 124, 126, 133). After the ingested ice slurry reaches the stomach/gastrointestinal region it will absorb a considerable amount of internal heat, lowering the temperature locally (96). It is also likely that the ingestion of ice may also lead to a decrease in brain temperature (155), that may persist throughout exercise, increasing the likelihood of an improvement regarding thermal sensation in the latter stages. This may extend the time required to achieve a critically high brain temperature, allowing subjects to exercise for longer periods of time or greater intensities (53, 114, 156, 157), by increasing and/or maintaining central drive and motivation (158). In addition, internal cooling via ice slurry ingestion may improve exercise performance in the heat by stimulating internal thermoreceptors. In humans, thermoreceptors have been identified in the stomach and small intestine (159) It has been shown that the glossopharyngeal nerve conducts impulses for temperature sensation from the posterior third of the tongue and upper pharynx to the brain (160). Thus, ice slurry ingestion may directly affect T_{core} afferents and leads to a beneficial effect on the inhibitory feedback, ultimately influencing exercise performance. A decrease in T_{core} may affect exercise performance by increasing the margin between the initial core temperature and temperatures at which athletic performance is affected. A lower core body temperature at a given point of exercise had a similar effect to that which occurs with acclimation and enabled athletes to exercise at higher intensities during self-paced exercise (or for a longer duration during constant pace exercise) (161). In fact, a decrease in T_{core} is likely to promote a reduction in sweat rate necessary for heat dissipation, delaying progressive fluid losses and dehydration (162). Progressive dehydration precipitates a cascade of events including a decrease in plasma volume and an increase in plasma osmolality (5), a decrease in sweat rate and evaporative heat loss (163) and a decrease in cardiac filling (164). The blood flow redistribution and other thermoregulatory demands of exercising in hot and/or humid environments represents a significant stress to the cardiovascular system, limiting performance, as maintaining a similar relative intensity requires the reduction of absolute intensity (i.e., work load) (165).

Putative reasons for the lack of performance improvements with internal cooling strategies in some studies may be related with a lower core-to-skin temperature gradient (121, 123), no reduction on core body temperature (T_{core}), (i.e., lower heat

storage capacity) (119), self-paced intermittent protocols (99, 122, 127, 132), short duration protocols (97, 131), moderate environmental conditions (97, 139), no changes in thermal sensation and rate of perceived exertion (107, 115, 139), or gastrointestinal discomfort due to an excessive amount of ice slurry (125). Actually, a lower core-to-skin temperature gradient found in two studies (121, 123) did not promote a sufficient convective heat flux from the center to the periphery, hindering thermoregulation mechanisms to cope with the heat and thus not benefiting performance. This can happen when the environmental temperature is much higher than skin temperature, and also when T_{core} decreases very fast at the beginning of the exercise (93). On the other hand, cooling strategies may provide the most gains in physical performance in stressful environmental conditions (higher temperatures and humidity levels and/or longer duration and greater intensity exercises) (95), that were not observed in some of the included studies (97, 99, 122, 127, 131, 132, 139). As increases in core temperature are proportional to exercise intensity (166), it is easier to avoid an increase in heat strain in exercises protocols whose intensity is not great, or at least is interspersed with moments of lower intensity recovery periods (intermittent efforts), minimizing the benefits of administering an ice or cold beverage. At the same time, in other studies (107, 115, 139), authors report that, despite the improvement observed in the thermoregulatory parameters (such as T_{core}), physical performance was not increased due to a lack of improvement in performance perceptual parameters (thermal sensation and rate of perceived exertion). This could indicate that behavioral thermoregulation and thermal autonomic responses to exercising in the heat are equally important, and that the success of cooling strategies may depend on its effectiveness in improving both variables. Finally, it is known that the maximum rate of intestinal absorption is 0.5 L/hour when cycling at 85% VO_{2max} and the intake of large volumes of fluids may not be advantageous (167). This could be the reason why administrating 1 L of ice-slurry at once found no improvements on physical performance, despite a reduction on $T_{core.}$ in one study (125).

Timing and administration mode

Ingesting ice or cold beverages seemed to be more effective in improving physical performance than just rinsing, which may be explained by the higher density of thermal receptors in the gastrointestinal tract (12).

The amount of ice or cold beverages administered before and/or during exercise was quite different between trials. Also, some authors opted for giving an absolute amount, while others preferred to adjust the volume to athletes' body weight ($g{\cdot}kg^{-1}$) due to an improved gastrointestinal tolerance (94, 130, 133). However, performance benefits were observed with a wide range of volumes, which suggest that the amount ingested is not a key factor regarding the ergogenic potential of these beverages.

Ice or cold beverages showed better results when administered during or before and during exercise (93, 95, 107, 108, 112, 116, 120, 126, 133). Per-cooling protocols involved the ingestion of ice slurry/crushed ice/ice slushy/cold beverages several times throughout exercise, while in the pre-cooling protocols these beverages were ingested once, typically between 10 to 35 min before the beginning of exercise (94, 96, 98, 99, 104, 114, 115, 121–125, 127, 129, 131, 134). Some recent studies suggested that the advantages gained from cooling during exercise may outweigh those of pre-cooling, due to a high thermal strain in the latter stages of the exercise (168). Although, T_{core} seems to decrease more with pre-cooling (physical thermoregulation), per-cooling seems to be more efficient in reducing thermal sensation and thermal comfort (behavioral thermoregulation) in more advanced phases of the exercise (12). So, the effectiveness of pre-cooling with ice slurry may be limited and its beneficial effects may be attenuated after 20–30 min (169).

Regarding beverage's temperature, it was found that ingesting 190 mL of ice slurry menthol flavored beverage (0.025%, 0.17°C) in the warm-up and 5 times during running, significantly decreased completion time (3.3%) when comparing with the same amount of cold menthol flavored beverage (0.025%, 3.1°C) (130). This agrees with another study (50) reporting that the ingestion of 1.25 $g{\cdot}kg^{-1}$ of ice (0.5°C) every 5 min, six times before exercise, significantly improved time to exhaustion (19.8%), when comparing with the same amount of cold water (4°C). This difference may have occurred due to different effects on T_{core}, since crushed ice ingestion leads to a greater reduction in body temperature due to the additional energy that is required to change solid ice to liquid water, allowing for a significantly larger amount of heat absorption and thus more work to be completed (53).

Sex differences

The only study (of five) with participants from both sexes that reported a significant improvement in physical performance with internal thermal cooling techniques in a hot and humid outdoor environment, enhancing the ecological validity of the findings, did not independently report the effect of ice slurry ingestion 30 min before exercise (8.0 $g{\cdot}kg^{-1}$ at −1.4°C) for men and women (104). In other studies, the consumption of either cold water during exercise (190 mL at 1.3°C) (102) and of crushed ice 30 minutes before exercise (6.8 $g{\cdot}kg^{-1}$ at −0.5°C) (99), did not change physical performance. However, it should be mentioned that one of that studies (102) comprised swimming and exercise in water, which increases the heat dissipation capacity due to greater forced convective and conductive transfer heat transfer from the skin (170), facilitating the maintenance of T_{core} at a lower level and thus reducing the likelihood of cooling benefits. And int the other study (99), the intermittent exercise protocol used was less affected by cooling strategies. Similarly, mouth rinse

of cold water 1 min before the start of exercise (25 mL at 4°C) did not show effect on relative power output in both male and female subjects (105). Though, it is important to refer that applying a thermal cooling technique only 1 min before a 3-min exercise may not be enough time to notice the benefits of these methods, neither for men nor for women. Finally, another trial (98) showed a non-significant decrease in completion time (2.2%) in a 800-kJ cycling time trial in women after the ingestion of crushed ice (7.0 $g \cdot kg^{-1}$) 30 min before. It is expected that an athlete with a higher percentage of fat mass requires less energy to change their mean body temperature when comparing with an athlete with a lower percentage of fat mass (171). Therefore, as the rate of heat storage may be greater in females than in males, this might hinder the ergogenic effect due to an increased heat storage capacity, which could explain why ice ingestion may exert greater benefits in men.

Influence of environmental conditions

Unlike menthol, slightly better results were found on physical performance when using ice or cold beverages under a WBGT <28°C, compared to the studies carried out in a WBGT ≥ 28°C. However, it is important to notice that all the studies completed with a WBGT ≥ 28°C that showed significant improvements in physical performance (n = 13) involved continuous endurance exercise protocols (94–96, 107, 108, 112, 114, 116, 120, 124, 126, 133, 137). Concerning the other sixteen studies carried out under a WBGT ≥28°C, and that did not reveal significant enhancements, six involved intermittent exercise protocols (67, 93, 99, 113, 119, 122, 127) and ten continuous endurance ones (98, 105, 109, 114, 115, 121, 123, 125, 129, 131). Of these ten studies that involved endurance exercise, eight had a pre-cooling mode (98, 105, 115, 121, 123, 125, 129, 131), which, as seen in point 4.2.2, will eventually not be the most interesting cooling timing for thermal methods.

Like menthol, more important than the environmental conditions seems to be the type of exercise protocol. As mentioned before, in continuous exercise, the heat stored is higher and T_{core} and thermal sensation rise faster. Thus, this type of exercise, regardless of environmental conditions, will theoretically benefit better from thermal cooling methods than intermittent exercises, especially those of shorter duration.

This may indicate that the benefits of cooling on physical performance are not only verified for high or extreme environmental conditions, and that, even in less demanding conditions, for higher and continuous exercise intensities, it could still be beneficial the application of internal cooling techniques. Nevertheless, further studies involving simultaneously internal cooling thermal techniques, continuous endurance exercise and per or pre + per-cooling modes, both in more and less adverse environmental conditions, will be needed for a better understanding.

Mixed thermal and non-thermal techniques

Combining thermal with non-thermal strategies seems to display better results than an isolated approach. As an example, superior results on completion time were reported when thermal and non-thermal techniques were combined (ice slurry menthol flavored – 0.01%), instead of used in isolation (108). Similarly, the combination of pre-cooling with ice slurry ingestion and per-cooling with menthol solution mouth rinse improved time to fatigue compared to per-cooling with menthol alone (128). A possible reason to justify these results is the reduction of the mood disturbance, in particular ratings of tension, depression, and confusion, when thermal and non-thermal methods are simultaneously applied (128). However, a synergistic effect of thermal and non-thermal interventions should be considered in future research, for a better understanding.

Menthol solution *vs.* ice or cold beverage

Although two of the three studies that compared the effect of menthol with ice or cold beverages found greater benefits in physical performance with the non-thermal method, it is important to note that in one of them (105) the pre-cooling (either with menthol or with a cold beverage) was carried out just 1 min before the trial and that the trial itself only lasted 3 min, not sufficient to see a decrease in T_{core}. In the other study (129), while menthol was applied as per-cooling, ice slurry was ingested 30 min before exercise. Thus, in this case, we may be observing the benefits of applying a cooling method during the exercise (which, as previously mentioned, seems to be more advantageous than pre-cooling), rather than comparing the effectiveness of a thermal method with a non-thermal one.

Limitations and strengths

This systematic review has some limitations. Firstly, most studies included a relatively small sample size. Secondly, men and women seem to have different thermoregulatory responses to exercise in the heat, which may have influenced the effectiveness of cooling methods on physical performance, especially regarding mixed-sample studies. Another possible limitation is related with the fact that trials were performed in an ambient environment with large temperature and relative humidity amplitudes (i.e., 22-38°C and 20-80%). Moreover, exercise protocols noticeably varied between studies, namely regarding duration and intensity, possible generating conflicting results. Furthermore, authors opted to not collect adverse effects

in the selected studies. In fact, in all the included studies, a familiarization session was performed to test participants tolerance to L-menthol or to ice/cold beverages, and any participant has been excluded from any study based on intolerances or adverse effects. Additionally, the included studies did not evaluate immediate/long term complications resulting from the intervention, so for those reasons adverse effects were not reported in the data synthesis. Other limitation is that with 43 studies and 472 subjects included, a meta-analysis could potentially have been carried out. However, due to the high heterogeneity of the methodologies used, not only regarding the systematic differences of exercise protocols, but also considering the variances in timing and frequency of cooling, in the beverages doses, in the population involved, and in the environmental conditions, the authors opted not to perform a quantitative analysis. Additionally, the risk of bias in the selective reporting and blinding categories was generally unclear. In fact, this may be difficult to assure when administering menthol or ice/cold beverages, due to its distinctive sensory effect. Whilst it is challenging to conduct double-blind experiments in this type of studies, improvements in research design, mainly with menthol, should be attained. Finally, publication bias was not accessed in this systematic review.

Regarding the strengths of this article, we first highlight the fact that, to the best of our knowledge, this is the first systematic review that exclusively focuses on internal cooling methods. The literature is mainly focused on external techniques. Secondly, only studies with a randomized crossover design were included, increasing the internal validity of the results (172). Furthermore, this review involved studies with several types of exercise protocols, which allows for a wider broad understanding of the internal cooling effect on physical performance in different sports disciplines, considering their physiological particularities. It is also important to notice that this analysis, unlike most others that focus exclusively on pre or per-cooling modes, was able to conclude about the cooling timing role on physical performance.

Future lines of research

Sport competitions are taking place more often in hot environments, so the implementation of cooling techniques before and during competition will become even more important for athletes to cope with the heat. More solid evidence is needed about the implication of cooling strategies on short and intense and even intermittent efforts, especially in outdoor settings and that approach the real conditions and rules of different sports, including team sports. Moreover, studying both male and female individuals is of upmost importance, due to sex-related differences in thermal sensation and heat storage capacity. Since the majority of the collected evidence in this topic refers to physically active individuals or recreational athletes, protocols that involve elite athletes are also required. Likewise, it is also important to understand the effect of different environmental conditions on the response to internal cooling, so that the recommendations can take this factor into account.

Practical applications

In addition to acclimatization or in situations where this is not feasible, athletes competing in outdoor higher temperatures and humidity contexts should adopt prophylactic strategies to avoid adverse effects resulting from a temperature rise of body temperature on physical performance. Such strategies may be practical and cost-effective, namely the ingestion of an ice slurry 30 min before exercise and the ingestion or mouth rinsing of an ice slurry menthol flavored beverage (0.01%) during exercise. In endurance exercise competitions, per-cooling strategies can be divided into multiple moments during the race, including hydration breaks, half-time or drinking stations.

Conclusion

Rinsing a menthol solution (0.01%) improves physical performance during continuous endurance exercise. Conversely, the ingestion of ice or cold beverages does not seem to increase performance. However, slightly greater results were found for ice beverages, in per-cooling or pre and per-cooling continuous endurance trials. Co-administration of menthol with or within ice beverages seems to exert a synergistic effect by improving physical performance. Although not entirely clear, even in environmental conditions that are not extreme, internal cooling strategies may exert an ergogenic effect. Further studies exploring both intermittent and outdoor exercise protocols, involving elite male and female athletes and performed under not extreme environmental conditions are warranted.

Data availability statement

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

Author contributions

MR and VT were responsible for conception and design. MR and PB conducted search procedure, data analysis, and methodological quality analysis of the included studies. All authors made substantial contributions to conception, design, and interpretation of the data, drafting of the manuscript, and in giving final approval of the final version, read, and agreed to the published version of the manuscript.

Acknowledgments

We are grateful to the medical research librarian Marta Azevedo who provided help on search strategy for this systematic review.

Conflict of interest

FJT is currently the Innovation Manager of a Biotechnology company (Bettery S.A.) that produces dietary supplements. No funds or resources of this company were used in this publication.

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.2022.959516/full#supplementary-material

References

1. Guy JH, Deakin GB, Edwards AM, Miller CM, Pyne DB. Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. *Sports Med.* (2015) 45:303–11. doi: 10.1007/s40279-014-0277-4

2. Sunderland C, Nevill ME. High-intensity intermittent running and field hockey skill performance in the heat. *J Sports Sci.* (2005) 23:531–40. doi: 10.1080/02640410410001730197

3. Tan CL, Knight ZA. Regulation of body temperature by the nervous system. *Neuron.* (2018) 98:31–48.

4. Flouris AD, Schlader ZJ. Human behavioral thermoregulation during exercise in the heat. *Scand J Med Scin Sports.* (2015) 25(Suppl. 1):52–64.

5. Sawka MN, Leon LR, Montain SJ, Sonna LA. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Compr Physiol.* (2011) 1:1883–928. doi: 10.1002/cphy.c100082

6. Choo HC, Nosaka K, Peiffer JJ, Ihsan M, Abbiss CR. Ergogenic effects of precooling with cold water immersion and ice ingestion: a meta-analysis. *Eur J Sport Sci.* (2018) 18:170–81. doi: 10.1080/17461391.2017.1405077

7. Rizzo L, Thompson MW. Cardiovascular adjustments to heat stress during prolonged exercise. *J Sports Med Phys Fitness.* (2018) 58:727–43.

8. Brothers RM, Bhella PS, Shibata S, Wingo JE, Levine BD, Crandall CG. Cardiac systolic and diastolic function during whole body heat stress. *Am J Physiol Heart Circ Physiol.* (2009) 296:H1150–6.

9. Nybo L, Nielsen B. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol.* (2001) 91:1055–60.

10. Bongers CC, Thijssen DH, Veltmeijer MT, Hopman MT, Eijsvogels TM. Precooling and percooling (cooling during exercise) both improve performance in the heat: a meta-analytical review. *Br J Sports Med.* (2015) 49:377–84. doi: 10.1136/bjsports-2013-092928

11. Ruddock A, Robbins B, Tew G, Bourke L, Purvis A. Practical cooling strategies during continuous exercise in hot environments: a systematic review and meta-analysis. *Sports Med.* (2017) 47:517–32. doi: 10.1007/s40279-016-0592-z

12. Bongers CC, Hopman MT, Eijsvogels TM. Cooling interventions for athletes: an overview of effectiveness, physiological mechanisms, and practical considerations. *Temperature.* (2017) 4:60–78. doi: 10.1080/23328940.2016.1277003

13. Stevens CJ, Taylor L, Dascombe BJ. Cooling during exercise: an overlooked strategy for enhancing endurance performance in the heat. *Sports Med.* (2017) 47:829–41. doi: 10.1007/s40279-016-0625-7

14. Siegel R, Laursen PB. Keeping your cool: possible mechanisms for enhanced exercise performance in the heat with internal cooling methods. *Sports Med.* (2012) 42:89–98. doi: 10.2165/11596870-000000000-00000

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

16. Flood TR. Menthol use for performance in hot environments. *Curr Sports Med Rep.* (2018) 17:135–9.

17. Patel T, Ishiuji Y, Yosipovitch G. Menthol: a refreshing look at this ancient compound. *J Am Acad Dermatol.* (2007) 57:873–8. doi: 10.1016/j.jaad.2007.04.008

18. Tyler CJ, Sunderland C, Cheung SS. The effect of cooling prior to and during exercise on exercise performance and capacity in the heat: a meta-analysis. *Br J Sports Med.* (2015) 49:7–13. doi: 10.1136/bjsports-2012-091739

19. Douzi W, Dugue B, Vinches L, Al Sayed C, Halle S, Bosquet L, et al. Cooling during exercise enhances performances, but the cooled body areas matter: a systematic review with meta-analyses. *Scand J Med Sci Sports.* (2019) 29:1660–76. doi: 10.1111/sms.13521

20. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* (2015) 4:1.

21. Pollock A, Berge E. How to do a systematic review. *Int J Stroke.* (2017) 13:174749301774379.

22. Sibbald B, Roberts C. Understanding controlled trials. Crossover trials. *BMJ.* (1998) 316:1719.

23. Higgins JPAD. Assessing risk of bias in included studies. In: Higgins JP, Green S editors. *Cochrane Hand-book for Systematic Reviews of Interventions: Cochrane Book Series*. Chichester: Wiley (2008).

24. Meamarbashi A, Rajabi A. The effects of peppermint on exercise performance. *J Int Soc Sports Nutr.* (2013) 10:15

25. Hue O, Chabert C, Collado A, Hermand E. Menthol as an adjuvant to help athletes cope with a tropical climate: tracks from heat experiments with special focus on guadeloupe investigations. *Front Physiol.* (2019) 10:1360. doi: 10.3389/fphys.2019.01360

26. Choo HC, Peiffer JJ, Pang JWJ, Tan FHY, Aziz AR, Ihsan M, et al. Effect of regular precooling on adaptation to training in the heat. *Eur J Appl Physiol.* (2020) 120:1143–54. doi: 10.1007/s00421-020-04353-1

27. Hue O, Monjo R, Riera F. Imposed cold-water ingestion during open water swimming in internationally ranked swimmers. *Int J Sports Med.* (2015) 36:941–6. doi: 10.1055/s-0035-1548812

28. Zimmermann M, Landers G, Wallman K, Kent G. Precooling with crushed ice: as effective as heat acclimation at improving cycling time-trial performance in the heat. *Int J Sports Physiol Perform.* (2018) 13:228–34. doi: 10.1123/ijspp.2016-0766

29. Burdon CA, Easthope CS, Johnson NA, Chapman PG, O'Connor H. The influence of ice slushy on voluntary contraction force following exercise-induced hyperthermia. *Appl Physiol Nutr Metab.* (2014) 39:781–6. doi: 10.1139/apnm-2013-0394

30. Chalmers S, Siegler J, Lovell R, Lynch G, Gregson W, Marshall P, et al. Brief in-play cooling breaks reduce thermal strain during football in hot conditions. *J Sci Med Sport.* (2019) 22:912–7.

31. Deshayes TA, De La Flore A, Gosselin J, Beliveau J, Jeker D, Goulet EDB. The impact of an ice slurry-induced gastrointestinal heat sink on gastrointestinal and rectal temperatures following exercise. *Sports.* (2019) 7:198. doi: 10.3390/sports7090198

32. Doubt TJ, Hsieh SS. Additive effects of caine and cold water during submaximal leg exercise. *Med Sci Sports Exerc.* (1991) 23:435–42. doi: 10.1580/1080-6032(2000)011[0005:teogam]2.3.co;2

33. James CA, Richardson AJ, Watt PW, Gibson OR, Maxwell NS. Physiological responses to incremental exercise in the heat following internal and external precooling. *Scand J Med Sci Sports.* (2015) 25(Suppl. 1):190–9. doi: 10.1111/sms.12376

34. Lamarche DT, Meade RD, McGinn R, Poirier MP, Friesen BJ, Kenny GP. Temperature of ingested water during exercise does not affect body heat storage. *Med Sci Sports Exerc.* (2015) 47:1272–80.

35. Lamarche DT, Meade RD, McGinn R, Poirier MP, Friesen BJ, Kenny GP. On the maintenance of human heat balance during cold and warm fluid ingestion response. *Med Sci Sports Exerc.* (2015) 47:1318. doi: 10.1249/MSS.0000000000000638

36. Lee JKW, Yeo ZW, Nio AQX, Koh ACH, Teo YS, Goh LF, et al. Cold drink attenuates heat strain during work-rest cycles. *Int J Sports Med.* (2013) 34:1037–42. doi: 10.1055/s-0033-1337906

37. Morris NB, Coombs G, Jay O. Ice slurry ingestion leads to a lower net heat loss during exercise in the heat. *Med Sci Sports Exerc.* (2016) 48:114–22. doi: 10.1249/MSS.0000000000000746

38. Morris NR, Chaseling GK, Bain AR, Jay O. Temperature of water ingested before exercise alters the onset of physiological heat loss responses. *Am J Physiol Regul Integr Comp Physiol.* (2019) 316:R13–20. doi: 10.1152/ajpregu.00028.2018

39. Onitsuka S, Zheng X, Hasegawa H. Ice slurry ingestion before and during exercise inhibit the increase in core and deep-forehead temperatures in the second half of the exercise in a hot environment. *J Thermal Biol.* (2020) 94:102760. doi: 10.1016/j.jtherbio.2020.102760

40. Saldaris JM, Landers GJ, Lay BS. Enhanced decision making and working memory during exercise in the heat with crushed ice ingestion. *Int J Sports Physiol Perform.* (2020) 15:503–10. doi: 10.1123/ijspp.2019-0234

41. Wimer GS, Lamb DR, Sherman WM, Swanson SC. Temperature of ingested water and thermoregulation during moderate-intensity exercise. *Can J Appl Physiol.* (1997) 22:479–93.

42. Mur-Gimeno E, Sebio-Garcia R, Solé J, Lleida A, Moras G. Short-term effects of two different recovery strategies on muscle contractile properties in healthy active men: a randomised cross-over study. *J Sports Sci.* (2022) 40:646–54. doi: 10.1080/02640414.2021.2010978

43. Stevens CJ, Ross MLR, Vogel RM. Development of a "cooling" menthol energy gel for endurance athletes: effect of menthol concentration on acceptability and preferences. *Int J Sport Nutr Exerc Metab.* (2021) 31:40–5. doi: 10.1123/ijsnem.2020-0190

44. Lee Y, Yun S, Hwang J, Lee H, Jung A, Lee DT. Effect of cold water intake during exercise in heat on cognitive function. *Exerc Sci.* (2021) 30:122–8. doi: 10.1017/S002966510800877X

45. Naito T, Haramura M, Muraishi K, Yamazaki M, Takahashi H. Cooling during short-term heat acclimation enhances aerobic capacity but not sweat capacity. *Eur J Sport Sci.* (2022) 22:579–88. doi: 10.1080/17461391.2021.1884751

46. Alhadad SB, Low ICC, Lee JKW. Thermoregulatory responses to ice slurry ingestion during low and moderate intensity exercises with restrictive heat loss. *J Sci Med Sport.* (2021) 24:105–9. doi: 10.1016/j.jsams.2020.07.002

47. Ng J, Dobbs WC, Wingo JE. Effect of ice slurry ingestion on cardiovascular drift and V·O2max during heat stress. *Med Sci Sports Exerc.* (2019) 51:582–9.

48. Levels K, Teunissen LPJ, de Haan A, de Koning JJ, van Os B, Daanen HAM. Effect of warm-up and precooling on pacing during a 15-km cycling time trial in the heat. *Int J Sports Physiol Perform.* (2013) 8:307–11. doi: 10.1123/ijspp.8.3.307

49. Maunder E, Laursen PB, Kilding AE. Effect of ad libitum ice-slurry and cold-fluid ingestion on cycling time-trial performance in the heat. *Int J Sports Physiol Perform.* (2017) 12:99–105. doi: 10.1123/ijspp.2015-0764

50. Naito T, Iribe Y, Ogaki T. Ice ingestion with a long rest interval increases the endurance exercise capacity and reduces the core temperature in the heat. *J Physiol Anthropol.* (2017) 36:9. doi: 10.1186/s40101-016-0122-6

51. Naito T, Ogaki T. Comparison of the effects of cold water and ice ingestion on endurance cycling capacity in the heat. *J Sport Health Sci.* (2017) 6:111–7. doi: 10.1016/j.jshs.2015.12.002

52. Naito T, Sagayama H, Akazawa N, Haramura M, Tasaki M, Takahashi H. Ice slurry ingestion during break times attenuates the increase of core temperature in a simulation of physical demand of match-play tennis in the heat. *Temperature.* (2018) 5:371–9. doi: 10.1080/23328940.2018.1475989

53. Siegel R, Mate J, Brearley MB, Watson G, Nosaka K, Laursen PB. Ice slurry ingestion increases core temperature capacity and running time in the heat. *Med Sci Sports Exerc.* (2010) 42:717–25. doi: 10.1249/MSS.0b013e3181bf257a

54. Best R, Crosby S, Berger N, McDonald K. The effect of isolated and combined application of menthol and carbohydrate mouth rinses on 40 km time trial performance, physiological and perceptual measures in the heat. *Nutrients.* (2021) 13:4309. doi: 10.3390/nu13124309

55. Benjamin CL, Sekiguchi Y, Morrissey MC, Butler CR, Filep EM, Stearns RL, et al. The effects of hydration status and ice-water dousing on physiological and performance indices during a simulated soccer match in the heat. *J Sci Med Sport.* (2021) 24:723–8. doi: 10.1016/j.jsams.2021.05.013

56. Racinais S, Ihsan M, Taylor L, Cardinale M, Adami PE, Alonso JM, et al. Hydration and cooling in elite athletes: relationship with performance, body mass loss and body temperatures during the Doha 2019 IAAF World Athletics Championships. *Br J Sports Med.* (2021) 55:1335–41. doi: 10.1136/bjsports-2020-103613

57. Lee JKW, Yeo ZW, Nio AQX, Ang WH, Fan PWP. Ice slurry ingestion improves subsequent outdoor 10 km running performance in the heat. *Med Sci Sports Exerc.* (2011) 43:74.

58. Marino FE, Noakes TD. Cold fluids improve exercise performance in the heat by slowing the rate of heat gain. *Med Sci Sports Exerc.* (2009) 41:1681–2. doi: 10.1249/MSS.0b013e3181aa2748

59. Ng J, Wingo JE, Hallmark AV, Brown AJ, Powers SA. Voluntary intake of ice slurry beverages and exercise performance during heat stress. *Med Sci Sports Exerc.* (2017) 49:489.

60. Sekiguchi Y, Benjamin CL, Butler CR, Morrisey MC, Filep EM, Stearns RL, et al. The effect of hydration status and ice water dousing on heart rate variability prior to and during intermittent exercise in the heat. *Med Sci Sports Exerc.* (2020) 52:973.

61. Yun S, Lee Y, Lee H, Hwang JG, Jung AR, Lee DT. Effects of cold water intake during a short exercise in heat on cognitive function. *Med Sci Sports Exerc.* (2020) 52:964.

62. Ciuha U, Gronkvist M, Mekjavic IB, Eiken O. Strategies for increasing evaporative cooling during simulated desert patrol mission. *Ergonomics.* (2016) 59:298–309. doi: 10.1080/00140139.2015.1061142

63. Walker A, Driller M, Brearley M, Argus C, Rattray B. Cold-water immersion and iced-slush ingestion are effective at cooling firefighters following a simulated search and rescue task in a hot environment. *Appl Physiol Nutr Metab.* (2014) 39:1159–66. doi: 10.1139/apnm-2014-0038

64. Hurrie DMG, Talebian Nia M, Power K, Stecina K, Gardiner P, Lockyer E, et al. Spinal and corticospinal excitability in response to reductions in skin and core temperatures via whole-body cooling. *Appl Physiol Nutr Metab.* (2022) 47:195–205. doi: 10.1139/apnm-2021-0370

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

66. Brade C, Dawson B, Wallman K. Effect of precooling and acclimation on repeat-sprint performance in heat. *J Sports Sci.* (2013) 31:779–86.

67. Brade CJ, Dawson BT, Wallman KE. Effect of pre-cooling on repeat-sprint performance in seasonally acclimatised males during an outdoor simulated team-sport protocol in warm conditions. *J Sports Sci Med.* (2013) 12:565–70.

68. Clarke ND, Maclaren DP, Reilly T, Drust B. Carbohydrate ingestion and pre-cooling improves exercise capacity following soccer-specific intermittent exercise performed in the heat. *Eur J Appl Physiol.* (2011) 111:1447–55. doi: 10.1007/s00421-010-1771-5

69. Duffield R, Coutts A, McCall A, Burgess D. Pre-cooling for football training and competition in hot and humid conditions. *Eur J Sport Sci.* (2013) 13:58–67.

70. Emami A. The impact of pre-cooling and CoQ(10) supplementation on mediators of inflammatory cytokines in elite swimmers. *Nutr Cancer Int J.* (2020) 72:41–51. doi: 10.1080/01635581.2019.1614200

71. Garcia-Concepcion MA, Peinado AB, Hernandez P, Alvero-Cruz JR. Efficacy of different recovery strategies in elite football players. *Rev Int Med Cienc Act Fis Deporte.* (2015) 15:355–70. doi: 10.1123/ijspp.2022-0038

72. Hasegawa H, Takatori T, Komura T, Yamasaki M. Combined effects of pre-cooling and water ingestion on thermoregulation and physical capacity during exercise in a hot environment. *J Sports Sci.* (2006) 24:3–9. doi: 10.1080/02640410400022185

73. Lynch GP, Periard JD, Pluim BM, Brotherhood JR, Jay O. Optimal cooling strategies for players in Australian Tennis Open conditions. *J Sci Med Sport.* (2018) 21:232–7. doi: 10.1016/j.jsams.2017.05.017

74. Maia-Lima A, Ramos G, Moraes M, Pacheco DS, De Oliveira GA, De Barros CM, et al. Effects of precooling on 30-km cycling performance and pacing in hot and temperate environments. *Int J Sports Med.* (2017) 38:48–54. doi: 10.1055/s-0042-113465

75. Maley MJ, Minett GM, Bach AJE, Stewart KL, Stewart IB. Extending work tolerance time in the heat in protective ensembles with pre- and per-cooling methods. *Appl Ergonom.* (2020) 85:103064. doi: 10.1016/j.apergo.2020.103064

76. Minett GM, Duffield R, Kellett A, Portus M. Mixed-method pre-cooling reduces physiological demand without improving performance of medium-fast bowling in the heat. *J Sports Sci.* (2012) 30:907–15. doi: 10.1080/02640414.2012.679677

77. Minett GM, Duffield R, Kellett A, Portus M. Effects of mixed-method cooling on recovery of medium-fast bowling performance in hot conditions on consecutive days. *J Sports Sci.* (2012) 30:1387–96. doi: 10.1080/02640414.2012.709267

78. Minett GM, Duffield R, Marino FE, Portus M. Duration-dependant response of mixed-method pre-cooling for intermittent-sprint exercise in the heat. *Eur J Appl Physiol.* (2012) 112:3655–66. doi: 10.1007/s00421-012-2348-2

79. Munoz CX, Judelson DA, Coburn JW, Becker AJ, Carney KR, Schick MK, et al. Effects of drinking and external cooling on low intensity exercise and performance in hot-dry environments. *Med Sci Sports Exerc.* (2011) 43:126–7. doi: 10.1111/j.1600-0838.2012.01510.x

80. Pournemati P, Azarbayjani MA, Rezaee MB, Ziaee V, Pournemati P. The effect of inhaling peppermint odor and ethanol in women athletes. *Bratisl Lek Listy.* (2009) 110:782–7.

81. Ross ML, Garvican LA, Jeacocke NA, Laursen PB, Abbiss CR, Martin DT, et al. Novel precooling strategy enhances time trial cycling in the heat. *Med Sci Sports Exerc.* (2011) 43:123–33. doi: 10.1249/MSS.0b013e3181e93210

82. Ross MLR, Jeacocke NA, Laursen PB, Martin DT, Abbiss CR, Burke LM. Effects of lowering body temperature via hyperhydration, with and without glycerol ingestion and practical precooling on cycling time trial performance in hot and humid conditions. *J Int Soc Sports Nutr.* (2012) 9:55. doi: 10.1186/1550-2783-9-55

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

84. Emami A, Tofighi A, Asri-Rezaei S, Bazargani-Gilani B. The effect of short-term coenzyme Q10 supplementation and pre-cooling strategy on cardiac damage markers in elite swimmers. *Br J Nutr.* (2018) 119:381–90. doi: 10.1017/S0007114517003774

85. Keller S, Kohne S, Bloch W, Schumann M. Comparison of two different cooling systems in alleviating thermal and physiological strain during prolonged exercise in the heat. *Ergonomics.* (2021) 64:129–38. doi: 10.1080/00140139.2020.1818835

86. Moss JN, Trangmar SJ, Mackenzie RWA, Tyler CJ. The effects of pre- and per-cooling interventions used in isolation and combination on subsequent 15-minute time-trial cycling performance in the heat. *J Sci Med Sport.* (2021) 24:800–5. doi: 10.1016/j.jsams.2021.04.006

87. Mazalan NS, Landers GJ, Wallman KE, Ecker UA. Combination of ice ingestion and head cooling enhances cognitive performance during endurance exercise in the heat. *J Sports Sci Med.* (2022) 21:23–32. doi: 10.52082/jssm.2022.23

88. Huang JH, Lin CI, Liao CY, Hsieh MH, Lin HS, Chang CK. Intermittent cooling reduces perceived exertion but has no effect on baseball hitting or defense performance in a hot environment. *J Sports Sci Med.* (2022) 21:137–44. doi: 10.52082/jssm.2022.137

89. Naito T, Nakamura M, Muraishi K, Eda N, Ando K, Takemura A, et al. In-play optimal cooling for outdoor match-play tennis in the heat. *Eur J Sport Sci.* (2022) 22:326–35. doi: 10.1080/17461391.2020.1870160

90. Wiewelhove T, Conradt F, Rawlins S, Deacon J, Meyer T, Kellmann M, et al. Effects of in-play cooling during simulated tennis match play in the heat on performance, physiological and perceptual measures. *J Sports Med Phys Fitness.* (2021) 61:372–9.

91. James CA, Richardson AJ, Watt PW, Willmott AGB, Gibson OR, Maxwell NS. Short-term heat acclimation and precooling, independently and combined, improve 5-km time trial performance in the heat. *J Strength Cond Res.* (2018) 32:1366–75. doi: 10.1519/JSC.0000000000001979

92. Hornery DJ, Farrow D, Mujika I, Young WB. Caffeine, carbohydrate, and cooling use during prolonged simulated tennis. *Int J Sports Physiol Perform.* (2007) 2:423–38. doi: 10.1123/ijspp.2.4.423

93. Naito T, Haramura M, Muraishi K, Yamazaki M, Takahashi H. Impact of ice slurry ingestion during break-times on repeated-sprint exercise in the heat. *Sports Med Int Open.* (2020) 4:E45–52. doi: 10.1055/a-1139-1761

94. Byrne C, Owen C, Cosnefroy A, Lee JKW. Self-paced exercise performance in the heat after pre-exercise cold-fluid ingestion. *J Athl Train.* (2011) 46:592–9. doi: 10.4085/1062-6050-46.6.592

95. Burdon C, O'Connor H, Gifford J, Shirreffs S, Chapman P, Johnson N. Effect of drink temperature on core temperature and endurance cycling performance in warm, humid conditions. *J Sports Sci.* (2010) 28:1147–56. doi: 10.1080/02640414.2010.489197

96. Ihsan M, Landers G, Brearley M, Peeling P. Beneficial effects of ice ingestion as a precooling strategy on 40-km cycling time-trial performance. *Int J Sports Physiol Perform.* (2010) 5:140–51. doi: 10.1123/ijspp.5.2.140

97. LaFata D, Carlson-Phillips A, Sims ST, Russell EM. The effect of a cold beverage during an exercise session combining both strength and energy systems development training on core temperature and markers of performance. *J Int Soc Sports Nutr.* (2012) 9:44. doi: 10.1186/1550-2783-9-44

98. Zimmermann M, Landers GJ, Waltman KE. Crushed ice ingestion does not improve female cycling time trial performance in the heat. *Int J Sport Nutr Exerc Metab.* (2017) 27:67–75.

99. Zimmermann MR, Landers GJ. The effect of ice ingestion on female athletes performing intermittent exercise in hot conditions. *Eur J Sport Sci.* (2015) 15:407–13. doi: 10.1080/17461391.2014.965751

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

101. Parton AJ, Waldron M, Clifford T, Jeffries O. Thermo-behavioural responses to orally applied l-menthol exhibit sex-specific differences during exercise in a hot environment. *Physiol Behav.* (2021) 229:113250. doi: 10.1016/j.physbeh.2020.113250

102. Hue O, Monjo R, Lazzaro M, Baillot M, Hellard P, Marlin L, et al. The effect of time of day on cold water ingestion by high-level swimmers in a tropical climate. *Int J Sports Physiol Perform.* (2013) 8:442–51. doi: 10.1123/ijspp.8.4.442

103. Gibson OR, Wrightson JG, Hayes M. Intermittent sprint performance in the heat is not altered by augmenting thermal perception via L-menthol or capsaicin mouth rinses. *Eur J Appl Physiol.* (2019) 119:653–64.

104. Yeo ZW, Fan PW, Nio AQ, Byrne C, Lee JK. Ice slurry on outdoor running performance in heat. *Int J Sports Med.* (2012) 33:859–66.

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

106. Best R, Temm D, Hucker H, McDonald K. Repeated menthol mouth swilling affects neither strength nor power performance. *Sports.* (2020) 8:90. doi: 10.3390/sports8060090

107. Lee JKW, Shirreffs SM, Maughan RJ. Cold drink ingestion improves exercise endurance capacity in the heat. *Med Sci Sports Exerc.* (2008) 40:1637–44.

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

109. De Carvalho MV, Deandrade MT, Ramos GP, Maia-Lima A, Pereira ER, Mendes TT, et al. The temperature of water ingested ad libitum does not influence performance during a 40-km self-paced cycling trial in the heat. *J Sports Med Phys Fitness.* (2015) 55:1473–9.

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

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

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

113. Siegel R, Maté J, Watson G, Nosaka K, Laursen PB. The influence of ice slurry ingestion on maximal voluntary contraction following exercise-induced hyperthermia. *Eur J Appl Physiol.* (2011) 111:2517–24. doi: 10.1007/s00421-011-1876-5

114. Siegel R, Mate J, Watson G, Nosaka K, Laursen PB. Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *J Sports Sci.* (2012) 30:155–65. doi: 10.1080/02640414.2011.625968

115. Zimmermann M, Landers G, Wallman KE, Saldaris J. The effects of crushed ice ingestion prior to steady state exercise in the heat. *Int J Sport Nutr Exerc Metab.* (2017) 27:220–7.

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

117. Podlogar,T, Bolčič T, Cirnski S, Verdel N, Debevec T. Commercially available carbohydrate drink with menthol fails to improve thermal perception or cycling exercise capacity in males. *Eur J Sport Sci.* (2021) 21:1–9.

118. Lee JK, Shirreffs SM. The influence of drink temperature on thermoregulatory responses during prolonged exercise in a moderate environment. *J Sports Sci.* (2007) 25:975–85. doi: 10.1080/02640410600959947

119. Aldous JWF, Chrismas BCR, Akubat I, Stringer CA, Abt G, Taylor L. Mixed-methods pre-match cooling improves simulated soccer performance in the heat. *Eur J Sport Sci.* (2019) 19:156–65. doi: 10.1080/17461391.2018.1498542

120. Barwood MJ, Goodall S, Bateman J. The effect of hot and cold drinks on thermoregulation, perception, and performance: the role of the gut in thermoreception. *Eur J Appl Physiol.* (2018) 118:2643–54. doi: 10.1007/s00421-018-3987-8

121. Choo HC, Peiffer JJ, Lopes-Silva JP, Mesquita RNO, Amano T, Kondo N, et al. Effect of ice slushy ingestion and cold water immersion on thermoregulatory behavior. *PLoS One.* (2019) 14:e0212966. doi: 10.1371/journal.pone.0212966

122. Gerrett N, Jackson S, Yates J, Thomas G. Ice slurry ingestion does not enhance self-paced intermittent exercise in the heat. *Scand J Med Sci Sports.* (2017) 27:1202–12.

123. Riera F, Trong TT, Rinaldi K, Hue O. Precooling does not enhance the effect on performance of midcooling with ice-slush/menthol. *Int J Sports Med.* (2016) 37:1025–31.

124. Saldaris JM, Landers GJ, Lay BS. The effect of crushed ice ingestion on endurance performance and decision-making in hot and humid conditions. *Int J Perform Anal Sport.* (2019) 19:393–401.

125. Stanley J, Leveritt M, Peake JM. Thermoregulatory responses to ice-slush beverage ingestion and exercise in the heat. *Eur J Appl Physiol.* (2010) 110:1163–73. doi: 10.1007/s00421-010-1607-3

126. Stevens CJ, Dascombe B, Boyko A, Sculley D, Callister R. Ice slurry ingestion during cycling improves Olympic distance triathlon performance in the heat. *J Sports Sci.* (2013) 31:1271–9. doi: 10.1080/02640414.2013.779740

127. Thomas G, Cullen T, Davies M, Hetherton C, Duncan B, Gerrett N. Independent or simultaneous lowering of core and skin temperature has no impact on self-paced intermittent running performancein hot conditions. *Eur J Appl Physiol.* (2019) 119:1841–53.

128. Saldaris JM, Landers GJ, Lay BS. Physical and perceptual cooling: improving cognitive function, mood disturbance and time to fatigue in the heat. *Scand J Med Sci Sports.* (2020) 30:801–11. doi: 10.1111/sms.13623

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

130. Tran Trong T, Riera F, Rinaldi K, Briki W, Hue O. Ingestion of a cold temperature/menthol beverage increases outdoor exercise performance in a hot, humid environment. *PLoS One.* (2015) 10:e0123815. doi: 10.1371/journal.pone.0123815

131. Xu M, Wu Z, Dong Y, Qu C, Xu Y, Qin F, et al. A mixed-method approach of pre-cooling enhances high-intensity running performance in the heat. *J Sports Sci Med.* (2021) 20:26–34. doi: 10.52082/jssm.2021.26

132. Brade C, Dawson B, Wallman K. Effects of different precooling techniques on repeat sprint ability in team sport athletes. *Eur J Sport Sci.* (2014) 14:S84–91. doi: 10.1080/17461391.2011.651491

133. Burdon CA, Hoon MW, Johnson NA, Chapman PG, O'Connor HT. The effect of ice slushy ingestion and mouthwash on thermoregulation and endurance performance in the heat. *Int J Sport Nutr Exerc Metab.* (2013) 23:458–69. doi: 10.1123/ijsnem.23.5.458

134. Beaven CM, Kilduff LP, Cook CJ. Lower-Limb passive heat maintenance combined with pre-cooling improves repeated sprint ability. *Front Physiol.* (2018) 9:1064. doi: 10.3389/fphys.2018.01064

135. Gibson OR, James CA, Mee JA, Willmott AGB, Turner G, Hayes M, et al. Heat alleviation strategies for athletic performance: a review and practitioner guidelines. *Temperature.* (2019) 7:3–36. doi: 10.1080/23328940.2019.1666624

136. University of Connecticut. *Wet Bulb Globe Temperature Monitoring.* (2017). Available online at: https://ksi.uconn.edu/prevention/wet-bulb-globe-temperature-monitoring/# (accessed May 16, 2022).

137. Armstrong LE, Casa DJ, Millard-Stafford M, Moran DS, Pyne SW, Roberts WO. American college of sports medicine position stand. Exertional heat illness during training and competition. *Med Sci Sports Exerc.* (2007) 39:556–72. doi: 10.1249/MSS.0b013e31802fa199

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

139. Lee JKW, Maughan RJ, Shirreffs SM. The influence of serial feeding of drinks at different temperatures on thermoregulatory responses during cycling. *J Sports Sci.* (2008) 26:583–90. doi: 10.1080/02640410701697388

140. Eccles R. Menthol and related cooling compounds. *J Pharm Pharmacol.* (1994) 46:618–30.

141. Stevens CJ, Mauger AR, Hassmèn P, Taylor L. Endurance performance is influenced by perceptions of pain and temperature: theory, applications and safety considerations. *Sports Med.* (2018) 48:525–37.

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

143. Bharate SS, Bharate SB. Modulation of thermoreceptor TRPM8 by cooling compounds. *ACS Chem Neurosci.* (2012) 3:248–67.

144. Bridge MW, Weller AS, Rayson M, Jones DA. Responses to exercise in the heat related to measures of hypothalamic serotonergic and dopaminergic function. *Eur J Appl Physiol.* (2003) 89:451–9. doi: 10.1007/s00421-003-0800-z

145. Brisson GR, Péronnet F, Perrault H, Boisvert P, Massicotte D, Gareau R. Prolactinotrophic effect of endogenous and exogenous heat loads in human male adults. *J Appl Physiol.* (1991) 70:1351–5. doi: 10.1152/jappl.1991.70.3.1351

146. Mündel T, Bunn SJ, Hooper PL, Jones DA. The effects of face cooling during hyperthermic exercise in man: evidence for an integrated thermal, neuroendocrine and behavioural response. *Exp Physiol.* (2007) 92:187–95. doi: 10.1113/expphysiol.2006.034934

147. Hayes M, Smith D, Castle P, Watt PW, Ross EZ, Maxwell NS, et al. Peak power output provides the most reliable measure of performance in prolonged intermittent-sprint cycling. *J Sports Sci.* (2012) 31:565–72. doi: 10.1080/02640414.2012.744077

148. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc.* (2004) 36:2107–11.

149. Leijon SCM, Neves AF, Breza JM, Simon SA, Chaudhari N, Roper SD. Oral thermosensing by murine trigeminal neurons: modulation by capsaicin, menthol and mustard oil. *J Physiol.* (2019) 597:2045–61. doi: 10.1113/JP277385

150. Keringer P, Farkas N, Gede N, Hegyi P, Rumbus Z, Lohinai Z, et al. Menthol can be safely applied to improve thermal perception during physical exercise: a meta-analysis of randomized controlled trials. *Sci Rep.* (2020) 10:13636. doi: 10.1038/s41598-020-70499-9

151. Mora-Rodriguez R, Del Coso J, Estevez E. Thermoregulatory responses to constant versus variable-intensity exercise in the heat. *Med Sci Sports Exerc.* (2008) 40:1945–52. doi: 10.1249/MSS.0b013e31817f9843

152. Ekblom B, Greenleaf CJ, Greenleaf JE, Hermansen L. Temperature regulation during continuous and intermittent exercise in man. *Acta Physiol Scand.* (1971) 81:1–10.

153. Kraning KK II, Gonzalez RR. Physiological consequences of intermittent exercise during compensable and uncompensable heat stress. *J Appl Physiol.* (1991) 71:2138–45. doi: 10.1152/jappl.1991.71.6.2138

154. Rivera-Brown AM, Ramirez-Marrero FA, Frontanés J, Rosario RA, Hernández OJ, Frontera WR. Sweating and core temperature in athletes training in continuous and intermittent sports in tropical climate. *Arch Med Deporte.* (2019) 36:86–91.

155. Onitsuka S, Nakamura D, Onishi T, Arimitsu T, Takahashi H, Hasegawa H. Ice slurry ingestion reduces human brain temperature measured using non-invasive magnetic resonance spectroscopy. *Sci Rep.* (2018) 8:2757. doi: 10.1038/s41598-018-21086-6

156. Merrick MA, Jutte LS, Smith ME. Cold modalities with different thermodynamic properties produce different surface and intramuscular temperatures. *J Athl Train.* (2003) 38:28–33.

157. Vanden Hoek TL, Kasza KE, Beiser DG, Abella BS, Franklin JE, Oras JJ, et al. Induced hypothermia by central venous infusion: saline ice slurry versus chilled saline. *Crit Care Med.* (2004) 32(Suppl. 9):S425–31. doi: 10.1097/01.ccm.0000134259.59793.b8

158. Guest S, Grabenhorst F, Essick G, Chen Y, Young M, McGlone F, et al. Human cortical representation of oral temperature. *Physiol Behav.* (2007) 92:975–84.

159. Villanova N, Azpiroz F, Malagelada JR. Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. *J Physiol.* (1997) 502(Pt. 1):215–22. doi: 10.1111/j.1469-7793.1997.215bl.x

160. Pallett JP. *Textbook of Neurological Nursing*. Toronto, ON: Little, Brown & Co. Ltd (1985).

161. Jones PR, Barton C, Morrissey D, Maffulli N, Hemmings S. Pre-cooling for endurance exercise performance in the heat: a systematic review. *BMC Med.* (2012) 10:166. doi: 10.1186/1741-7015-10-166

162. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American college of sports medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* (2007) 39:377–90.

163. Montain SJ, Latzka WA, Sawka MN. Control of thermoregulatory sweating is altered by hydration level and exercise intensity. *J Appl Physiol.* (1995) 79:1434–9. doi: 10.1152/jappl.1995.79.5.1434

164. González-Alonso J, Mora-Rodríguez R, Below PR, Coyle EF. Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol.* (1995) 79:1487–96.

165. Périard JD, Racinais S. Self-paced exercise in hot and cool conditions is associated with the maintenance of %V̇O2peak within a narrow range. *J Appl Physiol.* (2015) 118:1258–65. doi: 10.1152/japplphysiol.00084.2015

166. Nielsen B. Thermoregulatory responses to arm work, leg work and intermittent leg work. *Acta Physiol Scand.* (1968) 72:25–32. doi: 10.1111/j.1748-1716.1968.tb03822.x

167. Jeukendrup AE, Jentjens RL, Moseley L. Nutritional considerations in triathlon. *Sports Med.* (2005) 35:163–81.

168. Douzi W, Dupuy O, Theurot D, Smolander J, Dugué B. Per-cooling (using cooling systems during physical exercise) enhances physical and cognitive performances in hot environments. a narrative review. *Int J Environ Res Public Health.* (2020) 17:1031. doi: 10.3390/ijerph17031031

169. Pryor RR, Suyama J, Guyette FX, Reis SE, Hostler D. The effects of ice slurry ingestion before exertion in Wildland firefighting gear. *Prehosp Emerg Care.* (2015) 19:241–6. doi: 10.3109/10903127.2014.959221

170. Nielsen B, Davies CT. Temperature regulation during exercise in water and air. *Acta Physiol Scand.* (1976) 98:500–8.

171. Jay O, Gariepy LM, Reardon FD, Webb P, Ducharme MB, Ramsay T, et al. A three-compartment thermometry model for the improved estimation of changes in body heat content. *Am J Physiol Regul Integr Comp Physiol.* (2007) 292:R167–75. doi: 10.1152/ajpregu.00338.2006

172. Sedgwick P. Bias in randomised controlled trials: comparison of crossover group and parallel group designs. *BMJ.* (2015) 351:h4283.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 02 November 2022
DOI 10.3389/fnut.2022.1016310

OPEN ACCESS

EDITED BY
Scott Forbes,
Brandon University, Canada

REVIEWED BY
Paul A. Roberson,
University of Colorado Anschutz Medical Campus, United States
Jeremy R. Townsend,
Lipscomb University, United States

*CORRESPONDENCE
Abbie E. Smith-Ryan
abbsmith@email.unc.edu

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 11 August 2022
ACCEPTED 29 September 2022
PUBLISHED 02 November 2022

CITATION
Cabre HE, Gordon AN, Patterson ND and Smith-Ryan AE (2022) Evaluation of pre-workout and recovery formulations on body composition and performance after a 6-week high-intensity training program. *Front. Nutr.* 9:1016310. doi: 10.3389/fnut.2022.1016310

Evaluation of pre-workout and recovery formulations on body composition and performance after a 6-week high-intensity training program

Hannah E. Cabre[1,2], Amanda N. Gordon[1], Noah D. Patterson[1] and Abbie E. Smith-Ryan[1,2,3]*

[1]Applied Physiology Laboratory, Department of Exercise and Sport Science, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Human Movement Science Curriculum, Department of Health Science-UNC School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [3]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Introduction: Activities such as high-intensity resistance training (HIRT) and high-intensity interval training (HIIT) may be more time-efficient modes to stimulate rapid changes in performance and body composition. There is little research evaluating the combined effects of HIRT and HIIT on body composition and strength, particularly when paired with nutritional supplementation.

Purpose: To evaluate the chronic effects of pre- and post-workout supplementation on body composition and strength, and to understand sex-specific responses.

Materials and methods: 64 untrained males (n = 23) and females (n = 41) (mean ± standard deviation; age: 33.2 ± 10.0 years; %fat: 31.6 ± 7.4%) were randomized to either (1) pre-post supplementation [SUP (n = 25); pre = multi-ingredient caffeine/HMB/vit D; post = whey protein/carbohydrates/glucosamine/vitamins], (2) placebo [PL (n = 24); non-caloric], or (3) control [CON (n = 15)]. All participants completed one repetition max (1RM) strength testing for leg press and bench press at baseline and week 6. Estimates of fat mass (FM) and lean mass (LM) were measured *via* dual energy x-ray absorptiometry. Participants in the SUP or PL group completed a 6-week supervised exercise intervention consisting of a full-body HIRT workout (3 × 6–8 reps) followed by a HIIT treadmill run (6 × 1 min run: 1 min rest) twice per week. Outcomes were evaluated by separate repeated measure ANOVAs (2 × 3).

Results: There were no differences in FM between groups or sex (p = 0.133–0.851). LM increased from baseline to post-testing for all groups [Mean difference [MD(Post-Pre) ± Standard Error (SE) = 0.78 ± 0.12 kg; p < 0.001]. While not significant (p = 0.081), SUP gained more LM

compared to PL [MD(SUP-PL) ± SE = 3.5 ± 3.3 kg] and CON [MD(SUP-CON) ± SE = 5.2 ± 3.8 kg]. LM increased over time for both males (0.84 ± 0.24 kg; p = 0.003) and females (0.73 ± 0.14 kg; $p < 0.001$). The SUP group resulted in a significant increase in 1RM leg press compared to the CON group (89.9 ± 30.8 kg; p = 0.015), with no significant differences compared to PL (p = 0.409). The SUP group had greater increases in 1RM bench press compared to the CON group (9.8 ± 1.8 kg; $p < 0.001$), with no significant differences compared to PL (p = 0.99). Both sexes increased upper- (5.5 ± 0.7 kg; $p < 0.001$) and lower-body strength (69.8 ± 4.5 kg $p < 0.001$) with training.

Conclusion: Nutrient supplementation timing appears to augment body composition changes and strength compared to control. Pre-/post-nutrient timing may support greater increases in LM and lower- and upper-body strength in both men and women.

Clinical trial registration: [https://clinicaltrials.gov/ct2/show/NCT04230824?cond=NCT04230824&draw=2&rank=1], identifier [NCT04230824].

KEYWORDS

interval exercise, dietary supplement, resistance training, untrained, sex differences, protein, ergogenic aid

Introduction

In light of the COVID-19 pandemic, working adults reported having less time due to greater work and home-life demands, highlighting that the current physical activity guidelines may be unattainable (1). As such, 80% of American adults do not meet the recommended physical activity guidelines, with lack of motivation and time being the most commonly cited barriers (2, 3), further emphasizing the importance of identifying practical and feasible exercise and nutrition strategies. Activities such as high-intensity resistance training (HIRT) and high-intensity interval training (HIIT) have emerged as more time-efficient modes to stimulate rapid changes in cardiometabolic health (4). These exercise strategies may improve engagement, while eliciting similar improvements in body composition compared to traditional exercises (5, 6), particularly when paired with nutritional support (5, 7, 8).

Broadly, HIRT requires participants to lift a heavy load with short recovery between sets reducing the total training time (9, 10). Compared to traditional resistance training, HIRT has been shown to significantly increase resting energy expenditure after exercise, and may improve fat oxidation (9). These adaptations may be beneficial for strength and body composition, particularly increases lean mass (LM) and decreases in fat mass (FM) (9, 10). Furthermore, HIRT mimics aerobic HIIT training, alternating repeated bouts of exercise at near maximal intensity (∼90%) interspersed with periods of rest or low intensity exercise (11). Prior research on HIIT training has largely focused on the rapid aerobic and metabolic adaptations that occur, yet more recent research has demonstrated advantageous improvements in LM and muscle size in as little as three weeks (6). While concurrent aerobic exercise and resistance training typically result in decreased hypertrophy and strength (12), HIIT may be an effective aerobic method for maintaining strength and LM (13). However, there is little research evaluating the combined effects of HIRT and HIIT on body composition and strength, or the additive effects with planned nutritional supplementation around exercise (14).

Nutrient timing may augment adaptations from HIRT and HIIT by enhancing energy availability and the adaptive responses to exercise (14). While there is conflicting information surrounding the impact of nutritional composition and timing on exercise (7, 15–18), data collectively supports nutrient consumption surrounding exercise augments exercise adaptations compared to withholding nutrients (17). Specifically, existing data support a potential synergistic effect of nutrient timing and HIRT + HIIT, respectively. When protein consumption prior to or post-HIRT was compared to no nutrient consumption in women, both groups consuming protein demonstrated greater increases in LM and strength (7). An investigation evaluating a multi-ingredient pre-workout supplement (caffeine, creatine, and amino acids) consumed prior to HIIT resulted in significant improvements in LM and anaerobic capacity (19). Taken together, nutrients paired

with high-intensity exercise may act synergistically to promote greater changes in body composition and strength. However, the timing of nutrients is variable in the literature with most studies focusing on either pre- or post-nutrient consumption separately rather than a combinatory approach (14).

Other nutritional ingredients have gained popularity such as β-hydroxy-β-methyl butyrate (HMB), vitamin D, and fish oil due to their positive effect on tissue repair (20) and inflammation (21, 22), possibly leading to improved recovery from intense exercise and maintenance of LM. However, whether these results translate to concurrent HIRT + HIIT training is unclear. Furthermore, sex-based differences exist in muscle and mitochondrial biogenesis in response to interval training (23). These differences are important considerations when evaluating body composition and strength outcomes, but investigations on the sex effects of exercise + nutrition is nearly nonexistent. Therefore, the purpose of this study aimed to evaluate the chronic effect of pre-and post-workout supplementation combined with a concurrent HIRT + HIIT exercise intervention, compared to placebo and control, on body composition, performance (VO_2max, 1RM strength, counter movement jump), and recovery (creatine kinase, isoprostanes) in inactive males and females; an exploratory aim was to investigate sex differences in body composition and performance. It was hypothesized that the nutrient timing would lead to greater improvements in body composition, performance, and recovery compared to placebo, and control.

Materials and methods

Subjects

Sixty-four healthy, untrained males (n = 23) and females (n =41) (mean ± standard deviation (SD); age: 33.2 ± 10.0 years, height: 169.8 ± 10.2 cm, weight: 73.6 ± 15.5 kg, BMI: 25.2 ± 3.7 kg/m^2) (**Table 1**) were recruited to participate in this study. Full CONSORT information is reported in **Figure 1**. All participants were healthy, non-smokers, between the ages of 18–52 years, with a BMI between 18.5 and 35 kg·m^{-2}, and did not participate in more than 3 h per week of exercise, resistance training, and/or interval training. Participants were not consistently consuming any prescription medications for blood pressure or supplements that would influence study outcomes such as beta-alanine, creatine, beta-hydroxy-beta-methylbutyrate, carnosine, vitamin D (>1,000 IU/day), protein powder, or fish oil (>1,000 mg/day). Participants were excluded if they had a weight gain or loss of 3.6 kg within the 30 days prior to enrollment, were pregnant or planned to become pregnant as confirmed by urine HCG, or were sensitive/allergic to any of the ingredients in the test product (**Table 2**). A health history questionnaire was used to confirm inclusion/exclusion criteria. All methodology was approved by the University's Institutional Review Board, and all participants provided verbal and written informed consent prior to participation.

Experimental design

This study was a randomized, double-blind, placebo-controlled trial. Participants were asked to abstain from food and caloric beverages (12 h), caffeine (12 h), alcohol (24 h), and physical activity (24 h) prior to baseline and post-testing sessions. At baseline, participants completed a maximal graded exercise test on a treadmill to volitional exhaustion, and a maximal strength protocol to determine the appropriate intensities for the exercise training. Body composition measures, blood markers of muscle damage/recovery, and countermovement jumps were also evaluated. Participants were then randomized in a 2:2:1 fashion to a (1) active ingredient supplement (SUP; exercise intervention with pre-post exercise supplementation), (2) placebo (PL; exercise intervention with non-caloric placebo provided before and after exercise; Crystal Light), or (3) control group (CON; no exercise or treatment assigned). Participants in the SUP treatment or PL group completed a 6-week supervised exercise intervention consisting of a full-body high-intensity resistance training workout followed by a high-intensity interval treadmill run twice per week. All groups participated in post-testing session identical to baseline testing within 48–120 h after the final training session, refraining from exercise at least 24 h prior (**Figure 2**). The number of participants per day for the post-testing visits are as followed: 48 h n = 10; 72 h n = 13; 96 h n = 6; 120 h n = 19.

Dietary intake

All participants were asked to complete a three-day food record (two weekdays and one weekend day) at baseline prior to training. The dietary information was entered into Food Processor (ESHA Research; Version 10) to account for nutrient intake including total calories (kcal/day), carbohydrates (g/day), protein (g/day), and fat (g/day). Participants were asked to maintain dietary habits for the duration of the study. Estimated daily caloric needs per participant were calculated using the Harris Benedict Equation with an activity factor of 1.375. The difference between estimated energy expenditure and actual dietary intake was calculated and stratified by above and below estimated total daily energy requirements, yet there were not significant differences in dietary intake.

Body composition

Participant body composition was evaluated utilizing a whole-body DXA scan (GE Lunar iDXA, GE Medical Systems

Ultrasound and Primary Care Diagnostics, Madison, WI, USA). Prior to each use, the device was calibrated according to the manufacturer's guidelines. Each participant's sex, birthdate, height, weight, and ethnicity were entered into the software (enCORE Software Version 16) prior to the scan. Participants wore loose athletic clothing and removed all metal and heavy plastic to reduce scan interference. Each participant was positioned supine in the center of the scanning table by a trained technician. Regions of interest were manually adjusted by the technician to determine lean mass (LM), fat mass (FM), and body fat percentage (%BF). DXA test-retest reliability from this laboratory for individuals of similar stature included intraclass correlation coefficient (ICC) = 0.99 and standard error of the measurement (SEM) = 1.07 kg for LM, ICC = 0.98 and SEM = 0.85 kg for FM, and ICC = 0.96 and SEM = 1.279% for %BF.

Maximal oxygen consumption (VO_2max)

To determine peak oxygen consumption (VO_2max) to establish exercise intensity, all participants completed a graded exercise test to volitional exhaustion on a treadmill (Woodway Treadmill Woodway USA, Inc., Waukesha, WI). For males, following a 3-min warm up at 5.6 km/h, intensity was increased to 9.0 km/h and was then increased by 1.1 km/h every 3 min until 18.0 km/h. For females, following a 3-min warm up at 5.6 km/h, intensity was increased to 7.2 km/h and was then increased by 1.1 km/h every 3 min until 16.3 km/h. Breath-by-breath respiratory gases were analyzed with fifteen-second averages using indirect calorimetry (Parvo Medics TrueMax 2400®, Salt Lake City, UT); the three highest oxygen consumption values were averaged and recorded as VO_2max (VO_2max; $ml \cdot kg^{-1} \cdot min^{-1}$). The test was considered maximal if it met a minimum of two of the following criteria: a plateau in heart rate (HR) or was within 10% of age-predicted HRmax; a plateau in VO_2 or no more than a 150 $ml \cdot min^{-1}$ increase; a respiratory exchange ratio value greater than 1.15 a.u. Peak speed was used to establish individual exercise intensity for the interval exercise training. Previous test-retest reliability for this VO_2max protocol resulted in an ICC = 0.98 and SEM = 1.17 ml/kg/min, respectively.

Strength testing

To determine one repetition maximum (1RM; kg) for leg press and bench press, each participant performed a set of 8–10 repetitions, with a weight that is approximately 50% of the anticipated 1RM as a warmup. The load was increased to 80% of the predicted 1RM, and participants were asked to perform of 4–6 repetitions. The weight was then increased to an estimated 1RM load, and the participants attempted a single repetition with the weight. After the completion of each successful 1RM attempt, the weight was increased until failure was reached, with 2–3 min of rest between each 1RM attempts. Leg press 1RM was determined first followed by 1RM for bench press. The leg press and bench press 1RM were used to estimate 75% to 85% of maximum load for the resistance training bouts. A systematic review on test-retest reliability for 1RM tests demonstrated an ICC = 0.97 and median coefficient of variation (CV) = 4.2% (24).

Multiple RM tests, specifically a 6RM, was used to predict participants' 1RM on four different accessory exercises. These exercises included an overhead shoulder press, a bicep curl, an overhead tricep extension, and an alternating stationary lunge,

TABLE 1 Participant characteristics presented as mean ± standard deviation.

Variable	Supplement group (n = 25; M = 11; F = 16)	Placebo group (n = 24; M = 10; F = 16)	Control group (n = 15; M = 5; F = 10)
Age (years)	31.3 ± 9.7	35.6 ± 10.2	32.9 ± 10.1
Height (cm)	171.7 ± 9.3	169.0 ± 11.0	167.8 ± 10.2
Weight (kg)	77.2 ± 16.2	71.3 ± 12.3	71.4 ± 18.7
BMI (kg/m^2)	26.1 ± 4.4	24.8 ± 2.2	24.4 ± 4.3
Average total calories (kcal/day)	1889.1 ± 558.6	2079.3 ± 437.6	2012.0 ± 498.3
Average protein (g/day)	85.0 ± 26.1	87.8 ± 25.1	95.2 ± 29.7
Average carbohydrate (g/day)	209.1 ± 97.5	228.1 ± 50.9	208.5 ± 78.3
Average fat (g/day)	75.3 ± 30.8	86.8 ± 22.1	82.0 ± 27.5
Relative protein intake (g/kg/day)	1.1 ± 0.3	1.3 ± 0.3	1.4 ± 0.4
Relative carbohydrate intake (g/kg/day)	2.8 ± 0.9	3.3 ± 0.8	3.2 ± 1.4
Relative fat intake (g/kg/day)	1.0 ± 0.4	1.24 ± 0.3	1.18 ± 0.4
Average HIRT training volume (kg)	67303.1 ± 29428.9	60483.4 ± 25350.0	–
Average HIIT training volume (km)	12.0 ± 3.2	12.4 ± 3.0	–

M, male; F, female; BMI, body mass index; kcal, calories; g, gram; kg, kilogram; HIRT, high intensity resistance training; HIIT, high intensity interval training; km, kilometers. There were no significant differences between groups for these outcomes ($p > 0.05$).

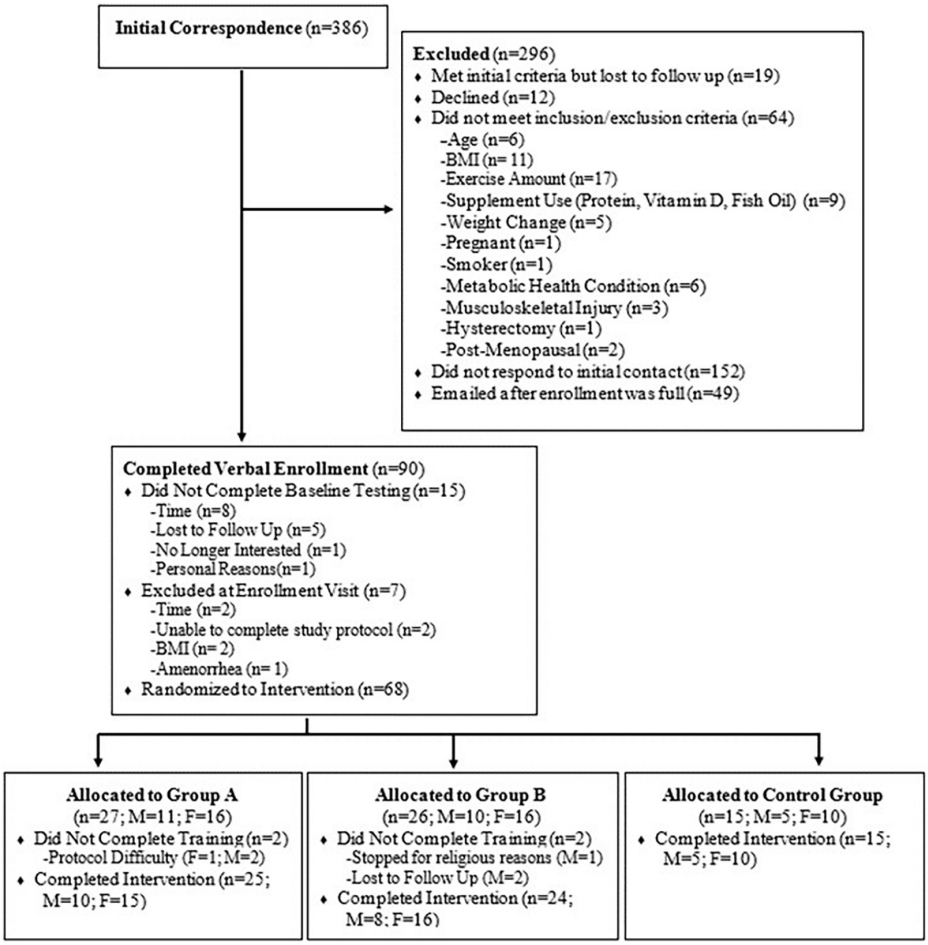


FIGURE 1
CONSORT recruitment.

all using dumbbells. The research staff determined what weight participants began with for each exercise, based on the prior training of the individual, aiming for 3–10 successful repetitions. Participants were allowed approximately 2 min of rest between each accessory exercise. The amount of weight used (rep weight) and the number of repetitions completed until fatigue (RTF) was put in the following equation to predict participants' 1RM (25):

$$1\,RM = \frac{rep\ weight}{0.522 + 0.419e^{-0.055*RTF}} \quad (1)$$

The projected 1RM value that was calculated from this equation was then used to estimate 75% to 85% of maximum load for the resistance training bouts.

Counter movement jump

Participants completed three maximal countermovement vertical jumps using a Just Jump^{TM} mat (Just Jump or Run, Probotics, Inc., Huntsville, AL, USA), each separated by 30 s of rest. Participants were positioned with feet shoulder-width apart and instructed to jump vertically, as high as possible, and return to the same position with both feet landing at the same time. Jump height (cm) was calculated automatically using the flight time from when the participant's feet left the mat until landing. The greatest jump height was determined as the CMJ. Test-retest reliability for 1RM tests demonstrated an ICC = 0.93 and Cronbach's alpha = 0.96 (26).

Blood analytes

A 12 ml venous blood sample was obtained from the antecubital region of the arm at baseline and during post-testing. The blood samples were obtained at the post-testing visit within ± 2 h of the time of day of baseline blood sample collection. Blood was sampled to determine the concentration of creatine kinase and isoprostanes. Blood samples for isoprostanes were immediately centrifuged at 3,000 rpm at 5°C for 10 min.

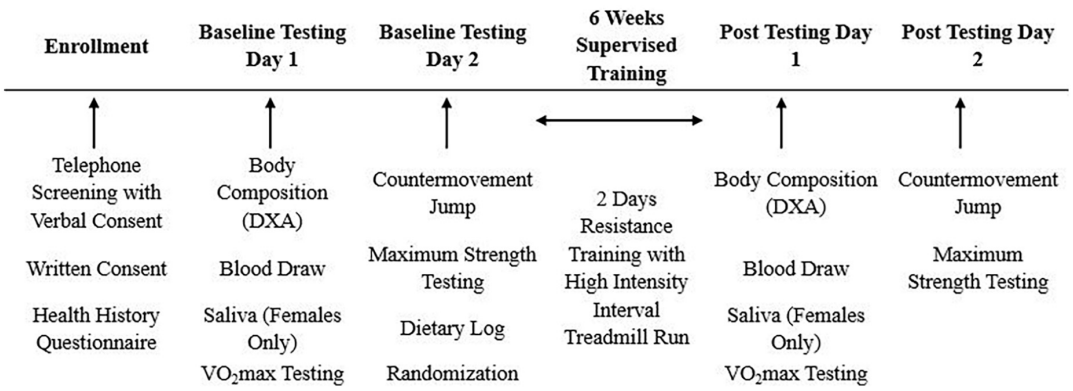


FIGURE 2
Experimental design.

Aliquots of serum for the isoprostanes were frozen at −80°C for batch analysis and were analyzed using commercially available, enzyme-linked assays (8 isoprostane ELISA Kit; ab175819; abcam, Cambridge, MA, USA). Blood samples for creatine kinase were allowed to coagulate at room temperature for 30 min and then were immediately centrifuged at 3,000 rpm at 5°C for 10 min. Samples for creatine kinase and isoprostanes were analyzed by LabCorp (Burlington, NC, USA). The average coefficient of variation in creatine kinase between duplicates samples was 3.65%, while isoprostanes was 3.54%.

Supplementation

Treatment randomization was assigned in a 2:2:1 group allocation for the SUP (Table 2 composition), PL, and CON, respectively, using Random Allocation Software (Sealed Envelope Software; Sealed Envelope Ltd., London, UK). Treatments were packaged and supplied in numerically labeled opaque containers by the Sponsor (Nu Skin, NSE Products, Inc., Provo, UT, USA) to maintain a double-blinded design. Participants were provided with their assigned treatment SUP pre-workout and post-workout, or non-caloric flavored powder blend PL (Crystal Light). Treatments were only consumed on training days (2 × per week). Participants were instructed to consume the pre-workout supplement with four- eight ounces of water 30 min prior to arriving for training visits. If participants did not consume the pre-workout supplement prior to the visit, they consumed the appropriate treatment at the laboratory and waited the 30 min before beginning the exercise training. The post-workout treatment was prepared with 4–8 ounces of water by research staff and was ingested by the participants in the laboratory within 15 min of cessation from exercise. Average group compliance (SUP n = 25; PL n = 24) was determined by dividing the total number of doses consumed by the total number of doses allotted. Compliance for the SUP group was 99.1%, and for the PL group was 98.8%.

Exercise intervention

Participants engaged in a progressive, supervised six-week high-intensity resistance training program as previously described (7, 9). Training took place two days per week in the laboratory, with at least 24 h, but not more than 10 days, separating each training visit. The initial weight for the leg press and bench press at the first training session was set at 80% of the participants' 1RM. The initial weight for the four accessory exercises was set at 75% of the participants' projected 1RM. Heart rate was continuously monitored using a Polar Heart Rate monitor and participants reported perceived

TABLE 2 Multi-ingredient active supplement descriptions.

Active pre-workout	
Ingredient	**Amount**
Caffeine	50 mg
Choline bitartrate	550 mg (226 mg choline)
Carbohydrate (palatinose)	5 g
β-hydroxy-β-methylbutyrate (HMB)	1.5 g
Vitamin D_3	500 IU
Active post-workout	
Whey protein	15 g
Caseinate protein	5 g
Carbohydrates	20 g (10 g palatinose and 10 g corn starch)
Vitamin C	200 mg
D-alpha tocopherol	45 IU
Vitamin D_3	1,000 IU
Glucosamine	1.5 g

Mg, milligrams; g, grams; IU, international units.

rate of exertion (Borg scale) after each exercise set. Resistance exercises were performed in the following order, under one-on-one supervision from laboratory staff: leg press, bench press, lunges, shoulder press, bicep curl, and triceps extension. Three sets of each exercise were completed for 6–8 repetitions, with 20–30 s rest between sets and 2:30 s rest in between each exercise. Load for each exercise was increased when participants successfully completed at least eight repetitions for each set, the weight was increased by 10% for lower body exercises and 5% for upper body exercises. Load was evaluated after each training session. Sessions were overseen and progressed by trained research staff. Following the resistance training, an interval exercise session occurred on the treadmill consisting of 5–6 bouts of 1-min of high-speed running at 90–100% peak speed during VO_2max, interspersed with a 1-min rest/walk period. If participants were unable to run on the treadmill, the interval training was completed on a cycle ergometer with the wattage determined by target heart rate. Training volume for HIRT was determined by product of sets × repetitions completed × external load used. Training volume for HIIT was determined by converting the miles per hour to kilometers per hour and multiplying speed × bout × duration (time) of bout (**Table 1**).

Statistical analysis

The Shapiro–Wilk test was used to determine if all data were normally distributed. Outliers were removed if the value was 3 standard deviations (SD) above or below the mean (n = 8 time points) (27). Baseline characteristics between groups were assessed with a one-way ANOVA. A series of 3 × 2 [group (SUP vs. PL vs. CON) × time (Baseline vs. Post)] repeated measures ANOVAs were used to evaluate group-by-time interaction effects on body composition (LM, FM, %BF), performance (VO_2max, CMJ, 1RM strength), and blood variables. Sex differences were evaluated with a 3 × 2 repeated measures ANOVA with the between subject factor as sex. Simple main effects were evaluated by performing independent or paired samples t-test to compare treatment groups at each specific time point, using Bonferroni adjustments to account for multiple comparisons. Analyses were performed using SPSS (Version 27.0; IBM, Somers, NY, USA) with statistical significance set *a priori* at $\alpha = 0.05$.

Results

Body composition

For total body mass, there was no significant group-by-time interaction (p = 0.124) or significant main effect for group (p = 0.302). There was a main effect for time {Mean difference [MD (Post-Pre)] ± Standard Error (SE) = −0.61 ± 0.22 kg; p = 004} (**Table 3**). For LM, there was no significant group-by-time interaction (p = 0.081) or significant main effect for group (p = 0.338). There was a main effect for time {Mean difference [MD (Post-Pre)] ± Standard Error (SE) = 0.78 ± 0.12 kg; $p < 0.001$}; the SUP group resulted in a greater increase in LM compared to the CON group (5.2 ± 3.8 kg; p = 0.510), although not significant. When separated by sex, there was no significant group-by-time interaction for males (p = 0.182) or females (p = 0.317). There was a main effect for time for males (0.84 ± 0.24 kg; p = 0.003) and females (0.73 ± 0.14 kg; $p < 0.001$). There was no main effect for group for males (p = 0.284) or females (p = 0.434). Individual effects for LM for males and females are presented in **Figures 3**, **4**.

For FM, there was no significant group-by-time interaction (p = 0.749), main effect for time (−0.19 ± 0.13 kg; p = 0.146), or main effect for group (p = 0.702). When separated by sex, there was no significant group-by-time interaction for males (p = 0.133) or females (p = 0.725), no main effect for time for males (0.29 ± 0.23 kg; p = 0.225) and females (0.18 ± 0.16 kg; p = 0.270), an no main effect for group for males (p = 0.643) or females (p = 0.925). Individual effects for FM for males and females are presented in **Figures 3**, **4**.

Performance

For absolute VO_2max, there was no significant group-by-time interaction (p = 0.160), main effect for time (0.46 ± 0.31 L/min; p = 0.141), or main effect for group (p = 0.260). When separated by sex, there was no significant group-by-time interaction for males (p = 0.311) or females (p = 0.185), or main effect for time for males (0.05 ± 0.07 L/min; p = 0.522) or females (0.42 ± 0.03 L/min; p = 0.128). There was no main effect for group for males (p = 0.572) or females (p = 0.052). For females, the *post-hoc* analysis demonstrated a significant difference between the SUP and CON group (0.4 ± 0.2 L/min; p = 0.047).

For relative VO_2max, there was no significant group-by-time interaction (p = 0.800). There was a significant main effect for time (−1.20 ± 0.46 mL/kg/min; p = 0.012), but no main effect for group (p = 0.374). VO_2max decreased from baseline to post-training (−1.2 ± 0.5 mL/kg/min; p = 0.012). When separated by sex, there was no significant group-by-time interaction for males (p = 0.951) or females (p = 0.359), or main effect for time for males (−1.5 ± 1.10 mL/kg/min; p = 0.185). There was a main effect for time in females (−1.0 ± 0.42 mL/kg/min; p = 0.022). There was no main effect for group for males (p = 0.740) or females (p = 0.352).

For 1RM leg strength (LP1RM), there was a significant group-by-time interaction ($p < 0.001$). There was a main effect for time (69.8 ± 4.5 kg $p < 0.001$), and a main effect for group (p = 0.018); the SUP group gained significantly more strength

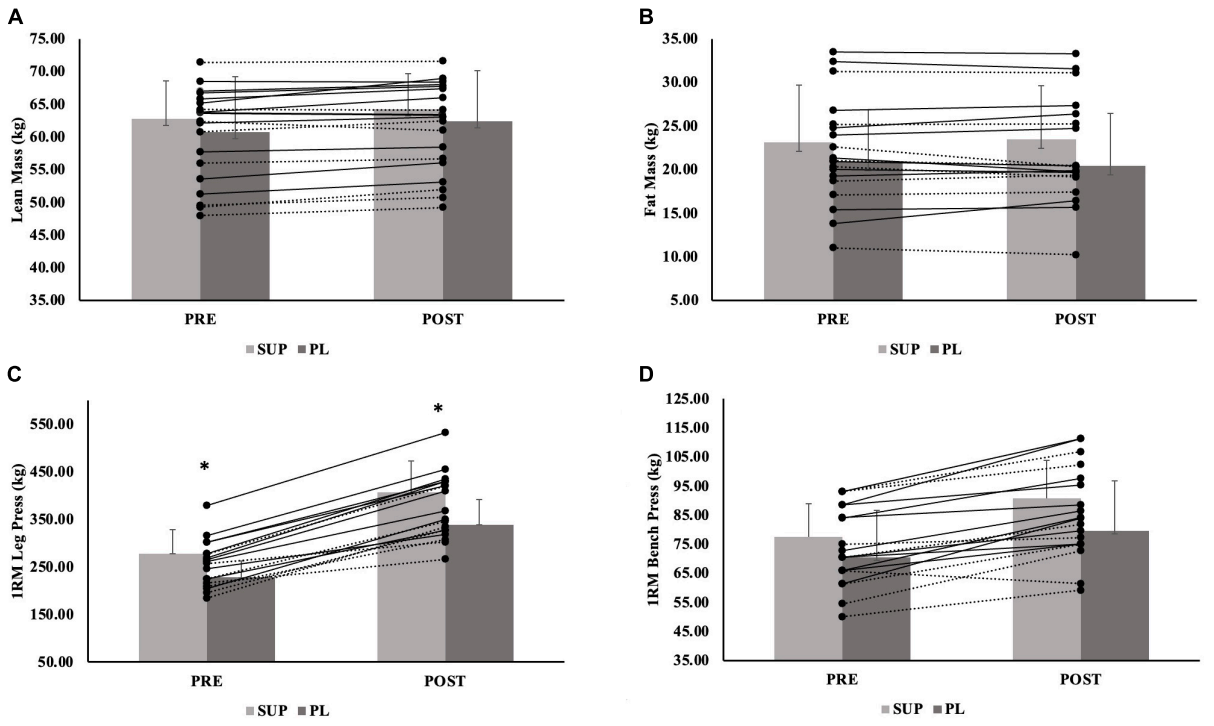


FIGURE 3
(A–D) Male individual responses for **(A)** lean mass, **(B)** fat mass, and **(C)** 1RM leg press, and **(D)** 1RM Bench Press. The lines represent the differences between baseline (PRE) and post-testing visit (POST) per participant. The solid lines represent males in the supplement group (n = 10). The dashed lines represent males in the PL group (n = 8). The gray bars represent treatment means for the supplement (SUP; light gray) and placebo (PL; dark gray) groups with average standard deviation (error bars). *Indicates significant main effect for group (p = 0.002).

than CON group (89.9 ± 30.8 kg; p = 0.015) (Table 3). The PL group also gained more 1RM leg strength than the CON group (51.9 ± 31.0 kg; p = 0.299), although not significant. When separated by sex, there was a significant group-by-time interaction for males (p < 0.001) and females (p < 0.001), main effect for time for males (82.9 ± 7.5 kg; p < 0.001) and females (61.6 ± 5.1 kg; p < 0.001), and main effect for group for males (p = 0.002) and females (p = 0.004). For males and females, the SUP group gained more strength than the CON group (149.7 ± 36.3 kg; p = 0.002 and 66.8 ± 19.6 kg; p = 0.005, respectively). For females, the PL group also gained more strength compared to the CON group (56.5 ± 19.4 kg; p = 0.018). Individual effects for LP1RM for males and females are presented in Figures 3, 4.

For 1RM upper body strength (BP1RM), there was a significant group-by-time interaction (p < 0.001) and main effect for time (5.5 ± 0.7 kg; p < 0.001), but no significant main effect for group (p = 0.439) (Table 3); the SUP group demonstrated greater increases than the CON group (10.5 ± 8.2 kg; p = 0.622), while not significant. When separated by sex, there was a significant group-by-time interaction for males (p = 0.002) and females (p < 0.001). There was a main effect for time for males (6.9 ± 1.5 kg; p < 0.001) and females (4.6 ± 0.6 kg; p < 0.001). There was no main effect for group for males (p = 0.450), but there was a main effect for females (p = 0.035). For females, the SUP group gained more strength compared to the CON group (6.6 ± 3.2 kg; p = 0.134), although not significant. The PL group gained more strength compared to the CON group (8.3 ± 3.1 kg; p = 0.035). Individual effects for BP1RM for males and females are presented in Figures 3, 4.

For CMJ, there was no significant group-by-time interaction (p = 0.886) (Table 3). There was a main effect for time (8.52 ± 0.40 cm; p = 0.040), but no main effect for group (p = 0.337). When separated by sex, there was no significant group-by-time interaction for males (p = 0.862) or females (p = 0.642). There was no main effect for time for males (−0.70 ± 5.5 cm; p = 0.899), but there was a main effect for time for females (1.4 ± 5.0 cm; p = 0.014). There was no main effect for group for males (p = 0.639) or females (p = 0.456).

Blood analytes

For CK, there was no significant group-by-time interaction (p = 0.938), no main effect for time (−8.3 ± 6.7 ng/ml; p = 0.222), and no main effect for group (p = 0.703) (Table 3). When separated by sex, there was no significant group-by-time interaction for males (p = 0.651) or females (p = 0.650). There

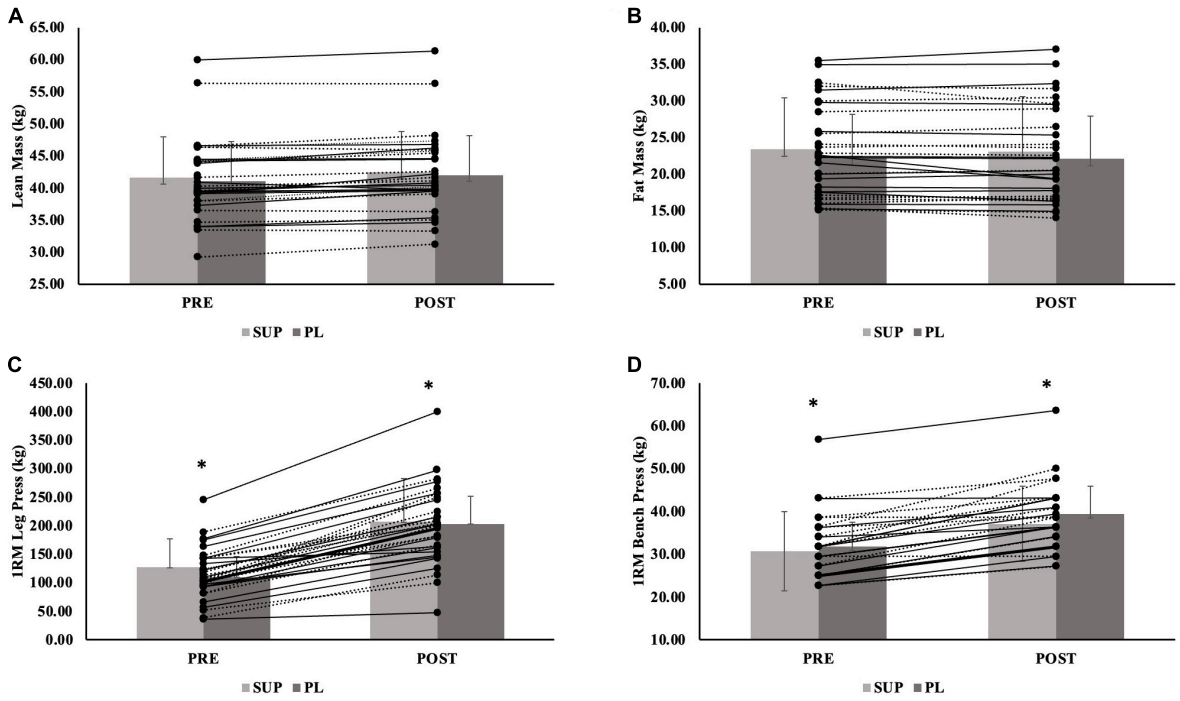


FIGURE 4
(A–D) Female individual responses for **(A)** lean mass, **(B)** fat mass, and **(C)** 1RM leg press, and **(D)** 1RM Bench Press. The lines represent the differences between baseline (PRE) and post-testing visit (POST) per participant. The gray bars represent treatment mean with average standard deviation. The solid lines represent females in the supplement group (n = 15). The dashed lines represent females in the placebo group (n = 16). The gray bars represent treatment means for the supplement (SUP; light gray) and placebo (PL; dark gray) groups with average standard deviation (error bars). *Indicates significant main effect for group for 1RM leg press (p = 0.004) and 1RM bench press (p = 0.035).

was no main effect for time for males (−0.2 ± 11.9 ng/ml; $p = 0.987$) or females (10.5 ± 8.4 ng/ml; $p = 0.221$). There was no main effect for group for males ($p = 0.192$) or females ($p = 0.714$).

For isoprostanes, there was no significant group-by-time interaction (p = 0.830) (Table 3). There was no main effect for time (5.88 ± 3.88 ng/ml; p = 0.135), and no main effect for group (p = 0.425). When separated by sex, there was no significant group-by-time interaction for males (p = 0.149) or females ($p = 0.678$), no main effect for time for males (0.7 ± 6.6 ng/ml; $p = 0.916$) or females (9.0 ± 4.7 ng/ml; $p = 0.062$), and no main effect for group for males ($p = 0.587$) or females ($p = 0.575$).

Discussion

The current study evaluated a twice weekly 40-min HIRT + HIIT exercise paired with nutrition supplementation. These data were collected during the COVID-19 pandemic in 2020, which required a reduced number of training days for feasibility. In general, the COVID-19 pandemic emphasized the importance of identifying feasible exercise strategies that are time efficient (28). The present study demonstrated that a twice weekly 40-min concurrent HIRT + HIIT exercise intervention can support increases in LM and strength in men and women. There was a significantly greater improvement in upper and lower body strength when nutrients were consumed before and after exercise. Females appeared to respond more favorably to supplementation, with greater increases in upper and lower body strength. The combined HIRT + HIIT exercise may be a feasible option to improve strength and LM, with only two sessions per week for six weeks. These improvements were further augmented when nutrients were consumed before and after exercise, particularly for women.

Meta-analyses suggest that the stimulation of muscle protein synthesis and increases in LM are influenced by nutrient timing, particularly protein intake (17). There is conflicting information on the actual timing of nutrients, whether pre- or post-exercise is more advantageous (7, 15–18), and what time frame is required for nutrient consumption to support exercise adaptations (14, 18, 29). However, data collectively supports that consuming nutrients around exercise may provide greater benefit than withholding nutrients (17). The present study supports existing findings; LM increased significantly from baseline to post-testing with the SUP group gaining more LM (+1.1 kg) compared to the PL group (+0.88 kg) and CON group (+0.37 kg). The multi-ingredient formula consumed in the present study contained ingredients that have been known to support muscle protein synthesis (30) and tissue repair (20)

TABLE 3 Body composition, performance, and blood analyte variables presented as mean ± standard deviation.

Body mass			
	Supplement	**Placebo**	**Control**
Pre-intervention (kg)	77.2 ± 16.2	71.3 ± 12.3	71.4 ± 18.7
Post-intervention (kg)	78.4 ± 17.1	71.9 ± 12.3	71.5 ± 18.4
Lean mass			
	Supplement	**Placebo**	**Control**
Pre-intervention (kg)	50.1 ± 12.2	46.6 ± 10.6	45.2 ± 12.1
Post-intervention (kg)	51.2 ± 12.4	47.5 ± 10.3	45.6 ± 12.0
Fat mass			
	Supplement	**Placebo**	**Control**
Pre-intervention (kg)	24.3 ± 8.3	21.8 ± 5.9	23.4 ± 9.7
Post-intervention (kg)	24.2 ± 8.2	21.6 ± 5.9	23.2 ± 9.9
Absolute VO_2max			
	Supplement	**Placebo**	**Control**
Pre-intervention (L/min)	2.9 ± 0.9	2.6 ± 0.9	2.4 ± 0.9
Post-intervention (L/min)	2.8 ± 0.9	2.6 ± 0.9	2.3 ± 0.8
Relative VO_2max			
	Supplement	**Placebo**	**Control**
Pre-intervention (ml/kg/min)	37.3 ± 8.1	36.3 ± 8.3	34.0 ± 8.2
Post-intervention (ml/kg/min)	36.1 ± 7.2	35.3 ± 8.2	32.4 ± 8.0
Lower body strength*			
	Supplement	**Placebo**	**Control**
Pre-intervention (kg)	187.5 ± 89.9	149.7 ± 67.0	141.8 ± 105.5
Post-intervention (kg)	286.9 ± 121.9†	248.7 ± 81.9	152.9 ± 106.1†
Upper body strength			
	Supplement	**Placebo**	**Control**
Pre-intervention (kg)	49.4 ± 25.4	44.7 ± 21.4	43.9 ± 28.5
Post-intervention (kg)	58.6 ± 28.8	52.8 ± 22.2	43.1 ± 27.2
Counter movement jump			
	Supplement	**Placebo**	**Control**
Pre-intervention (cm)	34.6 ± 9.5	32.3 ± 8.7	30.4 ± 10.5
Post-intervention (cm)	35.6 ± 9.8	33.3 ± 7.6	30.9 ± 10.3
Creatine kinase			
	Supplement (*n* = 22)	**Placebo**	**Control**
Pre-intervention (ng/ml)	103.6 ± 48.5	96.7 ± 42.0	100.2 ± 59.4
Post-intervention (ng/ml)	108.9 ± 45.4	104.7 ± 35.5	123.1 ± 109.3
Isoprostanes			
	Supplement	**Placebo**	**Control**
Pre-intervention (ng/ml)	89.1 ± 32.6	85.0 ± 31.3	97.4 ± 34.5
Post-intervention (ng/ml)	92.1 ± 34.5	93.0 ± 31.6	104.0 ± 28.3

*Indicates significant main effect for group ($p = 0.018$). †Indicates statistically significant difference between supplement and control group ($p = 0.015$) from *post-hoc* analysis.

[whey protein, casein protein, and β-hydroxy-β-methylbutyrate (HMB)], possibly supporting the greater, yet non-significant, increases in LM reported in the SUP group compared to CON. Furthermore, these increases in LM were observed without the addition of creatine monohydrate, a dietary supplement that has demonstrated improvements in LM especially when paired with resistance training (31). In a similar study evaluating nutrient timing around the same HIRT protocol implemented in women, consumption of nutrients (16 g CHO + 25 g PRO) before or after–exercise resulted in significantly greater increases in LM compared to no nutritional intake (PRE: +0.96 kg; Post: +0.64 kg; CON: +0.15 kg) (7). The present study resulted in similar improvements in LM in females (SUP: +0.9 kg; PL: +0.9 kg; CON: +0.4 kg). It is well-known that resistance training increases LM (32, 33), with an expected +2.8% gain in LM post 6-weeks of HIRT (10). Uniquely, in addition to HIRT, the present study included a concurrent aerobic HIIT bout, which has demonstrated increases in LM in as little as three weeks when performed by itself (6). Concurrent training with resistance training and HIIT has been suggested as an effective method for maintaining strength and LM (13). In support, the present study demonstrated a +2.2% gain in LM from baseline values within the SUP group, as well as a +1.9% gain within the PL group from baseline when concurrent training was employed. It appears that when HIRT is performed prior to HIIT, increases in LM and strength may result over time, particularly when nutrients are provided around the exercise.

Prior research has demonstrated that HIRT and HIIT elicit reductions in FM, possibly through increased fatty acids utilization during exercise (34, 35). However, studies including nutrient consumption surrounding high-intensity exercise have not demonstrated augmented FM loss (7, 8), most likely due to the lack of diet modification. In the present study, there were no changes in FM across time (−0.19 kg) or between groups. The results are consistent with previous studies utilizing similar exercise protocols, with or without nutritional supplementation, which have reported decreases in FM post-intervention ranging from −0.1 to −0.6 kg (8, 10, 36). The lack of significant changes in FM is primarily due to the absence of day-to-day dietary control. Fat loss with exercise is often not pronounced without caloric restriction or dietary intervention. The present study was not aimed at reducing calories or changing dietary intake, but rather providing specific nutrients surrounding a time effective exercise session. In contrast, the present study provided 220 calories twice per week with the pre- and post-supplementation, an amount that did not appear to influence body weight or FM. The nutritional supplement utilized in the present study included ingredients like caffeine and whey protein, which have previously supported FM loss while sparing LM during a caloric deficit (37, 38). Twice weekly supplementation, as well as the lack of planned caloric restriction likely impacted the lack of FM loss in the present study.

It is well-known that traditional RT provides a potent anabolic stimulus, which can result in increased muscle strength and maintenance of LM (39). Data collectively supports that RT improves muscle strength, quality, and may assist in prevention of chronic diseases (40). However, lack of time is often cited as

a major barrier for participation in RT exercises (41). HIRT is a time effective RT approach that has demonstrated significant and rapid improvements in maximal strength despite the relatively short time of effort (7, 10). Despite the short rest period between sets, participants were able to successfully complete 6–8 repetitions in each set. The present study supports existing findings with HIRT (7, 10, 13) demonstrating improvements in lower body strength baseline to post-testing in the SUP group (+99.4 kg) and PL group (+99.0 kg), compared to the CON group (+11.1 kg). While not significant, the SUP group (+9.2 kg) and PL group (+8.1 kg) demonstrated greater upper body strength increases compared to the and CON group (−0.8 kg). The HIRT intervention may be beneficial for increases in strength. Additionally, consumption of nutrients before and after exercise, particularly, protein, carbohydrates, and caffeine, may have provided a greater environment to support muscular adaptations associated with HIRT (42–44). A recent study evaluating six-weeks of HIRT training in healthy males and females resulted in significantly greater increases in lower (LP1RM: +49.9 kg) and upper body 1RM (BP1RM: +11.4 kg) strength when compared to traditional resistance training (LP1RM: +31.6 kg; BP1RM −7.9 kg), highlighting the impact of a short-term whole-body HIRT training approach for increasing strength in males and females. Existing data suggest that males and females respond similarly when beginning RT (45), yet females potentially experience greater increases in strength (46). Our findings support a potential sexually dimorphic response, with females in both the SUP and PL group significantly increasing LP1RM and BP1RM when compared to CON, while only males in the SUP group saw significant improvements in strength. As such, future research should explore sex differences in nutrient timing and HIRT adaptations, particularly as data in males cannot always be extrapolated to females.

In addition to maximal strength, the present study evaluated other performance outcomes such as aerobic fitness *via* VO_2max and lower body power *via* CMJ. HIIT training has been shown to augment cardiorespiratory fitness with data demonstrating VO_2max improvements in as little as six weeks (4, 47). In contrast to previous research, there was no change in absolute VO_2max values following the intervention, which is possibly due to the concurrent nature of the HIRT + HIIT on molecular pathways, with the HIIT taking place immediately after the HIRT exercises. Participants may have been fatigued from the HIRT session thereby limiting cardiorespiratory adaptations to HIIT. Additionally, due to the intense nature of the intervention, participants may not have fully recovered prior to when the post-testing was completed (∼48 h of the last training session). In addition to cardiorespiratory improvements, high-intensity exercise, whether aerobic or anaerobic, requires high locomotor speed and power (48). Maximal CMJ is a strong assessment of lower-body power test with good reliability (intraclass correlation, >0.989). There are limited data regarding the effects of HIRT and HIIT on CMJ (49, 50); the present study demonstrated positive effects of combined high-intensity strength and interval training on CMJ.

Creatine kinase and isoprostanes have consistently been used as indicators of muscle damage and oxidative stress, respectively (51, 52). It has been postulated that consumption of nutrients such as carbohydrate and protein surrounding high-intensity exercise may attenuate markers of muscle damage and oxidative stress (53, 54). While some research reports that protein-carbohydrate supplementation surrounding high-intensity exercise significantly reduces post-exercise serum CK levels (54, 55), other studies have not (56, 57). In the present study, there were no significant changes in CK across time or between groups. It remains unclear whether consumption of nutrients before or after high-intensity exercise mitigates markers of muscle damage (58). While the time of day was consistent between the baseline and post-testing visit, there may have been some variability in the post-testing values due to the difference in testing days after the last exercise session. The magnitude of oxidative stress following an acute bout of exercise is generally proportional to exercise intensity (59). However, there is limited research evaluating markers of oxidative stress, such as isoprostanes, after HIRT or the influence of nutrient timing on isoprostanes levels post-exercise. One previous study observing carbohydrate ingestion during RT in males reported acute isoprostanes levels were unaffected (53). Our findings may support this as there were no changes in isoprostanes across time or between groups. It appears that blood markers were not significantly impacted by nutrient timing in the present study.

The results of the present study reflect the impact of a minimal nutritional intervention with exercise, resulting in significant increases in LM and strength; nutrient consumption outside of the pre- and post-workout supplementation was not modified or monitored throughout the study. Participants' relative protein intake (g/kg) was lower (**Table 1**) than the recommended amount for increasing LM (1.6–2.0 g/kg) (17), and the addition of the 20 grams of protein twice a week would have only increased protein intake to 1.3 g/kg on the training days. A larger change in calorie and macronutrient composition, on more than two days per week, would have likely supported more pronounced effects on body composition. Additionally, some of the components of the multi-ingredient pre-workout formulation (e.g., caffeine, HMB, and carbohydrates) in the present study may have been lower compared to the recommended dosing amount (17). Future research may benefit from exploring a relative dose of the individual supplements vs. an absolute dose product as utilized in the present study. Although weight loss was not a primary outcome of the present study, results suggest future research targeting weight loss and FM loss may benefit from dietary modification in addition to nutrient supplementation and HIRT. The investigation of sex differences in body composition and performance was an exploratory aim of the present study. The sample size of males and females in each group may need to be larger in future

studies. Additionally, as a result of extensive research cleaning and spacing restrictions during the COVID-19 pandemic, the present intervention was only conducted on two days per week; inclusion of an additional day may have resulted in greater adaptations.

Conclusion

In conclusion, the twice weekly nutrition supplementation before and after the high-intensity exercise protocol appears to be an effective approach for increasing LM and strength, especially in females. These effects are likely attributed to nutrients supporting muscle recovery and providing anabolic stimuli for muscle growth in response to HIRT + HIIT (14). Females appeared to respond more favorably to nutrient consumption, demonstrating greater increases in upper and lower body strength. Future research should continue to explore sex differences in nutrient timing and HIRT adaptations, particularly as data in males cannot always be generalized to females. The present study suggests there are beneficial effects of the exercise and nutrition intervention despite minimal training time and lifestyle changes.

Data availability statement

Deidentified individual data that support the results will be shared upon a reasonable request beginning 12–36 months following publication provided the investigator who proposes to use the data has approval from an Institutional Review Board (IRB), Independent Ethics Committee (IEC), or Research Ethics Board (REB), as applicable, and executes a data use/sharing agreement with UNC. Requests to access the datasets should be directed to abbsmith@email.unc.edu.

Ethics statement

The studies involving human participants were reviewed and approved by the University of North Carolina Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AS-R designed the study. HC, AG, NP, and A-SR collected the data and contributed to the manuscript preparation. HC and AS-R analyzed and interpreted the data. All authors reviewed and approved final manuscript.

Funding

The authors declare that this study received funding from NSE Products, Inc. (NSEP). The funder developed the nutritional products evaluated in the study and was a part of the discussion around the study design. The funder was not involved in the data collection, analysis, interpretation of, or writing of the article.

Acknowledgments

We thank Shelly Hester, Ph.D. RD and Steve Wood, Ph.D. for their contributions to the nutrition supplement formulation and review of the manuscript.

Conflict of interest

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

Publisher's note

References

1. Constandt B, Thibaut E, De Bosscher V, Scheerder J, Ricour M, Willem A. Exercising in times of lockdown: an analysis of the impact of COVID-19 on levels and patterns of exercise among adults in Belgium. *Int J Environ Res Public Health.* (2020) 17:4144. doi: 10.3390/ijerph17114144

2. Gibala MJ, Little JP. Just HIT it! A time-efficient exercise strategy to improve muscle insulin sensitivity: perspectives. *J Physiol.* (2010) 588:3341–2. doi: 10.1113/jphysiol.2010.196303

3. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, et al. The physical activity guidelines for Americans. *JAMA.* (2018) 320:2020. doi: 10.1001/jama.2018.14854

4. Atakan MM, Güzel Y, Bulut S, Koşar SN, McConell GK, Turnagöl HH. Six high-intensity interval training sessions over 5 days increases maximal oxygen uptake, endurance capacity, and sub-maximal exercise fat oxidation as much as 6 high-intensity interval training sessions over 2 weeks. *J Sport Health Sci.* (2021) 10:478–87. doi: 10.1016/j.jshs.2020.06.008

5. Wingfield HL, Smith-Ryan AE, Melvin MN, Roelofs EJ, Trexler ET, Hackney AC, et al. The acute effect of exercise modality and nutrition manipulations on post-exercise resting energy expenditure and respiratory exchange ratio in women: a randomized trial. *Sports Med Open.* (2015) 1:11. doi: 10.1186/s40798-015-0010-3

6. Blue MNM, Smith-Ryan AE, Trexler ET, Hirsch KR. The effects of high intensity interval training on muscle size and quality in overweight and obese adults. *J Sci Med Sport.* (2018) 21:207–12. doi: 10.1016/j.jsams.2017.06.001

7. Pihoker AA, Peterjohn AM, Trexler ET, Hirsch KR, Blue MNM, Anderson KC, et al. The effects of nutrient timing on training adaptations in resistance-trained females. *J Sci Med Sport.* (2019) 22:472–7. doi: 10.1016/j.jsams.2018.09.236

8. Hirsch KR, Greenwalt CE, Cabre HE, Gould LM, Brewer GJ, Blue MNM, et al. Metabolic effects of high-intensity interval training and essential amino acids. *Eur J Appl Physiol.* (2021) 121:3297–311. doi: 10.1007/s00421-021-04792-4

9. Paoli A, Moro T, Marcolin G, Neri M, Bianco A, Palma A, et al. High-Intensity Interval Resistance Training (HIRT) influences resting energy expenditure and respiratory ratio in non-dieting individuals. *J Transl Med.* (2012) 10:237. doi: 10.1186/1479-5876-10-237

10. Moro T, Marcolin G, Bianco A, Bolzetta F, Berton L, Sergi G, et al. Effects of 6 weeks of traditional resistance training or high intensity interval resistance training on body composition, aerobic power and strength in healthy young subjects: a randomized parallel trial. *Int J Environ Res Public Health.* (2020) 17:4093. doi: 10.3390/ijerph17114093

11. Gibala MJ, Gillen JB, Percival ME. Physiological and health-related adaptations to low-volume interval training: influences of nutrition and sex. *Sports Med.* (2014) 44(Suppl. 2):127–37. doi: 10.1007/s40279-014-0259-6

12. Wilson JM, Marin PJ, Rhea MR, Wilson SMC, Loenneke JP, Anderson JC. Concurrent training: a meta-analysis examining interference of aerobic and resistance exercises. *J Strength Cond Res.* (2012) 26:2293–307. doi: 10.1519/JSC.0b013e31823a3e2d

13. Moro T, Tinsley G, Bianco A, Gottardi A, Gottardi GB, Faggian D, et al. High intensity interval resistance training (HIIRT) in older adults: effects on body composition, strength, anabolic hormones and blood lipids. *Exp Gerontol.* (2017) 98:91–8. doi: 10.1016/j.exger.2017.08.015

14. Forbes SC, Candow DG, Smith-Ryan AE, Hirsch KR, Roberts MD, VanDusseldorp TA, et al. Supplements and nutritional interventions to augment high-intensity interval training physiological and performance adaptations—a narrative review. *Nutrients.* (2020) 12:390. doi: 10.3390/nu12020390

15. Aragon AA, Schoenfeld BJ. Nutrient timing revisited: is there a post-exercise anabolic window? *J Int Soc Sports Nutr.* (2013) 10:5. doi: 10.1186/1550-2783-10-5

16. Arent SM, Cintineo HP, McFadden BA, Chandler AJ, Arent MA. Nutrient timing: a garage door of opportunity? *Nutrients.* (2020) 12:1948. doi: 10.3390/nu12071948

17. Kerksick CM, Arent S, Schoenfeld BJ, Stout JR, Campbell B, Wilborn CD, et al. International society of sports nutrition position stand: nutrient timing. *J Int Soc Sports Nutr.* (2017) 14:33. doi: 10.1186/s12970-017-0189-4

18. Schoenfeld BJ, Aragon AA, Krieger JW. The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *J Int Soc Sports Nutr.* (2013) 10:53. doi: 10.1186/1550-2783-10-53

19. Smith AE, Fukuda DH, Kendall KL, Stout JR. The effects of a pre-workout supplement containing caffeine, creatine, and amino acids during three weeks of high-intensity exercise on aerobic and anaerobic performance. *J Int Soc Sports Nutr.* (2010) 7:10. doi: 10.1186/1550-2783-7-10

20. Holeček M. Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions: HMB supplementation and muscle. *J Cachexia Sarcopenia Muscle.* (2017) 8:529–41. doi: 10.1002/jcsm.12208

21. Rondanelli M, Klersy C, Terracol G, Talluri J, Maugeri R, Guido D, et al. Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. *Am J Clin Nutr.* (2016) 103:830–40. doi: 10.3945/ajcn.115.113357

22. Sacheck JM, Blumberg JB. Role of vitamin E and oxidative stress in exercise. *Nutrition.* (2001) 17:809–14. doi: 10.1016/S0899-9007(01)00639-6

23. Scalzo RL, Peltonen GL, Binns SE, Shankaran M, Giordano GR, Hartley DA, et al. Greater muscle protein synthesis and mitochondrial biogenesis in males compared with females during sprint interval training. *FASEB J.* (2014) 28:2705–14. doi: 10.1096/fj.13-246595

24. Grgic J, Lazinica B, Schoenfeld BJ, Pedisic Z. Test–retest reliability of the One-Repetition Maximum (1RM) strength assessment: a systematic review. *Sports Med Open.* (2020) 6:31. doi: 10.1186/s40798-020-00260-z

25. Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller JC, et al. Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. *J Appl Physiol.* (1996) 81:2095–104. doi: 10.1152/jappl.1996.81.5.2095

26. Slinde F, Suber C, Suber L, Edwén CE, Svantesson U. Test-retest reliability of three different countermovement jumping tests. *J Strength Cond Res.* (2008) 22:640–4. doi: 10.1519/JSC.0b013e3181660475

27. Rousseeuw PJ, Hubert M. Robust statistics for outlier detection. *WIREs Data Min Knowl Discov.* (2011) 1:73–9. doi: 10.1002/widm.2

28. Brand, R, Timme S, Nosrat S. When pandemic hits: exercise frequency and subjective well-being during COVID-19 pandemic. *Front Psychol.* (2020) 11:570567. doi: 10.3389/fpsyg.2020.570567

29. Cribb PJ, Hayes A. Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy. *Med Sci Sports Exerc.* (2006) 38:1918–25. doi: 10.1249/01.mss.0000233790.08788.3e

30. Phillips S. *Protein Consumption and Resistance Exercise: Maximizing Anabolic Potential. Gatorade Sports Science Institute.* (2013). Available online at: https://www.gssiweb.org/sports-science-exchange/article/sse-107-protein-consumption-and-resistance-exercise-maximizing-anabolic-potential#articleTopic_5 (accessed April 12, 2022).

31. Wu SH, Chen KL, Hsu C, Chen HC, Chen JY, Yu SY, et al. Creatine supplementation for muscle growth: a scoping review of randomized clinical trials from 2012 to 2021. *Nutrients.* (2022) 14:1255. doi: 10.3390/nu14061255

32. Schoenfeld BJ, Ogborn D, Krieger JW. Effects of resistance training frequency on measures of muscle hypertrophy: a systematic review and meta-analysis. *Sports Med.* (2016) 46:1689–97. doi: 10.1007/s40279-016-0543-8

33. Krieger JW. Single vs. multiple sets of resistance exercise for muscle hypertrophy: a meta-analysis. *J Strength Cond Res.* (2010) 24:1150–9. doi: 10.1519/JSC.0b013e3181d4d436

34. Wewege M, van den Berg R, Ward RE, Keech A. The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis: exercise for improving body composition. *Obes Rev.* (2017) 18:635–46. doi: 10.1111/obr.12532

35. Schuenke M, Mikat R, McBride J. Effect of an acute period of resistance exercise on excess post-exercise oxygen consumption: implications for body mass management. *Eur J Appl Physiol.* (2002) 86:411–7. doi: 10.1007/s00421-001-0568-y

36. Gillen JB, Percival ME, Ludzki A, Tarnopolsky MA, Gibala Martin J. Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women: interval training improves body composition. *Obesity.* (2013) 21:2249–55. doi: 10.1002/oby.20379

37. Frestedt JL, Zenk JL, Kuskowski MA, Ward LS, Bastian ED. A whey-protein supplement increases fat loss and spares lean muscle in obese subjects: a randomized human clinical study. *Nutr Metab.* (2008) 5:8. doi: 10.1186/1743-7075-5-8

38. Tabrizi R, Saneei P, Lankarani KB, Akbari M, Kolahdooz F, Esmaillzadeh A, et al. The effects of caffeine intake on weight loss: a systematic review and dos-response meta-analysis of randomized controlled trials. *Crit Rev Food Sci Nutr.* (2019) 59:2688–96. doi: 10.1080/10408398.2018.1507996

39. Lyristakis PM, Wundersitz DWT, Zadow EK, Mnatzaganian G, Gordon BA. The influence of considering individual resistance training variables as a whole on muscle strength: a systematic review and meta-analysis protocol. *PLoS One.* (2022) 17:e0262674. doi: 10.1371/journal.pone.0262674

40. Westcott WL. Resistance training is medicine: effects of strength training on health. *Curr Sports Med Rep.* (2012) 11:209–16. doi: 10.1249/JSR.0b013e31825dabb8

41. Hurley KS, Flippin KJ, Blom LC, Bolin JE, Hoover DL, Judge LW. Practices, perceived benefits, and barriers to resistance training among women enrolled in college. *Int J Exerc Sci.* (2018) 11:226–38.

42. Andersen LL, Tufekovic G, Zebis MK, Crameri RM, Verlaan G, Kjær M, et al. The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. *Metabolism.* (2005) 54:151–6. doi: 10.1016/j.metabol.2004.07.012

43. Grgic J, Trexler ET, Lazinica B, Pedisic Z. Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis. *J Int Soc Sports Nutr.* (2018) 15:11. doi: 10.1186/s12970-018-0216-0

44. Baechle T, Earle R. *Essentials of Strength Training and Conditioning- National Strength and Conditioning Association*. Champaign, IL: Human Kinetics (2008).

45. Cureton KJ, Collins MA, Hill DW, McElhannon FM. Muscle hypertrophy in men and women. *Med Sci Sports Exerc.* (1988) 20:338–44. doi: 10.1249/00005768-198808000-00003

46. O'Hagan FT, Sale DG, MacDougall JD, Garner SH. Response to resistance training in young women and men. *Int J Sports Med.* (1995) 16:314–21. doi: 10.1055/s-2007-973012

47. Milanović Z, Sporiš G, Weston M. Effectiveness of High-Intensity Interval Training (HIT) and continuous endurance training for VO2max improvements: a systematic review and meta-analysis of controlled trials. *Sports Med.* (2015) 45:1469–81. doi: 10.1007/s40279-015-0365-0

48. Buchheit M, Laursen PB. High-intensity interval training, solutions to the programming puzzle: part II: anaerobic energy, neuromuscular load and practical applications. *Sports Med.* (2013) 43:927–54. doi: 10.1007/s40279-013-0066-5

49. Hammami M, Gaamouri N, Ramirez-Campillo R, Shephard RJ, Bragazzi NL, Chelly MS, et al. Effects of high-intensity interval training and plyometric exercise on the physical fitness of junior male handball players. *Eur Rev Med Pharmacol Sci.* (2021) 25:7380–9. doi: 10.26355/eurrev_202112_27434

50. Hermassi S, Chelly M, Fieseler G, Bartels T, Schulze S, Delank KS, et al. Short-term effects of combined high-intensity strength and sprint interval training on anthropometric characteristics and physical performance of elite team handball players. *Sportverletz Sportschaden.* (2017) 31:231–9. doi: 10.1055/s-0043-120884

51. Czerska M, Zieliński M, Gromadzińska J. Isoprostanes – A novel major group of oxidative stress markers. *Int J Occup Med Environ Health.* (2015) 29:179–90. doi: 10.13075/ijomeh.1896.00596

52. Koch AJ, Pereira R, Machado M. The creatine kinase response to resistance exercise. *J Musculoskelet Neuronal Interact.* (2014) 14:68–77.

53. McAnulty SR, McAnulty LS, Nieman DC, Morrow JD, Utter AC, Dumke CL. Effect of resistance exercise and carbohydrate ingestion on oxidative stress. *Free Radic Res.* (2005) 39:1219–24. doi: 10.1080/10725760500317536

54. Saunders MJ, Luden ND, Herrick JE. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. *J Strength Cond Res.* (2007) 21:678. doi: 10.1519/R-20506.1

55. Valentine RJ, Saunders MJ, Todd MK, St. Laurent TG. Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption. *Int J Sport Nutr Exerc Metab.* (2008) 18:363–78. doi: 10.1123/ijsnem.18.4.363

56. Breen L, Tipton KD, Jeukendrup AE. No effect of carbohydrate-protein on cycling performance and indices of recovery. *Med Sci Sports Exerc.* (2010) 42:1140–8. doi: 10.1249/MSS.0b013e3181c91f1a

57. Cermak NM, Solheim AS, Gardner MS, Tarnopolsky MA, Gibala MJ. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. *Med Sci Sports Exerc.* (2009) 41:2158–64. doi: 10.1249/MSS.0b013e3181ac10bf

58. Bogdanis GC, Stavrinou P, Fatouros IG, Philippou A, Chatzinikolaou A, Draganidis D, et al. Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans. *Food Chem Toxicol.* (2013) 61:171–7. doi: 10.1016/j.fct.2013.05.046

59. Quindry JC, Stone WL, King J, Broeder CE. The effects of acute exercise on neutrophils and plasma oxidative stress. *Med Sci Sports Exerc.* (2003) 35:1139–45. doi: 10.1249/01.MSS.0000074568.82597.0B

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 03 November 2022
DOI 10.3389/fnut.2022.1011836

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Găman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Himel Mondal,
Saheed Laxman Nayak Medical College, India
Vincent Kipkorir,
University of Nairobi, Kenya

*CORRESPONDENCE
Ahmad Saedisomeolia
Ahmad.saedisomeolia@mcgill.ca

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 04 August 2022
ACCEPTED 30 September 2022
PUBLISHED 03 November 2022

CITATION
Sadeghi A, Saedisomeolia A, Jalili-Baleh L, Khoobi M, Soleimani M, Fakhr Yasseri AM, Yekaninejad MS, Farzin A, Amini E and Nowroozi MR (2022) FruHis significantly increases the anti-benign prostatic hyperplasia effect of lycopene: A double-blinded randomized controlled clinical trial. *Front. Nutr.* 9:1011836. doi: 10.3389/fnut.2022.1011836

FruHis significantly increases the anti-benign prostatic hyperplasia effect of lycopene: A double-blinded randomized controlled clinical trial

Alireza Sadeghi[1], Ahmad Saedisomeolia[1,2]*, Leili Jalili-Baleh[3], Mehdi Khoobi[4], Mohammad Soleimani[5], Ali Mohammad Fakhr Yasseri[6], Mir Saeed Yekaninejad[7], Amirreza Farzin[8], Erfan Amini[8] and Mohammad Reza Nowroozi[8]

[1]Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences (TUMS), Tehran, Iran, [2]School of Human Nutrition, McGill University, Montreal, QC, Canada, [3]Department of Medical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran University of Medical Sciences, Tehran, Iran, [4]Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, [5]Department of Urology, Modares University Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [6]Endologist, Shariati Hospital, Alborz University of Medical Sciences, Karaj, Iran, [7]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, [8]Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Background: For decades, lycopene was considered the main compound of tomato protecting benign prostatic hyperplasia (BPH). Recent animal studies suggest that a newly discovered compound "FruHis" boosts lycopene for its action. This study aimed to determine whether FruHis enhances the action of lycopene to modify the laboratory parameters and clinical outcomes of patients with BPH.

Materials and methods: Current study was conducted on 52 BPH patients, who were randomly assigned into four groups of treatments: lycopene plus FruHis (n = 11, 25 mg/day lycopene and 10 mg/day FruHis), lycopene (n = 12, 25 mg/day lycopene), FruHis (n = 12, 10 mg/day FruHis), and placebo (n = 13). Patients received these supplements for 8 weeks.

Results: FruHis intake strengthened the reducing effects of lycopene on insulin-like growth factor-1 (IGF-1) (−54.47 ± 28.36 ng/mL in the lycopene + FruHis group vs. −30.24 ± 46.69 ng/mL in the lycopene group), total prostate-specific antigen (TPSA) (−1.49 ± 4.78 ng/mL in the lycopene + FruHis group vs. −0.64 ± 2.02 ng/mL in the lycopene group), and symptom score (−4.45 ± 4.03 in the lycopene + FruHis group vs. −1.66 ± 5.41 in the lycopene group) in BPH patients. Such findings were also seen for body mass index (BMI) and waist circumference (WC). However, except for IGF-1, these reductions were not statistically significant compared with the placebo, and the intakes of lycopene and FruHis alone, however, were

clinically important. Such effects of lycopene and FruHis were not seen for free PSA (FPSA) and FPSA/TPSA ratio.

Conclusion: Despite the non-significant effects of lycopene and FruHis, it seems that FruHis intake strengthens the beneficial effects of lycopene on IGF-1, TPSA, and symptom scores among BPH patients.

Clinical trial registration: [www.irct.ir], identifier [IRCT20190522043669N1].

KEYWORDS

lycopene, FruHis, tomato, benign prostatic hyperplasia, prostate specific antigen

Introduction

Benign prostatic hyperplasia (BPH) is prevalent among 50% of men with the age range of ≥50 years and 90% of ≥80-year-old men (1, 2). This disorder is characterized by a non-malignant enlargement of the prostate gland that is due to the proliferation of epithelial and stromal cells in this gland (2, 3). BPH is associated with an increased smooth muscle tone and obstruction of the proximal urethra leading to increased urinary frequency, nocturia, urinary incontinence, and voiding (slow and/or weak stream); all of these symptoms decrease the quality of life of patients considerably (3, 4).

Different types of pharmaceutical therapies such as alpha-blockers and 5-alpha-reductase inhibitors have been suggested to control BPH outcomes (5, 6). However, these medications may cause side events including erectile dysfunction, decreased libido, dizziness, and hypotension (5). Hence, "complementary" or "alternative therapies" with limited adverse events are taken into consideration long ago. Among these approaches, much attention has been paid to lycopene, a lipid-soluble antioxidant compound, which is one of the main dietary carotenoids mostly found in tomato and its products (7, 8). It has been reported that lycopene is the strongest antioxidant among carotenoids. It has been shown that lycopene has a role in the prevention and management of BPH mainly through its antioxidant activity, inhibition of cell cycle progression, induction of apoptosis, increasing of gap-junctional cell communication, and inhibition of insulin-like growth factor I signal transduction (9–11).

Recent studies have shown that other constituents of cooked tomatoes may contribute to BPH prevention (12). Also, tomato processing, particularly heat, may alter tomato constituents and thereby changes the BPH-protective effects of tomato (13). During heat-processing, the tomato loses the activity of its known natural antioxidants, such as ascorbate, whereas the total antioxidant activity of the heat-processed tomato does not change and may often increase (13–15). In addition, observational studies reported that intake of heat-processed tomato products, compared to raw tomato, was associated with a lower risk of BPH and prostate cancer (PC) (16). However, it is still unclear which constituents are produced during the thermal process of tomato and which reaction is involved in this regard.

One of the most common chemical reactions during heat-processing is the Maillard reaction, which is a process in which an amino acid attaches a reducing sugar. This process is responsible for the browning and specific flavoring of baked, roasted, and dried foods, such as toasted bread and fried potatoes (17). This reaction produces ketosamines, such as FruHis (attachment of Fructose to Histidine), that are indigestible and partially absorbed into the bloodstream (18). An experimental study on rats has shown that FruHis exerts antioxidant and anti-cancer properties (12). However, it is not clear whether FruHis provides higher PC-protective effects for heat-processed tomatoes, compared to raw tomatoes. The interaction between lycopene and FruHis against prostate tumorigenesis is another question. Until now, no study has answered these questions. Therefore, the current study was conducted to determine the effect of lycopene and FruHis supplementation, combined and alone, on laboratory parameters and clinical outcomes of men with BPH.

Materials and methods

Participants

This study was a double-blind randomized controlled clinical trial that was conducted by the Department of Cellular and Molecular Nutrition, Tehran University of Medical Sciences, Tehran, Iran, from January 2020 to November 2021. Outpatients with BPH were recruited from educational and therapeutic centers of Tehran University of Medical Sciences. BPH was diagnosed by an expert urologist according to patient history, digital rectal examination (DRE), and laboratory findings, including serum prostate-specific antigen (PSA). According to the sample size formula suggested for intervention studies, considering a type I error of 5% ($\alpha = 0.05$), type II error of 20% ($\beta = 0.20$, power = 80%), and serum PSA levels as the most conservative variable, we required sample size of 12 individuals for each group. However, 13 patients were enrolled

in the intervention groups to make sure that the study powers significantly after the probable dropouts of the patients.

Inclusion and exclusion criteria

Patients were considered eligible for study entry if they had an age range between 50 and 70 years and had confirmed BPH based on clinical diagnosis by an expert urologist using clinical examination, DRE, and paraclinical tests including serum PSA. Criteria of BPH diagnosis included a negative test of the digital rectal exam (DRE) and PSA levels between 4 and 10 ng/mL. To rule out prostatic cancer among suspicious cases, we assessed findings from the prostate biopsy and excluded patients with PSA levels of >10 ng/mL. Patients were not included if they (1) had PC or any other malignancies; (2) had incurable urinary tract infection or intractable urinary retention; (3) intended to receive surgical treatment for BPH; (4) consumed supplements containing lycopene during the past 6 months, and (5) had a history of allergy to tomato or its products. Moreover, we excluded individuals who changed the type or dosage of their medications (related to BPH) during the intervention, patients who consumed unusual amounts of tomato or its products during the study, those that were not willing to continue with the intervention process, patients who suffered from probable complications related to prescribed supplements, and individuals who consumed less than 80% of lycopene, FruHis, or placebo supplements during the trial. The flowchart of this study is shown in **Figure 1**.

Ethics

After the selection of patients, they were asked to complete a written informed consent to participate in the current study. The Ethics Committee of Tehran University of Medical Sciences has approved the study protocol of this clinical trial (IR.TUMS.VCR.REC.1397.951). Moreover, this clinical trial has been registered in the Iranian Registry of Clinical Trials[1] with the reference number IRCT20190522043669N1.

Study design and intervention

After selecting participants based on the mentioned criteria, first, they were stratified based on age groups (55–60 and 60–75 years) and BMI (18.5–24.9 and 25–30 years), and then, were randomly allocated to the four intervention groups, including lycopene plus FruHis, lycopene, FruHis, and placebo groups. In this way, we identified patients with the same condition in terms of age and BMI. Then, to conduct random allocation, an identification code was given to each participant, the codes

1 www.irct.ir

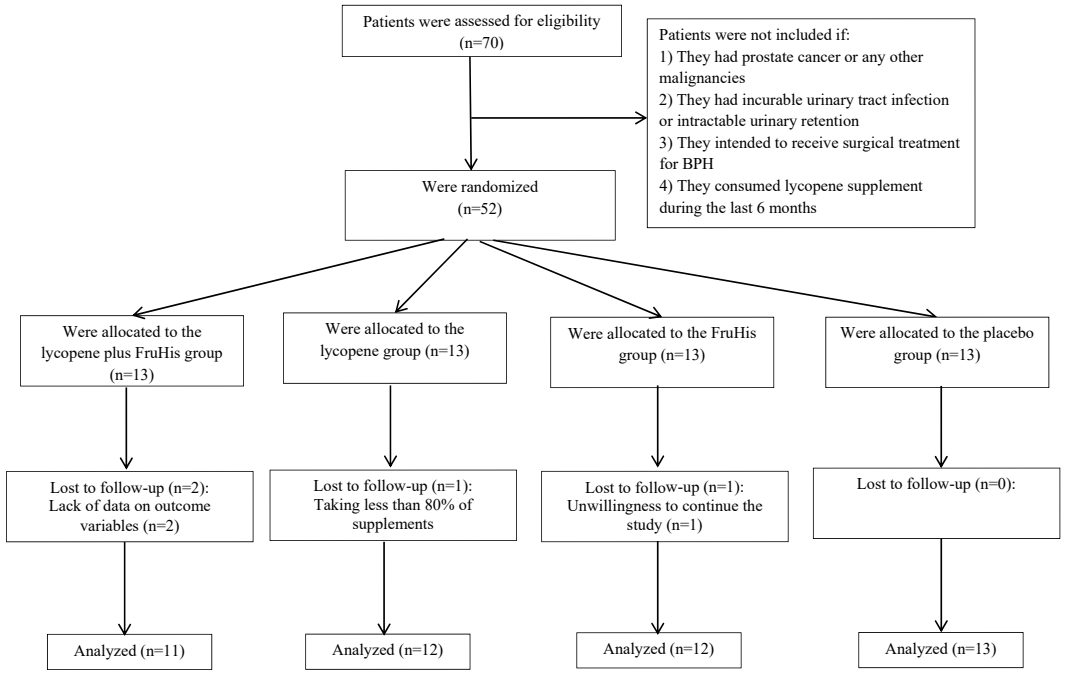


FIGURE 1
The flowchart of the current study.

of participants with the same age and BMI were stated in a lottery container, and finally, they were randomly assigned to the four intervention groups. Random allocation was performed by an independent person who was unaware of the aim of our study. Patients in the lycopene plus FruHis group received two capsules per day; one contained 25 mg/day of lycopene and one contained 10 mg/day of FruHis. Patients in the lycopene group received one capsule containing 25 mg/day of lycopene and one placebo capsule per day. Patients in the FruHis group received one capsule containing 10 mg/day of FruHis and one placebo capsule every day. In the placebo group, patients received two placebo capsules per day. Placebo capsules contained starch. At the baseline study, all capsules that should be consumed during the study were given to patients. The length of the intervention was 8 weeks because previous studies have shown that lycopene supplementation during 4–8 weeks with a dosage between 15 and 30 mg/day reduced BPH outcomes such as serum levels of PSA (19). However, there is no evidence of the best dosage of lycopene. In the current study, we aimed to answer the question: Does FruHis increase the beneficial effect of lycopene with the same dosage and intervention duration compared with the previous studies? Since we found no study on FruHis supplementation and given that the FruHis dosage in cooked tomato is less than lycopene (18), we administered 10 mg/day FruHis in the current trial.

Compliance to study protocol

To increase the compliance of participants, phone messages were sent to participants to remind the consumption of prescribed supplements on weekly base time points. In addition to the message, taking supplements was monitored by phone calls every other week. Participants were asked to return the empty capsule packs at end of the trial, to make sure that the boxes are empty. Participant's compliance was assessed using the following formula: (number of used capsules/all given capsules) × 100. Acceptable compliance with the study was considered as 80% or over. We assessed primary and secondary outcome variables at the study baseline and end of the trial.

To assess dietary intakes during the study, participants were asked to fill a 3-day dietary record (**Supplementary file**, section A) in the first 2 weeks and a 3-day dietary record in the last 2 weeks. The 3-day records included 2 weekdays and a weekend. Participants were asked to fulfill the dietary records based on household measures and then, the household measures were converted to grams using available booklets for domestic foods. The mean dietary intakes in the six dietary records were considered as usual dietary intakes during the study. Moreover, the dietary intakes (in grams) were converted to nutrient intakes using the US National Nutrient Databank modified for Iranian Foods (20, 21). In addition to dietary intake, the physical activity throughout the intervention period was assessed two times using 1-day physical activity records; one in the first 2 weeks and one in the last 2 weeks of the intervention (**Supplementary file**, section B). All the participants were trained to fill out their physical activity records. Participants were asked not to change their physical activity and dietary intake during the study.

Preparation of supplements

Lycopene and placebo supplements were provided by the Pourateb Pharmaceutical Company, Tehran, Iran. To prepare FruHis, the method developed by Mossine et al. (18, 22) was used. Briefly, FruHis is synthesized from an aqueous solution of food-grade L-histidine, glacial acetic acid, and D-glucose. Then, the solution was purified to provide non-hygroscopic crystalline powder, which was free of any detectable impurities. The melting point was determined on a Kofler hot stage apparatus. The IR spectra (KBr disks) were recorded by the Nicolet FT-IR Magna 550 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using the Varian-INOVA 500 MHz instrument. Mass analysis of the compound was determined with the Agilent Technology (HP), Electron Impact 70 eV. Nα-(1-Deoxy-D-fructos-1-yl)-L-histidine. Mp: 130–140 (dec.); IR (KBr, cm^{-1}) ν_{max}: 3,300 (NH), 2,930 (C-H), 1,621 (C = O); ^{1}H NMR (D_2O, 500 MHz) δ: 8.53 (s, 1H, Imidazolyl), 7.27 (s, 1H, Imidazolyl), 4.07–3.32 (m, β-pyranose and α-β-furanose forms). ^{13}C NMR (D_2O, 125 MHz) δ: 171.1, 133.8, 126.6, 117.8, 95.1, 73.9, 69.7, 69.1, 68.7, 63.8, 61.6, 24.3.

The appearance of the FruHis capsules, such as color, shape, size, and packaging, was identical to the lycopene and placebo capsules.

Assessment of variables

A researcher-made questionnaire was used to collect data on age, education, marital status, ethnicity, economic status, smoking, medical history, and nutritional supplement use at the study baseline. At the baseline and end of the trial, the primary outcome variables including serum levels of total PSA (TPSA), free PSA (FPSA), FPSA/TPSA ratio, insulin-like growth factor-1 (IGF-1), disease severity, quality of life (QoL), and secondary outcome variables including body mass index (BMI) and waist circumference (WC) were measured.

Biochemical assessments

After 8 h of fasting, 5 mL venous blood samples were collected from each participant at the beginning and end of the trial. After sampling, serum was extracted from the blood sample and then, was stored at −70°C until further analysis. TPSA and FPSA were measured using the commercial kits of chemiluminescence enzyme immunoassay (CLEIA) (Roche, Germany). Also, serum concentrations of IGF-1 were measured

using the commercial kits of enzyme-linked immunosorbent assay (ELISA) (Mediagnost, Germany).

Assessment of symptoms and quality of life

American Urological Association (AUA) symptom index was used to assess the obstructive and irritative voiding symptoms (23). The AUA symptom index contains seven items evaluating BPH symptoms including incomplete emptying, frequency, intermittency, urgency, weak stream, hesitancy, and nocturia. Each item can be scored from 0 to 5. By summing up the scores, a score between 1 and 35 is achieved. In this scoring outcome measure, greater scores show higher severity of BPH symptoms. The total score of BPH symptoms was considered as an outcome variable in the current study. In addition to the seven items mentioned above, the updated version of the AUA symptom index contains one disease-specific QoL question as follows (23): If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that? The response categories for this question were "delighted" (score 0), "pleased" (score 1), "mostly satisfied" (score 2), "mixed" (score 3), "mostly dissatisfied" (score 4), "unhappy" (score 5), and "terrible" (score 6). In the current study, we considered the score of QoL improvement as an outcome variable. QoL improvement was considered as changing the scores from 4–6 to 0–3 throughout the trial.

The World Health Organization (WHO) confirmed the reliability and validity of the updated version of the AUA symptom index and considered it the International Prostate Symptom Score (IPSS) (24). The IPSS was translated to Persian and the validity and reliability of this version were confirmed in Panahi et al. study (25).

Anthropometric measurements

All anthropometric measures were assessed according to the US National Institutes of Health protocols (26). Weight was measured using a digital scale at the state of minimum clothing without shoes to the nearest 100 g. Height was measured using a standard stadiometer, without shoes, to the nearest 0.5 cm. BMI was calculated as weight (kg)/height (m^2). WC was measured using a strip meter at mid-distance intervals between the super elliptic bone and the last gear, to the nearest 0.5 cm.

Statistical analysis

The normality of the distribution of outcome variables was assessed using the Kolmogorov–Smirnov test. Among baseline and dietary data, three variables including age and dietary intakes of vitamin D and selenium were not normal-distributed and therefore we used non-parametric tests for these variables. Among outcome variables, the distribution of TPSA and BMI was not normal. We normalized these variables using the log transformation to avoid using non-parametric tests for outcome variables. To examine differences in continuous baseline and dietary variables across the four intervention groups, one-way analysis of variance (ANOVA) (for normal-distributed variables) and Kruskal–Wallis (for non-normally distributed variables) were employed. In addition, the Chi-square test is used to assess the distribution of categorical variables across the four intervention groups. To assess the effect of interventions on outcome variables in each group, a paired-sample *t*-test was used. Moreover, to compare changes in outcome variables across the four intervention groups, the ANOVA test was employed. The two-by-two comparison was done using the Bonferroni test. In addition, multivariate analysis of covariance (ANCOVA), as a general linear model, was used to examine the effects of lycopene and FruHis supplementation on outcome variables. In this analysis, we controlled for baseline values of outcome variables to detect an independent effect. All statistical analyses were conducted using the SPSS software version 18 (SPSS, Inc. Chicago, IL, USA). $P < 0.05$ will be considered significant.

Results

Characteristics of the participants

From all recruited patients ($n = 52$), four were excluded due to taking less than 80% of supplements ($n = 1$), unwillingness to continue the study ($n = 1$), and lack of data on outcome variables ($n = 2$). In total, 48 patients with complete data were included in the statistical analysis: 11 patients in the lycopene plus FruHis group, 12 patients in the lycopene group, 12 patients in the FruHis group, and 13 patients in the placebo group. Regarding adherence to interventions, it is found that all patients included in the analysis consumed 100% of the capsules throughout the study period.

The baseline characteristics of participants in the four intervention groups are shown in **Table 1**. We found no significant difference between the four intervention groups in terms of demographic variables, smoking habits, anthropometric measures, disease history, and supplement consumption history. In addition, the daily dietary intakes of participants throughout the trial, particularly tomato and its products, were not different across the four intervention groups (**Table 2**).

Intervention

The influences of lycopene and FruHis supplementation on primary and secondary outcome variables are indicated in **Table 3**. Our results showed that placebo and FruHis alone

TABLE 1 Baseline characteristics of participants in the intervention groups.

Variables	Lycopene plus FruHis group (n = 11)	Lycopene group (n = 12)	FruHis group (n = 12)	Placebo group (n = 13)	P-value*
Age (year)	65.00 ± 9.64	62.16 ± 6.17	64.61 ± 9.13	65.15 ± 7.17	0.78
Weight (kg)	77.48 ± 14.57	77.90 ± 21.56	74.56 ± 11.73	80.14 ± 15.00	0.86
BMI (kg/m^2)	25.99 ± 3.52	25.98 ± 4.66	25.64 ± 3.78	26.27 ± 4.80	0.98
WC (cm)	97.72 ± 9.00	98.25 ± 12.85	100.25 ± 8.85	101.81 ± 9.47	0.77
University educated (%)	54.5	33.3	41.7	53.8	0.68
Fars ethnicity (%)	72.7	75.0	75.0	84.6	0.89
Economic status (weak) (%)	27.3	58.3	58.3	23.1	0.13
Current smoker (%)	9.1	41.7	0	30.8	0.05
Married (%)	100	91.7	91.7	76.9	0.30
PA (Met-h/wk)	2277.7 ± 423.3	2264.4 ± 605.5	2189.5 ± 310.7	2392.4 ± 442.3	0.78
Supplement use (%)	54.5	50.0	50.0	46.2	0.98
Disease history					
CVD (%)	45.5	33.3	25.0	30.8	0.76
Hypertension (%)	9.1	25.0	16.7	7.7	0.60
Diabetes (%)	45.5	33.3	16.7	15.4	0.30
Thyroid diseases (%)	9.1	8.3	25.0	15.4	0.63

Data are presented as mean (± SD) or percent. BMI, body mass index; WC, waist circumference; CVD, cardiovascular disease; PA, physical activity.
*Obtained from one-way analysis of variance (ANOVA) (for normal-distributed continuous variables) and Kruskal–Wallis (for non-normally distributed continuous variables) or Chi-square test (for categorical variables).

TABLE 2 Dietary intakes of participants throughout the trial in the intervention groups.

Variables	Lycopene plus FruHis group (n = 11)	Lycopene group (n = 12)	FruHis group (n = 12)	Placebo group (n = 13)	P-value*
Energy (Kcal)	1473 ± 275	1313 ± 329	1443 ± 234	1392 ± 476	0.71
Protein (g/day)	52.75 ± 11.31	48.08 ± 11.35	52.96 ± 11.69	52.41 ± 17.42	0.78
Carbohydrate (g/day)	189.10 ± 45.10	164.43 ± 45.67	177.32 ± 30.90	175.20 ± 61.27	0.66
Fat (g/day)	58.45 ± 14.68	53.15 ± 14.73	60.13 ± 10.62	55.23 ± 20.45	0.69
SFA (g/day)	29.46 ± 6.86	28.72 ± 8.85	26.85 ± 8.49	28.01 ± 8.60	0.89
PUFA (g/day)	42.37 ± 10.93	39.73 ± 10.11	39.64 ± 15.42	39.75 ± 9.87	0.93
Fiber (g/day)	10.83 ± 2.81	10.00 ± 3.02	10.67 ± 3.48	10.19 ± 3.22	0.90
Vitamin C (mg/day)	113.34 ± 44.82	95.54 ± 63.99	130.32 ± 62.23	133.01 ± 76.01	0.44
Vitamin E (mg/day)	3.96 ± 2.52	3.80 ± 1.41	3.66 ± 1.30	4.42 ± 2.10	0.76
Vitamin D (mg/day)	0.58 ± 1.19	0.45 ± 0.96	0.11 ± 0.18	0.51 ± 1.00	0.31
Iron (mg/day)	21.09 ± 5.59	18.56 ± 5.73	17.51 ± 6.43	19.85 ± 6.33	0.52
Selenium (mg/day)	0.03 ± 0.04	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.03	0.39
Tomato and its products (g/day)	20.06 ± 15.24	14.70 ± 14.05	21.21 ± 17.55	14.67 ± 11.99	0.56

Data are presented as mean (± SD). SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid.
*Obtained from one-way analysis of variance (ANOVA) (for normal-distributed variables) and Kruskal–Wallis (for non-normally distributed variables).

did not induce any change in TPSA, while lycopene induced a 13.2% reduction (-0.64 ± 2.02 ng/mL) in TPSA. However, when patients consumed a combination of FruHis and lycopene, FruHis increased the effect of lycopene from 13 to 30.3% (-1.49 ± 4.78 ng/mL) (Table 3 and Figure 2). This reduction was not significant compared with the placebo group and the intake of lycopene and FruHis alone. Also, we observed the same pattern for IGF-1, symptom score, BMI, and WC, when FruHis improved the effect of lycopene on IGF-1 from 11.3 to 18% (-54.47 ± 28.36 ng/mL in the lycopene + FruHis group vs. −30.24 ± 46.69 ng/mL in the lycopene group), on symptom score from 14.5 to 32% (-4.45 ± 4.03 in the lycopene + FruHis group vs. −1.66 ± 5.41 in the lycopene group), on BMI from 0.38 to 2.1% (-0.55 ± 0.90 kg/m^2 in the lycopene + FruHis group vs. −0.10 ± 0.30 kg/m^2 in the lycopene group), and on WC from 0.2 to 0.7% (-0.68 ± 0.46 cm in the lycopene + FruHis group vs. −0.20 ± 0.86 cm in the lycopene group) (Figure 2 and Table 3). Comparing the end

of the trial with baseline values, the observed changes in IGF-1, symptom score, and WC were statistically significant in the lycopene plus FruHis group (Table 3). However, between-group comparisons led to non-significant results. For FPSA, no significant change was seen in all intervention groups. In terms of FPSA/TPSA ratio, when comparing baseline and end-of-trial values, the effect of lycopene supplementation alone was more than the combination of lycopene and FruHis (5.44 ± 7.59% in the lycopene plus FruHis group vs. 2.77 ± 10.24% in the lycopene group); however, this difference was not statistically significant (Table 3). When the analyses were adjusted for baseline values of outcome variables, the reducing effect of lycopene plus FruHis supplementation on IGF-1 remained significant (Table 4).

In terms of QoL, an improvement was seen in the lycopene plus FruHis (18.2%), lycopene (33.3%), and FruHis (33.3%) groups without any remarkable changes in the placebo group (7.7%) (Figure 2).

TABLE 3 Means of outcome variables at baseline and end of the trial and changes during the trial in the intervention groups.

	Week 0	Week 8	Mean change	Change %	*P**	*P***
IGF-1 (ng/mL)						0.12
Lycopene plus FruHis group (n = 11)	302.66 ± 87.24	248.18 ± 73.17	−54.47 ± 28.36	18.0	<0.001	
Lycopene group (n = 12)	265.56 ± 51.29	235.31 ± 54.53	−30.24 ± 46.69	11.3	0.04	
FruHis group (n = 12)	318.04 ± 63.63	309.98 ± 55.17	−8.06 ± 38.00	2.5	0.47	
Placebo group (n = 13)	277.72 ± 85.63	265.35 ± 43.85	−12.37 ± 73.05	4.4	0.55	
FPSA (ng/mL)						0.47
Lycopene plus FruHis group (n = 11)	0.73 ± 0.48	0.70 ± 0.43	−0.02 ± 0.35	2.7	0.84	
Lycopene group (n = 12)	0.71 ± 0.19	0.91 ± 0.51	0.20 ± 0.57	28.1	0.24	
FruHis group (n = 12)	0.84 ± 0.71	0.93 ± 0.45	0.10 ± 0.35	11.9	0.34	
Placebo group (n = 13)	0.59 ± 0.32	0.59 ± 0.33	−0.004 ± 0.19	0.67	0.93	
TPSA (ng/mL)						0.51
Lycopene plus FruHis group (n = 11)	4.91 ± 5.27	3.42 ± 1.94	−1.49 ± 4.78	30.3	0.32	
Lycopene group (n = 12)	4.82 ± 1.68	4.18 ± 1.39	−0.64 ± 2.02	13.2	0.29	
FruHis group (n = 12)	4.77 ± 3.67	4.76 ± 2.69	−0.002 ± 1.59	0	0.99	
Placebo group (n = 13)	4.27 ± 2.82	4.19 ± 3.01	−0.07 ± 1.00	1.6	0.78	
FPSA/TPSA (%)						0.72
Lycopene plus FruHis group (n = 11)	18.76 ± 10.30	21.53 ± 5.82	2.77 ± 10.24	14.7	0.39	
Lycopene group (n = 12)	15.56 ± 3.99	21.00 ± 7.20	5.44 ± 7.59	35.0	0.03	
FruHis group (n = 12)	18.59 ± 7.85	21.24 ± 7.28	2.64 ± 4.06	14.2	0.04	
Placebo group (n = 13)	15.58 ± 6.67	17.69 ± 11.36	2.11 ± 8.20	13.5	0.37	
Symptom score[a]						0.12
Lycopene plus FruHis group (n = 11)	13.90 ± 7.73	9.45 ± 7.72	−4.45 ± 4.03	32.0	0.004	
Lycopene group (n = 12)	11.41 ± 5.08	9.75 ± 6.28	−1.66 ± 5.41	14.5	0.30	
FruHis group (n = 12)	18.25 ± 8.82	12.12 ± 8.64	−6.12 ± 7.27	33.5	0.01	
Placebo group (n = 13)	11.07 ± 7.91	9.81 ± 7.15	−1.26 ± 5.39	11.3	0.41	
BMI (kg/m^2)						0.17
Lycopene plus FruHis group (n = 11)	25.99 ± 3.52	25.43 ± 3.29	−0.55 ± 0.90	2.1	0.06	
Lycopene group (n = 12)	25.98 ± 4.66	25.87 ± 4.70	−0.10 ± 0.30	0.38	0.24	
FruHis group (n = 12)	25.95 ± 3.83	25.86 ± 3.90	−0.08 ± 0.20	0.30	0.23	
Placebo group (n = 13)	27.12 ± 4.69	26.91 ± 4.69	−0.21 ± 0.50	0.77	0.19	
WC (cm)						0.38
Lycopene plus FruHis group (n = 11)	98.40 ± 9.29	97.72 ± 9.00	−0.68 ± 0.46	0.69	0.001	
Lycopene group (n = 12)	98.45 ± 12.61	98.25 ± 12.85	−0.20 ± 0.86	0.20	0.42	
FruHis group (n = 12)	100.40 ± 9.04	100.25 ± 8.85	−0.15 ± 0.41	0.15	0.27	
Placebo group (n = 13)	102.27 ± 9.42	101.81 ± 9.47	−0.45 ± 1.12	0.44	0.21	

Data are presented as mean (± SD). IGF, insulin-like growth factor; FPSA, free prostate-specific antigen; TPSA, total prostate-specific antigen; IPSS, international prostate symptom score; BMI, body mass index; WC, waist circumference.

[a]Obtained from IPSS.

*Obtained from the paired sample t-test.

**Obtained from the one-way analysis of variance (ANOVA). The two-by-two comparison was done using the Bonferroni test.

TABLE 4 Adjusted mean changes of outcome variables throughout the trial in the intervention groups.

Variables	Lycopene plus FruHis group (n = 11)	Lycopene group (n = 12)	FruHis group (n = 12)	Placebo group (n = 13)	P*
IGF-1	−49.17 ± 11.94[a]	−41.09 ± 11.57	3.92 ± 11.61	−17.92 ± 10.99	0.01
FPSA	−0.016 ± 0.10	0.20 ± 0.09	0.15 ± 0.10	−0.05 ± 0.09	0.20
TPSA	−1.36 ± 0.54	−0.56 ± 0.52	0.04 ± 0.52	−0.30 ± 0.50	0.42
FPSA/TPSA (%)	3.49 ± 2.18	4.81 ± 2.09	3.29 ± 2.09	1.49 ± 2.01	0.71
Symptom score[a]	−4.36 ± 1.59	−2.30 ± 1.54	−4.78 ± 1.60	−1.99 ± 1.49	0.51
BMI	−0.56 ± 0.16	−0.11 ± 0.16	−0.08 ± 0.17	−0.20 ± 0.16	0.17
WC	−0.68 ± 0.24	−0.21 ± 0.23	−0.14 ± 0.25	−0.44 ± 0.24	0.38

Data are presented as mean (± SE) adjusted for baseline values of outcome variables. IGF, insulin-like growth factor; FPSA, free prostate-specific antigen; TPSA, total prostate-specific antigen; IPSS, international prostate symptom score; BMI, body mass index; WC, waist circumference.
[a]Obtained from IPSS and significant compared with the FruHis group.
*Obtained from one-way analysis of covariance (ANCOVA). The two-by-two comparison was done using the Bonferroni test.

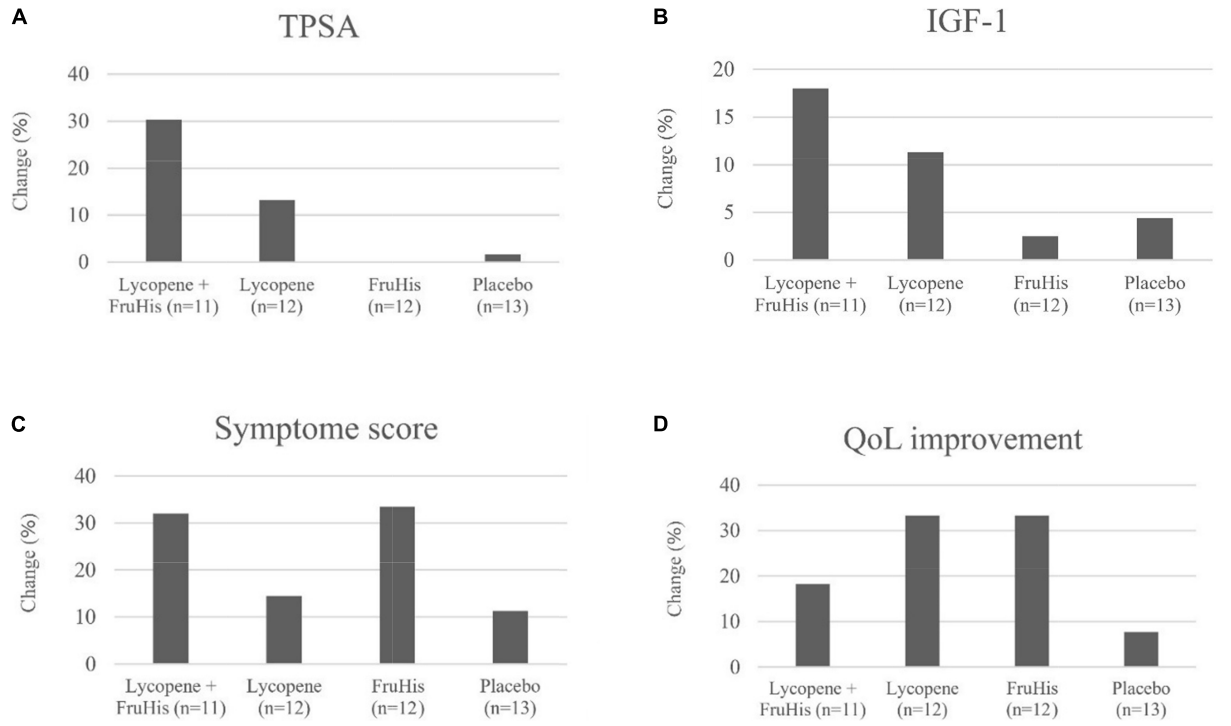


FIGURE 2
The effects of lycopene and FruHis supplementation, combined and alone, on TPSA **(A)**, IGF-1 **(B)**, symptom score **(C)**, and QoL improvement **(D)** among patients with BPH. QoL improvement was considered as changing the scores of 4–6 to 0–3 (based on QoL question in IPSS) throughout the trial. IPSS, international prostate symptom score; QoL, quality of life; TPSA, total prostate-specific antigen; IGF, insulin-like growth factor; BPH, benign prostatic hyperplasia.

Discussion

Based on the current literature review, this was the first study examining the effects of lycopene and FruHis supplementation, combined and alone, on laboratory parameters, disease symptoms, and QoL of patients with BPH. We found that intake of FruHis increases the beneficial effects of lycopene on TPSA, IGF-1, symptom score, BMI, and WC in patients with BPH. However, this increase for mentioned variables, except for IGF-1, was statistically non-significant compared with the placebo and intakes of lycopene and FruHis alone.

Benign prostatic hyperplasia is one of the most common diseases among men that is associated with chronic and progressive lower urinary tract symptoms (LUTS) or chronic complications, resulting in many health complications for men (27–29). In recent decades, many researchers have shown

the beneficial effects of tomato or lycopene consumption on clinical outcomes of BPH patients (30). However, there is evidence indicating other constituents of tomato, particularly heat-processed tomato products, may have a role in the beneficial effect (12). Obviously, due to the heating process, there will be an increased bioaccessibility and bioavailability of lycopene and other lipid-soluble compounds compared to raw tomatoes. FruHis is a ketosamine that is produced during the heat processing of tomato products by attaching histidine to fructose (18). However, according to the previous studies, it is not clear if FruHis is responsible for the anti-BPH effects of tomatoes. In the current study, we found that when FruHis was combined with lycopene, it induces a non-significant decrease in TPSA from 13.2 to 30.3%. This effect is in the line with previous findings on heat-processed tomato products containing a high amount of FruHis and lycopene. Paur et al. reported that 3 weeks of interventions with tomato products reduces serum concentrations of PSA in patients with BPH (31). Moreover, in a review article, Basu et al. concluded that consumption of processed tomato products has a higher PSA-reducing effect compared to the intake of lycopene supplements alone (32). It should be noticed that there are several studies indicating a beneficial effect of lycopene supplementation alone on PSA levels. In a randomized clinical trial, Schwarz et al. reported that 24 weeks of lycopene supplementation (15 mg/day) significantly reduces TPSA in men with BPH (33). The same significant reduction was reported by another study on men with high-grade prostatic intraepithelial neoplasia (HGPIN) after an intervention with 8 mg/day of lycopene for 48 weeks (34). In the current study, it must be noticed that the reducing effect of lycopene + FruHis supplementation on TPSA was not statistically significant compared to the placebo group. This might be explained by the low sample size of the current pilot study. In addition, the low dosage of lycopene and FruHis might be another reason. As seen in **Table 1**, the mean BMI of study participants was >25 kg/m^2, and therefore, 25 mg/day of lycopene and 10 mg/day FruHis might be inadequate for patients with overweight. In line with this claim, Cumar et al. assessed the effects of supplementation with 30 and 45 mg/day of lycopene on BPH patients with overweight and reported a non-significant effect compared with a placebo group (35). In addition, age is another factor affecting the efficacy of lycopene. Older patients may have a lower capacity for lycopene digestion and absorption, and therefore, need higher amounts of lycopene compared with younger patients.

In the current study, we found that FruHis supplementation increases the reducing effect of lycopene on IGF-1 concentrations from 11.3 to 18%. In the same line with our findings, Riso et al. reported a significant reducing effect on IGF-1 levels in healthy subjects due to the consumption of tomato drinks with a high amount of lycopene and probable content of FruHis (36). However, this significant effect was not seen in the study by Gann et al. who investigated the influence of lycopene-rich tomato extract on IGF-1 (37). The lack of significant effect in the Gann et al. study might be explained by a probable low amount of FruHis in the tomato extract administered. Overall, it seems that the combined intake of lycopene and FruHis, similar to the intake of tomato products containing both lycopene and FruHis, has a better reducing effect on IGF-1 compared to their intakes alone. In the current study, the baseline values of IGF-1 in the lycopene plus FruHis group were higher than the values in the lycopene group. This might be a reason for the greater reduction of IGF-1 in the combination group compared with the lycopene alone. However, when we adjusted the analyses for the baseline values of IGF-1, the significant effect of lycopene plus FruHis intake on IGF-1 remained significant.

The exact mechanisms through which lycopene and FruHis affect serum IGF-1 and TPSA levels are unknown. Although the physiological effects of FruHis have not been studied yet, there are some studies on lycopene (38, 39). It is proposed that lycopene contributes to some physiological pathways through inhibition of cell cycle progression, interleukin-6 expression, and androgen activation and signaling (38, 39). Hence, lycopene interferes with estrogen and androgen signaling, which has been proven to influence the production of IGF-1 and TPSA (40, 41). Also, oxidative stress contributes to the pathophysiology of BPH (42). Therefore, the antioxidant properties of lycopene may have another mechanism involved in the protective effects of lycopene on BPH. In the current study, FruHis intake strengthened the beneficial effects of lycopene. In an experimental study on rat prostate tumorigenesis, it had been shown that FruHis exerts antioxidant and anti-cancer properties (12). Since both lycopene and FruHis have antioxidant and anti-cancer effects, FruHis intake may have a synergistic effect on the beneficial effects of lycopene.

We also found that a combination of lycopene and FruHis intake, compared to lycopene intake alone, had a better reducing effect on symptom scores in BPH men. In addition, we found an improvement in QoL in the lycopene plus FruHis group compared to the placebo group. However, these positive effects were not statistically significant, and the size of changes is clinically notable. In agreement with our findings, Cormio et al. reported that consumption of whole tomato food supplement (WTFS), which may contain both lycopene and FruHis, decreases the symptom score from 9.05 to 7.15 and improves QoL, about one score, in patients with BPH (43). In the mentioned study, these changes were not significant compared to the control group. Both our study and Cormio et al. study had a low sample size and short duration of intervention that which may explain the non-significant positive effects of lycopene plus FruHis compared to the control group.

It must be kept in mind that our findings might be affected, to some extent, by the baseline characteristics of participants. For instance, patients in the lycopene group were more likely to

be smokers and have hypertension compared with the lycopene plus FruHis group. This may explain the lower reduction of IGF-1 in the lycopene group compared with the lycopene plus FruHis group. Cigarette smoking can increase oxidative stress and inflammation and attenuate the beneficial effects of lycopene (44). In addition, a significant association between hypertension and IGF-1 levels was reported in previous studies (45). Therefore, our findings on the higher beneficial effects of lycopene plus FruHis intake, compared with the intake of lycopene alone, should be considered with caution. In addition, patients in the lycopene plus FruHis group had a higher prevalence of diabetes compared with other intervention groups. There is evidence indicating significant associations of diabetes with serum levels of PSA and the risk of PC (46, 47). The high prevalence of diabetes in the combined intervention may attenuate the reducing effects of lycopene plus FruHis intake on TPSA levels and this may explain why the TPSA-reducing effect of the combined treatment was not significant compared with the placebo. Further studies are required to confirm our findings.

Strengths and limitations

This study had some strengths. In this study, we performed a comprehensive assessment (laboratory parameters, anthropometric measures, disease symptoms, and QoL) of patients with BPH before and after the trial. Participants in the four intervention groups were matched in terms of age and BMI and they were randomly allocated to the groups. Moreover, adherence to the interventions was high in our study, against the limitation by COVID-19 pandemic. Some limitations of our study should be taken into account. The most important limitation of this study is the low sample size. The main reason for the low sample size was limited funding as well as patient accessibility limitations due to the COVID-19 pandemic which made us unable to include a greater sample size. However, the number of patients included in the current study was reasonable for a pilot study. It should be noted that four patients were excluded during the trial and this dropout may affect the power of our study to detect significant effects. Also, the dropout among the intervention groups was different so the lycopene plus FruHis group contained 11 patients compared with the placebo group with 13 patients. In addition, due to limited funding, we could not assess tissue biomarkers of lycopene and FruHis to evaluate the influence of supplementation on tissue circulation of these compounds. In the current study, we prescribed lycopene and FruHis supplement as the two constituents of tomato products. It must be kept in mind that tomato products might have other effective constituents that influence BPH outcomes. Therefore, the effects of cooked tomato consumption, as a dietary intake on BPH outcomes might be different compared to the supplementation of lycopene and FruHis.

Conclusion

FruHis intake strengthens the reducing effects of lycopene on IGF-1, TPSA, symptom score, BMI, and WC among patients with BPH. Except for IGF-1, these reductions were not statistically significant compared with the placebo, and the intakes of lycopene and FruHis alone, however, were clinically important. Such findings were not seen for FPSA and FPSA/TPSA ratios. Further clinical trials are needed to assess the effects of different dosages of lycopene and FruHis on BPH outcomes. In addition, future studies should examine the influence of other constituents in tomato/its products on BPH outcomes.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.951). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AlS, AhS, MN, and MS designed the study. AlS, AmF, and EA collected the data. MY analyzed the data. AlS, AhS, and AlF drafted the manuscript. LJ-B and MK synthesized FruHis. All authors provided critical input and approved the final version of the manuscript.

Funding

This study was financially supported by a grant from the Tehran University of Medical Sciences, Tehran, Iran with code 97-03-161-40103.

Acknowledgments

We would like to express our heartfelt thanks to all the volunteers who participated in the study.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1011836/full#supplementary-material

References

1. Zeng XT, Jin YH, Liu TZ, Chen FM, Ding DG, Fu M, et al. Clinical practice guideline for transurethral plasmakinetic resection of prostate for benign prostatic hyperplasia (2021 Edition). *Mil Med Res.* (2022) 9:14. doi: 10.1186/s40779-022-00371-6

2. Chughtai B, Forde JC, Thomas DD, Laor L, Hossack T, Woo HH, et al. Benign prostatic hyperplasia. *Nat Rev Dis Primers.* (2016) 2:16031.

3. Devlin CM, Simms MS, Maitland NJ. Benign prostatic hyperplasia – what do we know? *BJU Int.* (2021) 127:389–99.

4. Tong Y, Xie K, Li S. Self-care and quality of life in elderly Chinese patients with benign prostatic hyperplasia. *Nurs Sci Q.* (2020) 33:79–84. doi: 10.1177/0894318419883417

5. Tacklind J, Fink HA, Macdonald R, Rutks I, Wilt TJ. Finasteride for benign prostatic hyperplasia. *Cochrane Database Syst Rev.* (2010) 2010:Cd006015.

6. Wilt, TJ, Howe RW, Rutks IR, MacDonald R. Terazosin for benign prostatic hyperplasia. *Cochrane Database Syst Rev.* (2002):Cd003851.

7. Bacanli M, Başaran N, Başaran AA. Lycopene: Is it beneficial to human health as an antioxidant? *Turk J Pharm Sci.* (2017) 14:311–8.

8. Qi WJ, Sheng WS, Peng C, Xiaodong M, Yao TZ. Investigating into anti-cancer potential of lycopene: Molecular targets. *Biomed Pharmacother.* (2021) 138:111546.

9. Wertz K, Siler U, Goralczyk R. Lycopene: Modes of action to promote prostate health. *Arch Biochem Biophys.* (2004) 430:127–34.

10. Linnewiel-Hermoni K, Khanin M, Danilenko M, Zango G, Amosi Y, Levy J, et al. The anti-cancer effects of carotenoids and other phytonutrients resides in their combined activity. *Arch Biochem Biophys.* (2015) 572:28–35. doi: 10.1016/j.abb.2015.02.018

11. Sharoni Y, Danilenko M, Dubi N, Ben-Dor A, Levy J. Carotenoids and transcription. *Arch Biochem Biophys.* (2004) 430:89–96.

12. Mossine VV, Chopra P, Mawhinney TP. Interaction of tomato lycopene and ketosamine against rat prostate tumorigenesis. *Cancer Res.* (2008) 68:4384–91. doi: 10.1158/0008-5472.CAN-08-0108

13. Jacob K, Garcia-Alonso FJ, Ros G, Periago MJ. Stability of carotenoids, phenolic compounds, ascorbic acid and antioxidant capacity of tomatoes during thermal processing. *Arch Latinoam Nutr.* (2010) 60:192–8.

14. Nicoli MC, Anese M, Parpinel MT, Franceschi S, Lerici CR. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett.* (1997) 114:71–4.

15. Gahler S, Otto K, Böhm V. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *J Agric Food Chem.* (2003) 51:7962–8.

16. Giovannucci E. A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp Biol Med.* (2002) 227:852–9.

17. Finot PA. Historical perspective of the Maillard reaction in food science. *Ann N Y Acad Sci.* (2005) 1043:1–8. doi: 10.1196/annals.1333.001

18. Mossine VV, Mawhinney TP. Nalpha-(1-deoxy-D-fructos-1-yl)-L-histidine ("D-Fructose-L-histidine"): A potent copper chelator from tomato powder. *J Agric Food Chem.* (2007) 55:10373–81. doi: 10.1021/jf072092i

19. Grammatikopoulou MG, Gkiouras K, Papageorgiou S, Myrogiannis I, Mykoniatis I, Papamitsou T, et al. Dietary factors and supplements influencing prostate specific-antigen (PSA) Concentrations in men with prostate cancer and increased cancer risk: An evidence analysis review based on randomized controlled trials. *Nutrients.* (2020) 12:2985. doi: 10.3390/nu12102985

20. Martin CL, Montville JB, Steinfeldt LC, Omolewa-Tomobi G, Heendeniya KY, Adler ME, et al. *USDA food and nutrient database for dietary studies 2011–2012: Documentation and user guide.* Beltsville, MD: US Department of Agriculture (2012).

21. Motlagh AD, Tabatabaei M. *Iranain food composition table.* Tehran: Iran Donyaye Taghzieh Press (2007).

22. Mossine VV, Barnes CL, Mawhinney TP. Solubility and crystal structure of N-(1-deoxy-beta-D-fructopyranos-1-yl)-l-histidine monohydrate ('D-fructose-l-histidine'). *Carbohydr Res.* (2007) 342:131–8. doi: 10.1016/j.carres.2006.11.011

23. Liu CC, Wang CJ, Huang SP, Chou YH, Wu WJ, Huang CH. Relationships between American urological association symptom index, prostate volume, and disease-specific quality of life question in patients with benign prostatic hyperplasia. *Kaohsiung J Med Sci.* (2004) 20:273–8. doi: 10.1016/S1607-551X(09)70118-4

24. Bosch JL, Hop WC, Kirkels WJ, Schröder FH. The international prostate symptom score in a community-based sample of men between 55 and 74 years of age: Prevalence and correlation of symptoms with age, prostate volume, flow rate and residual urine volume. *Br J Urol.* (1995) 75:622–30. doi: 10.1111/j.1464-410x.1995.tb07421.x

25. Panahi A, Bidaki R, Mehraban D, Rezahosseini O. Validity and reliability of Persian version of international prostate symptom score. *Galen Med J.* (2013) 2:18–21.

26. Patry-Parisien J, Shields M, Bryan S. Comparison of waist circumference using the world health organization and national institutes of health protocols. *Health Rep.* (2012) 23:53–60.

27. Langan RC. Benign prostatic hyperplasia. *Primary Care.* (2019) 46:223–32.

28. Kim EH, Larson JA, Andriole GL. Management of benign prostatic hyperplasia. *Annu Rev Med.* (2016) 67:137–51.

29. Sadeghi A, Sadeghi O, Khodadost M, Pirouzi A, Hosseini B, Saedisomeolia A. Dietary glycemic index and glycemic load and the risk of prostate cancer: An updated systematic review and dose-response meta-analysis. *Nutr Cancer.* (2020) 72:5–14. doi: 10.1080/01635581.2019.1621356

30. Sharifi-Zahabi E, Soltani S, Malekahmadi M, Rezavand L, Clark CCT, Shidfar F. The effect of lycopene supplement from different sources on prostate specific antigen (PSA): A systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med.* (2022) 64:102801. doi: 10.1016/j.ctim.2022.102801

31. Paur I, Lilleby W, Bøhn SK, Hulander E, Klein W, Vlatkovic L, et al. Tomato-based randomized controlled trial in prostate cancer patients: Effect on PSA. *Clin Nutr.* (2017) 36:672–9.

32. Basu A, Imrhan V. Tomatoes versus lycopene in oxidative stress and carcinogenesis: Conclusions from clinical trials. *Eur J Clin Nutr.* (2007) 61:295–303. doi: 10.1038/sj.ejcn.1602510

33. Schwarz S, Obermüller-Jevic UC, Hellmis E, Koch W, Jacobi G, Biesalski HK. Lycopene inhibits disease progression in patients with benign prostate hyperplasia. *J Nutr.* (2008) 138:49–53. doi: 10.1093/jn/138.1.49

34. Mohanty NK, Saxena S, Singh UP, Goyal NK, Arora RP. Lycopene as a chemopreventive agent in the treatment of high-grade prostate intraepithelial neoplasia. *Urol Oncol.* (2005) 23:383–5.

35. Kumar NB, Besterman-Dahan K, Kang L, Pow-Sang J, Xu P, Allen K, et al. Results of a randomized clinical trial of the action of several doses of lycopene in localized prostate cancer: Administration prior to radical prostatectomy. *Clin Med Urol.* (2008) 1:1–14. doi: 10.4137/cmu.s718

36. Riso P, Brusamolino A, Martinetti A, Porrini M. Effect of a tomato drink intervention on insulin-like growth factor (IGF)-1 serum levels in healthy subjects. *Nutr Cancer.* (2006) 55:157–62. doi: 10.1207/s15327914nc5502_6

37. Gann PH, Deaton RJ, Rueter EE, van Breemen RB, Nonn L, Macias V, et al. A phase II randomized trial of lycopene-rich tomato extract among men with high-grade prostatic intraepithelial neoplasia. *Nutr Cancer.* (2015) 67:1104–12. doi: 10.1080/01635581.2015.1075560

38. Voskuil DW, Vrieling A, van't Veer LJ, Kampman E, Rookus MA. The insulin-like growth factor system in cancer prevention: Potential of dietary intervention strategies. *Cancer Epidemiol Biomark.* (2005) 14:195–203.

39. Xie Z, Yang F. The effects of lycopene supplementation on serum insulin-like growth factor 1 (IGF-1) levels and cardiovascular disease: A dose-response meta-analysis of clinical trials. *Complement Ther Med.* (2021) 56:102632. doi: 10.1016/j.ctim.2020.102632

40. Walfisch S, Walfisch Y, Kirilov E, Linde N, Mnitentag H, Agbaria R, et al. Tomato lycopene extract supplementation decreases insulin-like growth factor-I levels in colon cancer patients. *Eur J Cancer Prev.* (2007) 16:298–303. doi: 10.1097/01.cej.0000236251.09232.7b

41. Nahum A, Zeller L, Danilenko M, Prall OW, Watts CK, Sutherland RL, et al. Lycopene inhibition of IGF-induced cancer cell growth depends on the level of cyclin D1. *Eur J Nutr.* (2006) 45:275–82. doi: 10.1007/s00394-006-0595-x

42. Minciullo PL, Inferrera A, Navarra M, Calapai G, Magno C, Gangemi S. Oxidative stress in benign prostatic hyperplasia: A systematic review. *Urol Int.* (2015) 94:249–54.

43. Cormio L, Calò B, Falagario U, Iezzi M, Lamolinara A, Vitaglione P, et al. Improvement of urinary tract symptoms and quality of life in benign prostate hyperplasia patients associated with consumption of a newly developed whole tomato-based food supplement: A phase II prospective, randomized double-blinded, placebo-controlled study. *J Transl Med.* (2021) 19:24. doi: 10.1186/s12967-020-02684-3

44. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. *Mutat Res Rev Mutat Res.* (2021) 787:108365. doi: 10.1016/j.mrrev.2021.108365

45. Ohshima H, Adachi H, Enomoto M, Fukami A, Nakamura S, Nohara Y, et al. Association between growth hormone and hypertension in a general population. *Hypertens Res.* (2020) 43:1430–6. doi: 10.1038/s41440-020-0500-7

46. Ozbek E, Otunctemur A, Dursun M, Sahin S, Besiroglu H, Koklu I, et al. Diabetes mellitus and HbA1c levels associated with high grade prostate cancer. *Asian Pac J Cancer Prev.* (2014) 15:2555–8.

47. Naito M, Asai Y, Mori A, Fukada Y, Kuwabara M, Katase S, et al. Association of obesity and diabetes with serum prostate-specific antigen levels in Japanese males. *Nagoya J Med Sci.* (2012) 74:285–92.

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 07 December 2022
DOI 10.3389/fnut.2022.1003620

OPEN ACCESS

EDITED BY
Heitor O. Santos,
Federal University of Uberlandia, Brazil

REVIEWED BY
Patrick Benjamin Wilson,
Old Dominion University, United States
Elisa Marroquin,
Texas Christian University,
United States

*CORRESPONDENCE
Ricardo J. S. Costa
ricardo.costa@monash.edu

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 26 July 2022
ACCEPTED 17 November 2022
PUBLISHED 07 December 2022

CITATION
Rauch CE, Mika AS, McCubbin AJ, Huschtscha Z and Costa RJS (2022) Effect of prebiotics, probiotics, and synbiotics on gastrointestinal outcomes in healthy adults and active adults at rest and in response to exercise—A systematic literature review. *Front. Nutr.* 9:1003620. doi: 10.3389/fnut.2022.1003620

Effect of prebiotics, probiotics, and synbiotics on gastrointestinal outcomes in healthy adults and active adults at rest and in response to exercise—A systematic literature review

Christopher E. Rauch[1], Alice S. Mika[1], Alan J. McCubbin[1], Zoya Huschtscha[2] and Ricardo J. S. Costa[1]*

[1]Department of Nutrition Dietetics and Food, School of Clinical Sciences, Faculty of Medicine Nursing and Health Sciences, Monash University, Notting Hill, VIC, Australia, [2]Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC, Australia

Introduction: A systematic literature search was undertaken to assess the impact of pre-, pro-, and syn-biotic supplementation on measures of gastrointestinal status at rest and in response to acute exercise.

Methods: Six databases (Ovid MEDLINE, EMBASE, Cinahl, SportsDISCUS, Web of Science, and Scopus) were used. Included were human research studies in healthy sedentary adults, and healthy active adults, involving supplementation and control or placebo groups. Sedentary individuals with non-communicable disease risk or established gastrointestinal inflammatory or functional diseases/disorders were excluded.

Results: A total of n = 1,204 participants were included from n = 37 papers reported resting outcomes, and n = 13 reported exercise-induced gastrointestinal syndrome (EIGS) outcomes. No supplement improved gastrointestinal permeability or gastrointestinal symptoms (GIS), and systemic endotoxemia at rest. Only modest positive changes in inflammatory cytokine profiles were observed in n = 3/15 studies at rest. Prebiotic studies (n = 4/5) reported significantly increased resting fecal Bifidobacteria, but no consistent differences in other microbes. Probiotic studies (n = 4/9) increased the supplemented bacterial species-strain. Only arabinoxylan oligosaccharide supplementation increased total fecal short chain fatty acid (SCFA) and butyrate concentrations. In response to exercise, probiotics did not substantially influence epithelial injury and permeability, systemic endotoxin profile, or GIS. Two studies reported reduced systemic inflammatory cytokine responses to exercise. Probiotic supplementation did not substantially influence GIS during exercise.

Discussion: Synbiotic outcomes resembled probiotics, likely due to the minimal dose of prebiotic included. Methodological issues and high risk of bias were identified in several studies, using the Cochrane Risk of Bias Assessment Tool. A major limitation in the majority of included studies was

the lack of a comprehensive approach of well-validated biomarkers specific to gastrointestinal outcomes and many included studies featured small sample sizes. Prebiotic supplementation can influence gut microbial composition and SCFA concentration; whereas probiotics increase the supplemented species-strain, with minimal effect on SCFA, and no effect on any other gastrointestinal status marker at rest. Probiotic and synbiotic supplementation does not substantially reduce epithelial injury and permeability, systemic endotoxin and inflammatory cytokine profiles, or GIS in response to acute exercise.

KEYWORDS

exercise-induced gastrointestinal syndrome, running, exertional-heat stress, epithelial, permeability, endotoxin, cytokine, gastrointestinal symptoms

Introduction

Gastrointestinal disturbances and associated symptoms are relatively common occurrences in the general population, and range from minor inconvenience to severe clinical conditions (e.g., gastrointestinal inflammatory and functional diseases/disorders) (1). Athletes (i.e., elite and amateur) and recreationally active populations (i.e., health and fitness) are also susceptible to these gastrointestinal disturbances and symptoms, which include those occurring at rest, as well as substantial perturbations that occur specifically during and/or after exercise (2). The reported incidence of gastrointestinal symptoms (GIS), as a result of exercise, during and/or after competitive events varies from <5 to >85% in both the elite and recreational population (2), depending on the exertional extent of the event. It is now well established that various factors increase the magnitude of exertional stress, and subsequently increase the risk of substantial gastrointestinal disturbances and associated GIS. These extrinsic and intrinsic exacerbation factors have been described in Costa et al. (2, 3).

The pathophysiology of disturbances to gastrointestinal integrity, function, subsequent systemic responses (e.g., endotoxemia and systemic inflammation), and associated GIS that active individuals present in response to exercise is referred to as "exercise-induced gastrointestinal syndrome" (EIGS), and is characterized by two primary pathways (Figure 1), as described in Gaskell et al. (13). Briefly, the gastrointestinal-circulatory pathway describes the splanchnic hypoperfusion and intestinal ischemia that occurs due to a redistribution of blood flow to skeletal muscle and peripheral circulation (14, 15), resulting in intestinal epithelial injury and hyperpermeability, plus local and/or systemic inflammatory effects in response to translocated pathogens (16–18). The gastrointestinal-neuroendocrine pathway describes the stress response contribution to gastrointestinal integrity and functional disturbances, *via* an increase in stress hormone responses and sympathetic activation (2). Such stress response is synonymous with impaired gastrointestinal motility, transit, digestive function, and nutrient absorption (19–21).

It is commonly assumed by athletes and their support crew that administration of probiotics will confer benefits to the gastrointestinal tract, particularly at times of intensified training or leading into or during competition, when gastrointestinal disturbance is of particular concern due to the potential to compromise physical performance (6, 19). Recently published narrative or opinion piece reviews exploring prebiotics (i.e., non-digestible material that can be fermented by bacteria in the lower gastrointestinal tract), probiotics (i.e., live bacteria which survive transit to colonize the lower gastrointestinal tract), and synbiotics (a combination of pre- and pro-biotics) in active adults, have implied a beneficial effect on the gastrointestinal tract in response to exercise and improved performance; however the primary focus has been on exercise performance or immunological outcomes (22–29). Unlike these narrative or opinion-based reviews, recent systematic literature reviews (SLR) that focused and/or included pro- and/or syn-biotic supplementation, concluded inconsistent methodologies and/or findings that provided no convincing evidence of any substantial beneficial effects resulting from probiotic supplementation in healthy populations (30–32). Although it is important to note that these SLR did not comprehensively evaluate EIGS markers or changes to fecal bacterial taxa or SCFA. Nevertheless, it has recently been demonstrated that the microbial composition of the gastrointestinal tract, when using partial correlation analysis and controlling for potential confounding factors, is another factor that may influence an individual's susceptibility of developing EIGS and associated GIS (33). Exploratory work suggests that an increased relative abundance of various SCFA producing commensal bacterial groups may improve epithelial integrity and reduce GIS in response to prolonged strenuous exercise (33–36), through mechanisms that warrant further exploration and clarification. Proposed mechanisms may include: (i)

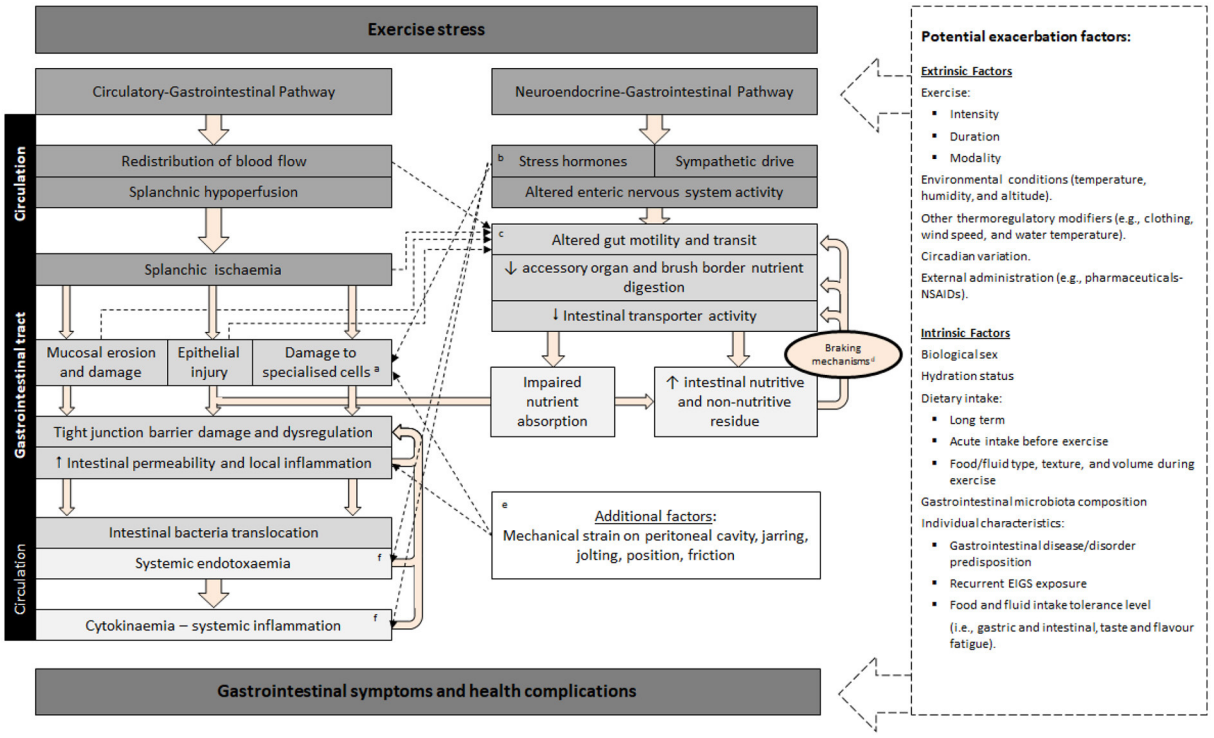


FIGURE 1
Schematic description of exercise-induced gastrointestinal syndrome (EIGS): Physiological changes in circulatory and neuroendocrine pathways at the onset of exercise resulting in perturbed gastrointestinal integrity and function, which may lead to gastrointestinal symptoms, with performance and clinical implications (2, 3). [a]Specialized antimicrobial protein-secreting (i.e., Paneth cells) and mucus-producing (goblet cells) cells, aid in preventing intestinal-originating pathogenic microorganisms entering systemic circulation. [b]Splanchnic hypoperfusion and subsequent intestinal ischemia and injury (including mucosal erosion) results from stress induced direct (e.g., enteric nervous system, and/or enteroendocrine cell) or indirect (e.g., braking mechanisms) alterations to gastrointestinal motility. [c]Increase in neuroendocrine activation and suppressed submucosal and myenteric plexus result in epithelial cell loss and subsequent perturbed tight junctions (4, 5). [d]Gastrointestinal brake mechanisms: Nutritive and non-nutritive residue along the small intestine, and inclusive of terminal ileum, results in neural and enteroendocrine negative feedback to gastric activity (6–10). [e]Aggressive acute or low grade, prolonged mechanical strain, is proposed to contribute toward disturbances to epithelial integrity (i.e., epithelial cell injury and tight-junction dysregulation) and subsequent "knock-on" effects for gastrointestinal functional responses (11). [f]Bacteria and bacterial endotoxin microorganism molecular patterns (MAMPs), and stress induced danger associated molecular patterns (DAMPs), are proposed to contribute toward the magnitude of systemic immune responses (e.g., systemic inflammatory profile) (12). Adapted from Costa et al. (2), with permission.

attenuation of exercise-associated hypoperfusion through the presence of nutrient content along the gastrointestinal tract and increased fermentation activity of commensal bacteria (6, 19, 37, 38); and/or, (ii) increased epithelial cell stability resulting from an increased luminal SCFA concentration (34, 35). The possible role of the gut microbiota as an intrinsic factor that alters the risk of EIGS pathophysiology, and subsequent GIS in response to exercise, suggests potential to manipulate this risk through the use of prebiotics, probiotics, and synbiotics. Mechanistically, particular probiotics (e.g., *Lactobacillus plantarum*) have demonstrated favorable effects on epithelial integrity (39, 40) and in clinical outcomes for patients presenting with infection and/or inflammation (41–43).

In regard to the application of variables to assess the impact of pre-, pro-, and/or syn-biotic supplementation on gastrointestinal status in response to exercise stress, various biomarkers have been employed (44). Changes in gastrointestinal integrity as a result of EIGS are commonly reported in research studies using intestinal fatty acid binding protein (I-FABP), a surrogate marker for intestinal epithelial injury; or urinary or plasma claudin-3 concentration, a surrogate marker for epithelial tight gap junction function or injury. Gastrointestinal permeability is commonly assessed by dual or multiple sugars tests including, urinary lactulose:mannitol or lactulose:rhamnose ratio for small intestinal permeability, and sucrose for gastroduodenal permeability. Translocation of pathogenic agents from the gastrointestinal lumen into systemic circulation are observed by measuring the plasma endotoxin response, including lipopolysaccharide (LPS), resulting lipopolysaccharide binding protein (LBP) response, and/or gram-negative endotoxin and anti-endotoxin antibodies such as IgM. Objective assessment of perturbations to gastrointestinal function may be measured *via*: (i) gastric antral sonography for gastric emptying, measuring ultrasound

half gastric emptying time or ultrasound full emptying time (45); (ii) electrogastrography (EGG), recording gastric myoelectrical activity (e.g., slow waves) using electrodes placed on the abdominal skin (21); and/or (iii) breath hydrogen response as a measure of carbohydrate malabsorption, as undigested material pass through the ilium where bacterial fermentation releases hydrogen and methane which diffuse through the lumen into the blood and excreted *via* the lungs (19, 46–49). *Via* the latter mechanism, orocecal transit time (OCTT) can be indirectly assessed *via* the administration of an indigestible carbohydrate, such as lactulose, recording the time to the resulting breath hydrogen peak (20). Participant reported data on defecation frequency and stool consistency using the Bristol Stool Rating Scale also offer supportive evidence on changes to gastrointestinal function (50).

As stated, several SLR have been published in respect to biotic supplementation interventions in active adults, but none have considered the methodological issues (e.g., adequate experimental design including exertional or exertional-heat stress with or without issues with sample collection timing, experimental control of confounding factors, limitations in EIGS biomarkers, validation and reliability of GIS assessment tool) or magnitude of response of key pathophysiological markers (e.g., clinical significance of responses) (44). To date, no systematic review has comprehensively examined in-depth the effect of short or long-term pre-, pro- and syn-biotics supplementation on gastrointestinal status outcomes in healthy active adults at rest and in response to acute exercise. Therefore, the aim of this current systematic literature review is to determine the beneficial, detrimental, or neutral effects of differing supplementation periods and dosages of pre-, pro- and syn-biotic supplementation, taken by healthy active adults, on gastrointestinal outcomes at rest and in response to exercise, with a specific focus on the defined markers characteristic of EIGS and associated GIS.

Methods

A systematic literature search was performed by three researchers (A.J.M, C.R, and Z.H), to determine the impact of varying pre-, pro-, and syn-biotic supplements and supplementation period on markers of gastrointestinal integrity (i.e., intestinal epithelial injury, permeability, and bacterial endotoxin translocation), gastrointestinal functional responses (i.e., gastric emptying, gastrointestinal transit, and myoelectrical activity), systemic inflammatory responses, gastrointestinal symptoms (i.e., incidence, severity, stool frequency, and consistency), and variables relating to the gut microbiota (i.e., bacterial composition and SCFA profile), both at rest and in response to exercise. The review was completed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement (51). The review was not pre-registered.

Search strategy

The literature search was undertaken of English-language, original research studies, from inception to beginning March 2022, using the databases Ovid MEDLINE, EMBASE, Cinahl, SportsDISCUS, Web of Science, and Scopus. Reference lists of review papers found from the search, and others known to the authors, were searched to identify any studies missed in the original search. Keywords applied in the literature search are shown in Table 1A, with search strategy logic for each database shown in Table 1B.

Eligibility criteria

Eligibility criteria were established a priori as per the Participant Intervention Comparator Outcomes Study (PICOS) design format (Table 2) (52). Original human research studies in healthy sedentary adults, and healthy active adults, involving supplementation and control or placebo groups, reporting quantified data on EIGS outcomes *in vivo* (i.e., gastrointestinal symptom description, stool frequency and consistency, intestinal integrity and permeability, systemic endotoxin and/or inflammatory cytokine profiles, gastrointestinal motility and/or other functional responses, fecal bacterial taxa and SCFA concentration) were considered for inclusion. Exclusion criteria included sedentary individuals with non-communicable disease risk or established gastrointestinal inflammatory or functional diseases/disorders, populations undergoing dietary modifications and/or supplementation, other than the pre-, pro-, or syn-biotic intervention, and a lack of a placebo or a control group. The inclusion and exclusion criteria were cross checked against the criteria reported within the reviewed studies. *Ex vivo* outcomes (i.e., antigen stimulated cytokine responses or other blood or tissue cultures) were excluded. After removal of duplicates, study titles and abstracts were reviewed by two researchers (Z.H and C.R) against the eligibility criteria, and verified by a third researcher (A.J.M) when required (i.e., disagreement between the primary reviewers) (Figure 2).

Data extraction

Data was extracted by two researchers (C.R. and Z.H.) and cross-checked by a third (A.J.M.). The extracted variables included the number of participants, sample size determination, age, biological sex, training status (i.e., years of experience and VO_{2max} where available), intervention

TABLE 1 General search strategy (A) and search strategy logic by database (B) for the systematic review on the effect of pre-, pro-, and synbiotics on gastrointestinal outcomes in healthy adults and healthy active adults.

(A) Field one (combine with OR): Population		Field two (combine with OR): Intervention and comparison		Field three (combine with OR): Outcome
Keywords: Exercise, Run*, Cycling, Cyclist, Physical Activity	AND	Keywords: probiotic, prebiotic, synbiotic	AND	Keywords:, intestinal injury and damage, I-FABP, intestinal fatty acid, tight junction, mucosal barrier, zonulin, claudin, endotoxin, LPS, LAL, lipopolysaccharide, gram negative bacteria, LBP, sCD14, intestinal permeability, lactulose, rhamnose, mannitol, urinary sugars, gastrointestinal motility, OCTT, EGG, gastrointestinal symptoms, gut discomfort, short chain fatty acids, SCFA, gastrointestinal microbiota and microbial composition.

(B) OVID EMBASE and Ovid MEDLINE(R) and Epub Ahead of Print

1. (Exercise or Run* or Cycling or Cyclist or "Physical Activity").mp. (mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word)
2. (probiotic or prebiotic or synbiotic).mp. (mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word)
3. ("intestinal injury" or "intestinal damage" or I-FABP or "intestinal fatty acid" or "tight junction" or "mucosal barrier" or zonulin or claudin or endotoxin or LPS or LAL or lipopolysaccharide or "gram negative bacteria" or LBP or sCD14 or "intestinal permeability" or lactulose or rhamnose or mannitol or "urinary sugars" or "gastrointestinal motility" or OCTT or EGG or "gastrointestinal symptoms" or "gut discomfort" or "short chain fatty acid*" or SCFA or microbiota or "microbial composition").mp. (mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word)
4. 1 and 2 and 3

SCOPUS

(TITLE-ABS-KEY (exercise OR run* OR cycling OR cyclist OR {physical activity}) AND TITLE-ABS-KEY (probiotic OR prebiotic OR synbiotic) AND TITLE-ABS-KEY ({intestinal injury} OR {intestinal damage} OR i-fabp OR {intestinal fatty acid} OR {tight junction} OR {mucosal barrier} OR zonulin OR claudin OR endotoxin OR lps OR lal OR lipopolysaccharide OR {gram negative bacteria} OR lbp OR scd14 OR {intestinal permeability} OR lactulose OR rhamnose OR mannitol OR {urinary sugars} OR {gastrointestinal motility} OR octt OR egg OR {gastrointestinal symptoms} OR {gut discomfort} OR {short chain fatty acid*} OR scfa OR microbiota OR {microbial composition}))

CINAHL plus and SPORTDiscus with full text

S7 S1 AND S2 AND S6
S6 S3 OR S4 OR S5
S5 gut discomfort OR short chain fatty acid* OR SCFA OR microbiota OR microbial composition
S4 gram negative bacteria OR LBP OR sCD14 OR intestinal permeability OR lactulose OR rhamnose OR mannitol OR urinary sugars OR gastrointestinal motility OR OCTT OR EGG OR gastrointestinal symptoms
S3 intestinal injury OR intestinal damage OR I-FABP OR intestinal fatty acid OR tight junction OR mucosal barrier OR zonulin OR claudin OR endotoxin or LPS or LAL or lipopolysaccharide
S2 probiotic OR prebiotic OR synbiotic
S1 exercise OR run* OR cycling OR cyclist* OR physical activity

Web of science

Exercise OR run* OR cycling OR cyclist OR "physical activity" (Topic) and probiotic or prebiotic or synbiotic (Topic) and "intestinal injury" or "intestinal damage" or I-FABP or "intestinal fatty acid" or "tight junction" or "mucosal barrier" or zonulin or claudin or endotoxin or LPS or LAL or lipopolysaccharide or "gram negative bacteria" or LBP or sCD14 or "intestinal permeability" or lactulose or rhamnose or mannitol or "urinary sugars" or "gastrointestinal motility" or OCTT or EGG or "gastrointestinal symptoms" or "gut discomfort" or "short chain fatty acid*" or SCFA or microbiota or "microbial composition" (Topic)

*Used to retrieve unlimited suffix variations.

(i.e., quantity, food source and composition of prebiotic, and/or bacterial species/strain of probiotic), exercise protocol used where relevant, ambient conditions, physiological and thermoregulatory strain responses during the exercise protocol where relevant). EIGS outcomes extracted included concentrations of: I-FABP (blood), claudin-3 (urinary or blood),

TABLE 2 PICOS table, showing the inclusion and exclusion criteria for study population, intervention, comparator, outcome/s, and study design.

PICOS	Inclusion	Exclusion
Population	Human Healthy community dwelling sedentary individuals. Sedentary individuals initiating a structured physical activity or exercise program. Recreational and competitive active adults (18–60 years). Male and female biological sex.	Animals and *in vitro* studies. Infants or children. Pregnancy or lactating. Sedentary individuals with non-communicable disease risk or established disease (i.e., cardiometabolic risk factors or established cardiovascular diseases, diabetes mellitus, and/or metabolic syndrome). Diagnosed disease or syndrome states (i.e., all clinical populations). Population adhering to dietary modifications and/or dietary supplementation, other than pre-/pro-/syn-biotic intervention.
Intervention	Acute and prolonged provisions of prebiotic/s, probiotic/s, and synbiotic blends (i.e., prebiotic + probiotic, with or without other nutrient inclusion) (e.g., vitamins, minerals, lipids, phytochemicals, and/or volatiles). With and without monitored and/or structures physical active and/or exercise program. Dietary control (monitoring or provisions).	Dietary interventions not containing acute and prolonged provisions of prebiotic/s, probiotic/s, and synbiotic blends. Acute and prolonged provisions of prebiotic/s, probiotic/s, and synbiotic blends that contain a pharmaceutics grade product or compound.
Comparator	Placebo group Control group	No placebo or control
Outcome	Gastrointestinal integrity markers: e.g., I-FABP, Claudin-3, dual sugars test for permeability, and other markers proposed to assess gastrointestinal epithelial integrity. Gastrointestinal functional markers: e.g., gastric aspiration, C^{13} breath test, OCTT, EGG, pH pill monitoring, H_2 and CH_4 malabsorption challenge. Systemic markers of compromised gastrointestinal integrity: e.g., CRP, systemic inflammatory response cytokine profile, systemic endotoxin profile (e.g., LPS, gram-negative endotoxin, ant-endotoxin antibody, sCD14, and/or LBP), systemic microbial identification (e.g., gene sequencing determination), immune cell functional responses and/or counts. Gastrointestinal signs and symptoms: e.g., stool habits and texture, QoL, and/or symptoms. Gastrointestinal microbiota: e.g., bacterial taxonomy (ASV or OTU) including α-diversity and relative abundance, bacterial functional markers including SCFA concentration (e.g., butyrate, propionate, and/or acetate).	
Study design	RCT or randomized crossover trial.	All other study designs

ASV, Amplicon sequence variant; CRP, c-reactive protein; EGG, electrogastrography; I-FABP, Intestinal fatty acid binding protein; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; OCTT, orocecal transit time; OTU, operational taxonomic units; QoL, quality of life; sCD14, soluble CD14; SCFA, short chain fatty acid; RCT, randomized control trial.

cytokine response (blood), dual sugars (urinary or blood) endotoxin response (blood LPS, LPB, gram negative endotoxin and anti-endotoxin antibodies). Gastrointestinal function measures extracted included: gastric antral sonography, EGG, breath hydrogen response for malabsorption, and OCTT. Other functional measures extracted include defecation frequency, stool consistency and GIS. Timepoints for data included resting pre- and post-exercise (i.e., immediately post-exercise or short-term recovery) where reported. Other timepoints reported were included and clearly specified, where resting pre- and post-exercise timepoints were not reported. Pre- to post-exercise Δ were calculated from extracted data. Between group differences and within group differences were extracted and tabulated. Where no baseline data was reported, this was included, but clearly specified. Data from tables was extracted and tabulated as presented. Graphical data was measured using WebPlotDigitizer (53) where appropriate. Only meaningful data was extracted, with heat mapping and other unclear data presentation methods excluded from extraction. Data was again checked during extraction, and inclusion/exclusion

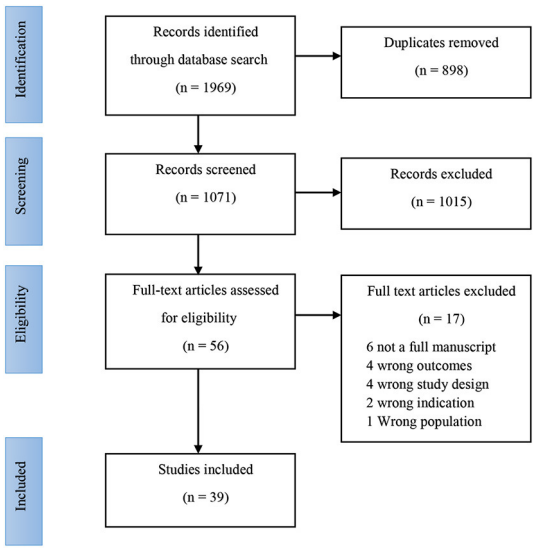


FIGURE 2
PRISMA diagram, showing the inclusion and exclusion of papers in the review.

criteria applied as appropriate. Disagreements between the primary reviewers were resolved by discussion and consensus. Data was separated into two groups according to the study protocol; outcomes measured only at rest after a period of supplementation, and outcomes measured in response to acute exercise, also following a period of supplementation. Due to the heterogeneous nature of the interventions, study methodologies and outcome measures, data were not considered appropriate for meta-analysis. Despite the lack of a meta-analysis, certainty of evidence was not deemed necessary as clinical or policy recommendations have not been made and the limitations of the data and findings have been discussed at length.

Risk of bias assessment

A risk of bias assessment was undertaken for identified studies, using the Cochrane Risk of Bias Assessment Tool (54). The tool is used to assess the likelihood of selection bias (i.e., random sequence generation and allocation concealment), performance bias (i.e., adequacy of participant blinding), detection bias (i.e., adequacy of researcher blinding), attrition bias (i.e., completeness of outcome data), reporting bias (completeness of outcome reporting), and other potential forms of bias.

Results

Search result

Results of the literature search are shown in Figure 2. The initial search returned 1,969 individual records, with 898 duplicates removed. No additional records were found from the reference lists of recent review papers on the topic. Title and abstract screening excluded 1,015 records, with full text screening excluding an additional 17 records. Data was therefore extracted from 39 records, and further categorized based on available outcome data. Outcomes are reported from a total of 1,204 participants. Due to the multifaceted nature of gastrointestinal status at rest and in response to exercise, results are presented according to each outcome type; including direct or indirect markers of intestinal injury and/or permeability, systemic endotoxin and/or inflammatory cytokine concentration, gastrointestinal functional responses, luminal microbial composition and SCFA concentration. Studies assessing resting gastrointestinal status to a period of pre-, pro-, and syn-biotic supplementation, $n = 37$ papers reported at least one of the gastrointestinal outcomes at rest, with or without GIS, before and after the biotic intervention period (Table 3). Of these, $n = 8$ provided a prebiotic intervention, $n = 24$ a probiotic intervention, and $n = 5$ a synbiotic intervention. Of the prebiotic studies, $n = 1$ provided the intervention substance in capsules, while all others incorporated the prebiotic ingredient into specifically formulated foods (i.e., bread, pasta, snack bar, or non-carbonated soft drink). Probiotic interventions varied from one to eight bacterial strains, given either in capsules, fermented dairy-based food or beverage, or sachets containing powder to be mixed in water. Synbiotic interventions consisted of either capsules, dairy based food, beverage or powder, containing between two to four probiotic strain mixtures, supplemented with either one or two prebiotic ingredients. The supplementation period ranged from 1 to 16 weeks. All of the included studies were conducted with adult participants (mean or median age < 45 years). $N = 21$ of the $n = 37$ studies characterized a specific exercise or sporting background in participants (i.e., rugby union, soccer, cycling, swimming, baseball, distance running, triathlon, or participants taken from a combination of team, endurance, and racquet sports). $N = 2$ studies were conducted with military recruits undergoing intense military training.

Intestinal epithelial injury at rest

No studies were identified as assessing intestinal epithelial injury at rest, before or after a period of pre-, pro-, or syn-biotic supplementation.

TABLE 3 Systematic review search results and study characteristics of included studies that attempted to determine the impact of prebiotic, probiotic and synbiotic supplementation on gastrointestinal outcomes at rest.

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Prebiotic studies					
Damen et al. (55)	$N = 27$ (10 M and 17 F), age: 25 (IQR 23–29) years, activity/fitness: not stated, study design: RXT	Not specified	Bread fortified with xylanase to produce Arabinoxylan oligosaccharides (AXOS) on baking. 180 g bread (7.2 g arabinoxylan) daily for 3 wk.	DC: Usual diet, not monitored. PA: Physical activity not stated	Gastrointestinal function GIS Bacterial taxa Fecal SCFA
Finegold et al. (56)	$n = 32$ (11M/21F), age (M 23–34 yrs, F 21–49 yrs) (activity not stated) RCT	Not specified	Xylooligosaccharides (XOS), up to 350 mg per cap. High (2.8 g) or low (1.4 g) dose from capsules, daily for 8 wk	Instructed to avoid high XOS/FOS/GOS foods and pre/probiotics and fermented foods. Twenty-four hour dietary recall used to compare between groups (data not reported).	Bacterial taxa Fecal SCFA GIS
François et al. (57)	$n = 63$ (M33/F30), age 42 yrs (activity not stated) RXT	Based on dataset of an earlier human intervention trial with WBE (58), an evaluable sample size of $n = 40$ was expected to provide 80% power (two-sided, $\alpha = 0{\cdot}05$) for detecting statistically significant difference in fecal bifidobacterial levels (primary outcome variable) between treatments.	Non-carbonated soft drink with wheat bran extract [containing Arabinoxylan oligosaccharides (AXOS)]. High (8 g/d) or Low (2.4 g/d) dose AXOS taken in a 70 mL drink, twice daily for 3 wk	Usual diet, 3 meals/day pattern, no pro/prebiotics. All food and beverage intake recorded for first 3 days of each study period. No activity monitoring stated.	Bacterial taxa Fecal SCFA GIS
Kleessen et al. (59)	$n = 45$ (10M/35F), age 23.5 yrs (activity not stated) RCT	Not specified	Snack bar with chicory inulin (CH) or Jerusalem artichoke inulin (JA), 7.7 g fructans per bar. 1 bar/d for 1 wk, 2 bars/d for 2 wk.	Asked to maintain usual habits (not monitored)	Bacterial taxa Fecal SCFA Gastrointestinal function GIS
Reimer et al. (60)	$N = 48$ (22M/28F at recruitment) Age 31.2 ± 9.1/30.5 ± 8.6 (Trial 1/ 2) (healthy untrained) RXT	The study was powered on the primary outcome of change in gut microbiota, which for the purposes of sample size calculation was based on changes in fecal Bifidobacterium from a previous trial (59). A sample size of $n = 25$ per group was determined to have 80% power to detect a difference between groups in fecal Bifidobacterium counts (CFU/g) assuming a 1.10-log difference with SD = 1.22 log, an $\alpha = 0.05$, and a dropout rate of ∼25%.	Snack bar with inulin type fructans (ITF) Int 1: moderate dose 7 g/d inulin + 2 g wheat based fiber Int 2: low dose 3 g/d inulin + 2 g oat based fiber Con 1: snack bar (0 g inulin, 0 g fiber) Con 2: snack bar (0 g inulin, 1 g fiber) 1 bar/d for 4 wk.	3 day weighed food record. Energy (kcal), protein, carbohydrate, fat @ baseline and within trials^{NS} Fiber time × treatment, trial 1 ($P = 0.001$), trial 2 ($P = 0.019$). Exercise across both trials, bw or within-group differences^{NS} (data not shown).	Bacterial taxa Fecal SCFA GIS Gastrointestinal function
Russo et al. (61)	$n = 15$ males, age 18.8 ± 0.7 yrs (activity not stated) RXT	Not specified	Inulin-enriched pasta: Int: 11.0 g/d fructans Pla: 1.4 g/d fructans 100 g/day pasta for 5 wk	All food provided, low inulin, amount calculated according to individual requirements. No activity monitoring stated.	Gastrointestinal function GIS
Russo et al. (45)	$n = 20$ males, age 18.8 ± 0.7 yrs (activity not stated) RXT	Not specified	Inulin-enriched pasta: Int: 11.0 g/d fructans Pla: 1.4 g/d fructans 100 g/day pasta for 5 wk	All food provided, low inulin, amount calculated according to individual requirements. No activity monitoring stated.	Gastrointestinal function

(Continued)

10.3389/fnut.2022.1003620

TABLE 3 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Russo et al. (62)	$n = 20$ males, age 18.8 ± 0.7 yrs (activity not stated) RXT	Sample size calculations based on data from Russo et al. (45) and Russo et al. (61). Probability that study would detect treatment difference with a 2-sided 0.05 significance level equal to 80% required enrolling only 17 subjects. This assumed true difference between treatments of 20% of urinary recovery of La, and standard deviation of the difference of 27%.	Inulin-enriched pasta: Int: 11.0 g/d fructans Pla: 1.4 g/d fructans 100 g/day pasta for 5 wk	All food provided, low inulin, amount calculated according to individual requirements. No activity monitoring stated.	Intestinal permeability
Probiotic studies					
Axelrod et al. (63)	$n = 7$ endurance runners, VO_{2max} 57.9 mL/kg/min RXT	A priori power analysis based on a previous investigation from healthy runners (64) estimated ∼6 needed to obtain statistical power at the recommended 0.80 level based upon mean, between-groups comparison effect size ($d = 1.2$).	*L. salivarius* UCC118, 2×10^8 CFU/cap 1 capsule daily for 4 wk	Normal lifestyle Activity not monitored	Intestinal permeability Cytokine responses Bacterial taxa
Batatinha et al. (65)	$n = 27$ male marathon runners, age: Int: 35.96±5.81; Pla: 40.46 ±7.79 Fitness status not stated RCT	Not specified	*B. animalis. Lactis* 10×10^9; *L. Acidophilus* 10×10^9 1 sachet daily for 30 days	Dietary control not stated. Training volume monitored, ^{NS} between groups.	Cytokine responses
Burton et al. (66)	$n = 13$ males age 24 (22–27) yrs (activity not stated) RXT	The desired sample number could not be determined because of the absence of previous clinical studies with a similar intervention.	Yogurt containing: *S. thermophilus* (10^9 CFU/g), *L. delbrueckii* spp. *bulgaricus* (10^8 CFU/g) *L. rhamnosus GG* (10^7 CFU/g) 400 g daily for 2 wk	Avoid dairy and fermented foods, 3 d food record each trial. 3 d control diet (provided) before each test day. Usual activity. Avoid intense activity 3 d prior to testing. ^{NS} difference between groups	Endotoxin responses Cytokine responses Bacterial taxa
Carbuhn et al. (67)	$n = 17$ female swimmers, age and fitness status not stated RCT	Not specified	*B. longum* 35624, 1×10^9 CFU bacteria per capsule 1 capsule daily for 6 wk	Refrain from foods rich in probiotics (ex. Kefir) and caffeine. Three day food log (^{NS}between groups). Standardized swim training program	Endotoxin responses Cytokine responses
Gill et al. (68)	$n = 8$ male runners and triathletes Age: 26 yrs VO_{2max} 59 ml/kg/min RXT	Based on the typical standard deviation of 0.7 EU/ml for circulatory endotoxin responses to exertional-stress (16, 69), and using standard alpha (0.05) and beta values (0.8) (www.dssresearch.com), a sample size of $n = 8$ is estimated to provide adequate statistical precision to detect a >10% difference in circulatory endotoxin concentration in response to EHS in the target population.	*L. casei*, $1.0 \times$ 10^{11} cells/bottle Commercial supplement, taken twice daily for 7 days	Dietary recall. ^{NS} between groups. Activity control not stated.	Cytokine responses Endotoxin responses
Gleeson et al. (70)	$n = 58$ (54M/30F recruited) athletes Age: Int: 32 ± 14, Pla: 25 ± 9 yrs Weekly training load: Int: 8.7 ± 4.1 h/week Pla: 9.3 ± 3.8 h/week RCT	Sample-size based on expected rate of 2.0 ± 1.0 URTI episodes (M ± SD) (71), a target 30% reduction in number of episodes, statistical power of 80%, and Type I error of 5%.	Fermented milk with L. casei Shirota, 6.5×10^9 live cells/drink 65 ml drink twice daily for 16 wk	Diet not monitored, no other probiotics or fermented dairy products. Training validated by International Physical Activity Questionnaire, ^{NS}between groups	Cytokine responses GIS

(Continued)

10.3389/fnut.2022.1003620

TABLE 3 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Haywood et al. (72)	$n = 30$ male rugby union players, age 24.7 ± 3.6 yrs RCT	In order to detect a 30% reduction in the number of infected days with 80% power and type 1 error of 5%, $n = 25$ participants were required.	Probiotica P3, Nutra-life: *L. gasseri*, 2.6×10^9 CFU/cap *B. bifidum*, 0.2×10^9 organisms/cap *B. longum*, 0.2×10^9 organisms/cap 1 capsule daily for 4 wk	Dietary control not stated. Standardized training program.	GIS
Hoffman et al. (73)	$n = 15$ male military recruits, age: Int: 20.0 ± 0.6, Pla: 20.2 ± 0.6 Fitness status not stated; military training. RCT	Because of the small sample size per group, it was decided a priori to initially analyze PRE-to-POST changes (Δ scores) using the non-parametric Mann-Whitney U test. In addition, to make additional inferences on the true effect of the dietary intervention, and account for the small sample size per group, magnitude-based inferential analysis was also used.	Staimune, Kerry Inc. (St Paul, MN). *Inactivated Bacillus coagulans; 1.0 × 10^9 CFU (powder form) Daily for 2 wk*	Participants were not permitted to use any additional dietary supplementation. All soldiers consumed their meals together, maintaining a similar dietary intake throughout the study. During study period, soldiers were garrisoned on base and participated in the same training tasks that included hand-to-hand combat skill development, working with and against various weapons and physical conditioning 5 times a week.	Cytokine responses
Huang et al. (74)	$n = 20$ male triathletes Age: Int: 21.6 ± 1.3 Pla: 21.9 ± 1.4 VO_{2max} mL/kg/min): Int: 55.5 ± 8.6 Pla: 56.6 ± 9.0 RCT	Not specified	*L. plantarum PS128, 2 capsules; 1.5 × 10^{10} CFU + 100 mg microcrystalline cellulose (per capsule)* 4 wk	Diet not monitored. Participants were required not to supplement with fermented food, probiotics, prebiotics, and antibiotics during the whole experimental process. Training not controlled. Maintain a regular lifestyle, avoiding any strenuous exercise, staying up late, smoking, or consuming alcoholic beverages.	Bacterial taxa Fecal SCFA
Klein et al. (75)	$n = 26$ (13M/13F), age 25 yrs (activity not stated) RXT	Power analysis performed using PASS 6.0 (NCSS Statistical Software, Kaysville, UT, USA). Based on data from the literature and study group to evaluate sample size. It resulted in a power of 90%.	300 g yogurt containing *B. lactis* 420×, 3.0×10^6 CFU/g *L. acidophilus* 74-2, 9.3×10^8 CFU/g 300 g daily for 5 wk	Food provided and additional foods aliquoted and analyzed (^{NS}between groups). Activity not stated.	Bacterial taxa Fecal SCFA
Lamprecht et al. (76)	$n = 23$ male triathletes, runners and cyclists Age: Int: 37.6 yrs, Pla: 38.2 yrs VO_{2max}: Int: 51.2 mL/kg/min Pla: 50.3 mL/kg/min RCT	Sample size calculation based on oxidation markers CP and MDA. Between 7 and 9 subjects estimated per group—depending on parameter, SD and effect size—to reach probability of error (alpha/2) of 5 and 80% power.	Ecologic® Performance: *B. bifidum W23, B lactis W51, E. faecium W54, L. acidophilus W22, L. brevis W63, L. lactis W58.* 2 × 2 g sachets daily for 14 wk, providing (10^{10} CFU/day	Habitual diet, food diary and repeated for 7 days prior to each exercise trial. Habitual training, no exercise 3 days prior to each exercise test.	Intestinal permeability Cytokine responses
Lee et al. (77)	$n = 16$ healthy untrained males Age Int 24.6 ± 2.8, Pla: 25.6 ± 4.1 VO_{2max}: Int: 47.3 ± 6.5, Pla: 46.6 ± 8.2 RCT	The required sample sizes for clinical trials based on expected change calculated using Harvard calculator (http://hedwig.mgh.harvard.edu/sample_size/size.html, accessed on 14 December 2020), assuming parallel design with 0.05 significance level, the change SD, power of 0.8, standard deviation of the difference with 3.2.	Synkefir: *L. paracasei DSM 32785 (LPC12), L. rhamnosus DSM 32786 (LRH10), L. helveticus DSM 32787 (LH43), L. fermentum DSM 32784 (LF26), and S. thermophilus DSM 32788 (ST30)* 20 g pouch daily for 28 days	All volunteers were prohibited from taking probiotics, prebiotic fermented products (yogurt or cheese), vitamins, minerals, herbal extracts, dietary supplements for exercise, or antibiotics to avoid unnecessary interference during the experiment. No significant difference on their daily nutritional intake and calories (data not shown). Activity not stated.	Bacterial taxa
Lin et al. (78)	$n = 21$ (14M, 7F) well trained middle and long distance runners. Fitness status not reported. Age: Pla: 21.2 ± 0.4 Int: 21.6 ± 0.7 RCT	Not specified	OLP-01, a human strain probiotic; Bifidobacterium longum subsp. Longum 3 capsules daily (1.5×10^{10} CFU/day) for 5 wk	Instructed not to consume nutritional supplements, yogurt, Yakult, other probiotic-related products, or antibiotics during the experiment. The team dietitian specified the diet and provided the same meal to ensure the consistency of the diet (Data not shown). Three weeks of regular training and 2 weeks of de-training. During the experiment, all the subjects cooperated with the team for work and rest (Data not shown).	Bacterial taxa

(Continued)

10.3389/fnut.2022.1003620

TABLE 3 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Pugh et al. (79)	$n = 24$ (20M/4F) marathon runners, age: Int: 34.8 ± 6.9 yrs Pla: 36.1 ± 7.5 yrs VO_{2max}: Int: 57.6 ± 8.0 mL/kg/min Pla: 56.4 ± 8.6 mL/kg/min RCT	Not specified	Proven Probiotics Ltd, Port Talbot, Wales: *L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34 > 25 billion CFU/cap 1 capsule daily for 4 wk	Dietary control not stated outside of acute exercise. Training diary kept during supplementation period (data not presented).	GIS
Sánchez Macarro et al. (80)	$n = 43$ healthy male volunteers who performed aerobic physical exercise. VO2max= 51.1 (8.8)mL/kg/min Age: Int: 25.3 ± 7.2, Pla: 27.1 ± 8.4 RCT	The sample size was calculated for an expected mean difference between groups in serum levels of MDA of 1.34 nmol/mL with SD of 1.6 nmol/L (81), with significance level of 5% and statistical power of 80%, assuming a drop-out rate of 10% since the primary analysis was performed in the PP data set, 20 evaluable participants for each treatment group were required.	Bifidobacterium longum CECT 7347 *Lactobacillus casei* CECT 9104, and *Lactobacillus rhamnosus* CECT 8361 1 capsule daily (10^9 CFU/day) for 6 wk	No dietary control: During the study period, there were no dietary restrictions, but medications that may affect the microbiome (e.g., antioxidants, statins) were not allowed. Physical activity not controlled	Bacterial taxa
Schreiber et al. (82)	$n = 27$ male cyclists, Age: Int: 25.9 ± 4.6 Pla: 29.5 ± 6.2 VO_{2max} (mL/kg/min) Int: 66.9 ± 6.4 Pla: 63.2 ± 5.0 ^{NS} difference between groups RCT	Not specified	*L. helveticus* Lafti L10, *B. animalis* ssp. lactis Lafti B94 *E. faecium* R0026, *B. longum* R0175 Bacillus subtilis R0179 15×10^9 CFU of a probiotic blend: 1 capsule daily for 90 days	Diet not controlled. Continued with their normal training routine throughout the study duration. "Participants' characteristics analysis revealed difference in training hours during the study period." Data not shown.	GIS Cytokine responses
Smarkusz-Zarzecka et al. (83)	$n = 66$ (46M/20F) runners, age: Int: F 37.21 ± 8.09 y M 40.85 ± 8.32 y Pla: F 33.33 ± 8.73 y M 38.61 ± 8.84 y VO_{2max} (mL/kg/min) Int: male: 38.22 ± 5.99 Female:34.02 ± 5.30 Pla: male: 42.34 ± 7.06 female:36.98 ±11.34 RCT	Not specified	Sanprobi Barrier, Sanprobi Ltd., Szczecin, Poland: *Bifidobacterium lactis W52, Lactobacillus brevis W63, Lactobacillus casei W56, Lactococcus lactis W19, Lactococcus lactis W58, Lactobacillus acidophilus W37, Bifidobacterium bifidum W23, Lactobacillus salivarius W24.* 2.5×10^9 CFU/g (1 capsule); 2 capsules, twice daily for 3 months.	Diet not controlled. Avoid physical activity for at least 24 h before the test.	Cytokine responses
Son et al. (84)	$n = 15$ bodybuilders (biological sex and fitness status not stated) Age: Int: 26.50 ± 5.01 Pla: 27.14 ± 5.93 RCT	Not specified	*L. acidophilus, L. casei, L. helveticus, Bifidobacterium bifidum* 1 capsule consisting of 10^{12} CFU For 60 days	The subjects were periodically monitored to ensure that nutritional intake was not altered during the supplement intake period; There was no significant difference in the characteristics of the study subjects before and after the probiotic intake period. Data not shown. Physical activity not monitored.	Bacterial taxa Fecal SCFA
Strasser et al. (85)	$n = 29$ (13M/16F) cyclists Age: Int: 25.7 ± 3.5 yrs, Pla: 26.6 ± 3.5 yrs VO_{2max}: Int: 55.1 ± 6.4 mL/kg/min Pla: 47.5 ± 7.1 mL/kg/min ($p < 0.01$) W_{max}: Int: 325 ± 54.2 W Pla: 274 ± 51.6 W ($p < 0.05$) RCT	Sample size calculation was based on changes in exercise-induced Trp levels (86) from baseline to end of 12-wk intervention. Between 10 and 12 subjects per group estimated, depending on SD and effect size, to reach probability of error (alpha/2) of 5 and 80% power. Allowing for a drop-out rate of 30%, 16 subjects per group were recruited.	Ecologic® Performance: *Bifidobacterium bifidum* W23 *Bifidobacterium lactis* W51 *Enterococcus faecium* W54 *Lactobacillus acidophilus* W22 *Lactobacillus brevis* W63 *Lactococcus lactis* W58 1×10^{10} CFU/sachet 1 sachet daily for 12 wk	No alcohol or fermented dairy products. 3 d food record at baseline and 12 wks. ^{NS}Between groups. Maintained normal training. Weekly training log. Int: 8.0 ± 2.3 h/wk Pla: 6.6 ± 4.3 h/wk ($p < 0.001$)	GIS

(Continued)

10.3389/fnut.2022.1003620

TABLE 3 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Tavares-Silva et al. (87)	$n = 14$ male runners Age: Pla: 38.28 ± 3.09 Int: 41.57 ± 3.20 VO_{2Peak} (kg/mL/min): Pla: 54.53 ± 6.88 Int: 56.92 ± 8.35 RCT	Not specified	Gelatinous capsules: *Lactobacillus acidophilus-LB-G80, Lactobacillus paracasei-LPc-G110, Lactococcus subp. lactis-LLL-G25, Bifidobacterium animalis subp. lactis-BL-G101, Bifidobacterium bifidum-BB-G90* 5×10^9 CFU 2.0 g/day, 1 capsule/d, 30 days	Dietary questionnaire 2x/wk + once on weekends: Kcal, carbohydrates, protein, lipids ^{NS}between groups. Physical activity not reported.	Cytokine responses
Townsend et al. (88)	$n = 25$ male baseball players Age: 20.1 ± 1.5 yrs 1RM Squat (baseline, mean): Int: 116.8 kg, Pla: 133.0 kg Deadlift 1RM (baseline mean): Int: 139.9 kg, Pla: 162.8 kg RCT	Not specified, however study reported as statistically under-powered to detect modest effects in some biomarkers.	*Bacillis subtilis* DE111, 1.24×10^9 CFU/cap 1 capsule daily for 12 wk	3 d food diary on wk 1, 9, 12 ^{NS}between groups. 12 wk triphasic, undulating, periodized resistance training program	Intestinal permeability Cytokine responses
Vaisberg et al. (89)	$n = 42$ male runners Age: Int: 39.6 yrs, Pla: 40.1 yrs VO_{2max} (mL/kg/min): Int: 57.64, Pla: 57.86 RCT	Not specified	*Lactobacillus casei Shirota,* 40×10^9 live cells/bottle 1 × 80 ml bottle daily for 30 days	Dietary control not stated. Instructed to maintain usual training exercise schedule—not reported.	Cytokine responses
West et al. (90)	$n = 88$ (62M/35F recruited) cyclists and triathletes Age: Int: M: 35.2 yrs, F: 36.5 yrs, Pla: M: 36.4 yrs, F 35.6 VO_{2max} (ml/kg/min): Int: M: 56.5, F: 53.0 Pla: M: 55.8, F: 51.6 RCT	A sample size of $n = 80$ required for identifying substantial changes in the incidence of illness (91). We assumed a rate of URTI symptoms of 60% in the placebo group, with sufficient power (86% at an alpha-level of 0.05) to detect a 50% reduction in symptoms.	*Lactobacillus fermentum* VRI-003 PCC®, 10^9 CFU/cap 1 capsule daily for 11 wk	4 day food diary. Usual diet, without probiotic foods. Training log kept	Cytokine responses Bacterial taxa GIS
Synbiotic studies					
Coman et al. (92)	$n = 10$ (3M/7F), age (range) 20–45 yrs (activity not stated) RCT	Not specified	Synbiotec S.r.l., Camerino, Italy: *L. rhamnosus* IMC 501[R] *L. paracasei* IMC 502[R] plus oat bran fiber 200 ml fermented milk, containing 1×10^9 CFU strain per portion. 200 ml Consumed daily for 4 wk	Not stated	Bacterial taxa GIS
Quero et al. (93)	$n = 27$ (14 sedentary males/13 professional male soccer players 2nd Div B level of the Spanish National League Age: Sedentary: Pla: 24.31 ± 3.94, Int: 23.04 ± 2.09 Athletes: Pla: 21.9 ± 2.77, Int: 20.66 ± 1.39 RCT	Not specified	Gasteel Plus® (Heel España S.A.U laboratories) *B. lactis* CBP-001010, *L. rhamnosus* CNCM I-4036, *B. longum* ES1, Fructooligosaccharides (200 mg) 1.5 mg of zinc, 8.25 µg of selenium, 0.75 µg of vitamin, and maltodextrin as an excipient. 1 stick containing ≥1×10^9 CFU daily for 30 days	Participants were prohibited from consuming probiotics, prebiotics, or fermented products (yogurt or other foods) and any medications that could interfere with the study protocol Subjects were asked to maintain, 2 weeks before and during the study, their regular lifestyle.	Cytokine responses

(Continued)

10.3389/fnut.2022.1003620

TABLE 3 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol (vs. placebo or control)	Dietary control (DC) Physical activity (PA)	Outcome/s reported
Roberts et al. (94)	$n = 20$ (18M/2F) long course triathletes Age 35 yrs VO_{2max}: Int: 47.6 mL/kg/min Pla: 50.5 ml/kg/min RCT	Power calculation assessment for sample size [G*power3, Dusseldorf (95)]; using $\alpha = 0.05$; $1 - \beta = 0.80$; based on observed data.	Bio-Acidophilus Forte, Biocare Ltd., Birmingham, UK): *L. acidophilus* CUL-60 (NCIMB 30157), 10^{10} CFU/cap *L. acidophillus* CUL-21 (NCIMB 30156), 10^{10} CFU/cap *B. bifidum* CUL-20 (NCIMB 30172), 9.5^{10} CFU/cap *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), 0.5^{10} CFU/cap Fructooligosaccharides, 55.8 mg per cap 1 capsule daily for 90 days	Habitual diet, food diary first and last wk of each month. ^{NS} between groups or over intervention time period. Prescribed triathlon training program, individualized. ^{NS}Between groups for training load throughout intervention period.	Intestinal permeability Endotoxin responses GIS
Valle et al. (96)	$n = 65$ (39M/26F) Military recruits Age: Int: 19·69 ± 1·25 Pla: 19·5 ± 1·22 RCT	Sample calculation in G * Power 3.1.9.2 software was based on the following data: 5% sample error, 95% CI and 0.72 effect size considering pre and post-intervention IgA values. The effect size was estimated based on the study by Olivares et al. (97).	*60 g ice cream containing:* *Lactobacillus acidophilus LA-5, 10.3 log CFU;* *Bifidobacterium animalis BB^{-1}2, 11.0 log CFU 2.3 g of inulin daily for 30 days*	We recommended participants not to consume any foods containing prebiotics and probiotics (e.g., probiotic yogurts, fermented milk) 15 d before the beginning of the research period, particularly over the weekend, when they are released to go home. This consumption was controlled during the week as all food was provided (data not shown). Not stated during the supplementation period however the participants were undergoing training in a military boarding school.	Bacterial taxa Fecal SCFA GIS
West et al. (98)	$N = 22$ male cyclists Age: Syn: 34.4 ± 3.5 yrs, Pre: 31.4 ± 4.9 yrs VO_{2max}: Syn: 57.9 ± 7.3 ml/kg/min Pre: 56.4 ± 4.9 ml/kg/min RCT	Sample size was determined based on variance analysis (standard deviations) from previous studies on the parameters of interest. To demonstrate a difference of 0.20 of the pooled between-subject standard deviation in the salivary immune parameters, which have previously shown the largest variance, a total of nine subjects per group were required to give 80% power at an α level of 0.05.	Synbiotic capsules (Biosource^{TM} Gut Balance, Probiotech Pharma): *L. paracasei* subs Paracasei (*L. casei* 431$^{®}$), 4.6×10^8 per cap *Bifidobacterium animalis* ssp. lactis (BB-12$^{®}$), 6×10^8 per cap *L. acidophilus* LA-5, 4.6×10^8 per cap *L. rhamnosus* GG, 4.6×10^8 per cap Raftiline, 90 mg per cap Raftilose GR, 10 mg per cap Prebiotic capsules: Acacia powder, 116 mg per cap 3 capsules daily for 3 wk	14 days run-in, no yogurt or products influencing microbiome. Training log kept: Training load/wk: (duration × intensity) Syn: 21.3 ± 18.5 Pro: 21.4 ± 16.8^{NS}	Cytokine responses Intestinal permeability Bacterial taxa Fecal SCFA GIS

RCT, Randomized control trial; RXT, randomized crossover trial; SCFA, short chain fatty acids; wk, weeks; NS, not significant.

10.3389/fnut.2022.1003620

Intestinal permeability at rest

$N = 6$ studies assessed markers of intestinal permeability at rest, before and after the supplementation period (Tables 3, 4). Reported markers included urinary lactulose:mannitol ratio (62, 94, 98), fecal (62, 63, 76) and serum zonulin (62, 88). One study observed a reduction in both urinary lactulose:mannitol ratio and serum zonulin, pre- to post-supplement period with inulin-enriched pasta ingestion, and no change was observed in the placebo trial (62). $N = 1$ study reported a 20% reduction in fecal zonulin following 14 weeks supplementation with a multi-strain probiotic (*B. bifidum* W23, *B. lactis* W51, *E. faecium* W54, *L. acidophilus* W22, *L. brevis* W63, and *L. lactis* W58), with the post-supplementation intervention value significantly lower than placebo that remained unchanged from baseline (76). No statistically significant differences were observed for other outcomes or interventions.

Systemic bacterial endotoxin profile at rest

$N = 4$ studies assessed systemic endotoxin responses pre- and post- supplementation period (Tables 3, 4). LPS and LBP were not influenced by 6 weeks supplementation with *B. longum* 35624 (88), or following 2 weeks multi-strain supplementation with *S. thermophilus*, *L. delbrueckii* spp. *bulgaricus* and *L. rhamnosus* GG (66). Gram negative endotoxin units (using a *Limulus* amoebocyte lysate endotoxin kit) (66) and anti-LPS endotoxin-core antibodies (i.e., IgG) were unaffected by 12 weeks of a synbiotic (multi-strain probiotic plus fructo-oligosaccharide) supplementation (94). Seven days of supplementation with *L. casei* (strain not specified) resulted in no change in plasma gram negative endotoxin concentration and no difference compared with placebo (68).

Systemic inflammatory cytokine profile at rest

$N = 14$ studies assessed systemic inflammatory cytokine responses or systemic inflammatory regulating factors, before and after the supplementation period (Tables 3, 4); of which, $n = 12$ used probiotics (63, 65–67, 69, 73, 76, 82, 83, 87–89) and $n = 2$ synbiotic (93, 98) as the intervention. Of these, $n = 3$ studies observed a positive effect of probiotic supplementation compared with placebo. An attenuated rise in C-C Motif Chemokine Ligand 2 (CCL2) was observed following 2 weeks supplementation with *S. thermophilus*, *L. delbrueckii* spp. *Bulgaricus*, and *L. rhamnosus* GG, compared with placebo (66). An attenuated rise in tumor necrosis factor alpha (TNF-α) was observed following 12 weeks supplementation with *B. subtilis*, compared with placebo (88). A significant reduction was observed in interleukin (IL)-2 and IL-10 compared with baseline in the intervention group only, and a significant drop in IL-4 was observed in the placebo group only compared with baseline, following 30 days of supplementing with *Lactobacillus acidophilus-LB-G80, Lactobacillus paracasei-LPc-G110, Lactococcus* subp. *lactis-LLL-G25, Bifidobacterium animalis* subp. *lactis-BL-G101* and *Bifidobacterium bifidum-BB-G90* (87). A multi-strain synbiotic (*L. paracasei* subs *Paracasei* (*L. casei* 431®), *B. animalis* ssp. *lactis* (BB-12®), *L. acidophilus* LA-5, *L. rhamnosus* GG, *Raftiline*, and *Raftilose* GR) for 3 weeks resulted in a 50% lower increase in circulating IL-16 concentration, compared to a prebiotic control (i.e., acacia gum) (98). One study with a multi-strain synbiotic (*B. lactis* CBP-001010, *L. rhamnosus* CNCM I-4036, *B. longum* ES1, and Fructooligosaccharides) for 30 days reported greater reduction in circulating IL-10 concentration in the placebo than the intervention group (93). Otherwise, no other effects on resting systemic inflammatory cytokines were reported.

Gastrointestinal functional markers at rest

$N = 4$ studies, all prebiotic supplementation interventions, reported outcomes relating to gastrointestinal functional responses at rest (55, 59–61) (Tables 3, 4). $N = 1$ study reported a reduction in frequency of bowel movements with the consumption of bread fortified with arabinoxylan oligosaccharides (AXOS) (55). Five weeks consumption of inulin enriched pasta increased ultrasound-measured gastric half emptying time at rest by a median 8.3 min, and full emptying time by a median 30 min, with no effect observed with placebo (45). Presumably the same intervention reported in a separate paper (61), a significantly greater increase in the median proportion of normal resting slow waves (i.e., normogastria) from pre- to post-intervention with electrogastrography (EGG) was observed. No other effects of supplementation intervention were observed on gastrointestinal functional markers.

Gastrointestinal symptoms at rest

The incidence of GIS throughout the period of supplementation was measured in $n = 16$ studies; of which, $n = 6$ utilized prebiotic (45, 55–57, 59, 60, 62), $n = 7$ probiotic (70, 72, 79, 82, 85, 90, 99), and $n = 3$ synbiotic (92, 94, 96) interventions (Tables 3, 4). Prebiotics supplementation did not influence GIS incidence at rest, other than a doubling of flatulence during 28 days of chicory or Jerusalem artichoke inulin supplementation, compared to placebo (59). A mild increase in flatulence was also seen following high dose AXOS bread consumption for 3 weeks, compared with placebo (57).

TABLE 4 Systematic review study outcomes of included studies that attempted to determine the impact of prebiotic, probiotic, and synbiotic supplementation on gastrointestinal outcomes at rest.

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Intestinal permeability				
Russo et al. (62)	$N = 20$ Study design: RXT	Prebiotic vs. placebo	Inulin-enriched pasta, 5 wk	Urinary lactulose/mannitol ratio: Int ↓ 0.02, Pla ↔ 0.00 ($p < 0.05$). Serum zonulin: Int ↓ 1.61 ng/ml, Pla ↑ 0.35 ng/ml ($p < 0.05$). *Fecal zonulin:* Int ↑ 0.01 μg/g, Pla ↔ 0.00 μg/g [NS]
Axelrod et al. (63)	$n = 7$ RXT	Probiotic vs. placebo	*L. salivarius* UCC118, 4 wk	Fecal zonulin: Int ↓ 0.18 mg/dL, Pla ↓ 0.2 mg/dL [NS]
Lamprecht et al. (76)	$n = 23$ RCT	Probiotic vs. placebo	*B. bifidum W23, B lactis W51, E. faecium W54, L. acidophilus W22, L. brevis W63, L. lactis W58,* 14 wk	Fecal zonulin: Int ↓ 8.8 ng/ml, Pla ↑ 1.6 ng/ml ($p = 0.019$)
Townsend et al. (88)	$n = 25$ RCT	Probiotic vs. placebo	*Bacillis subtilis* DE111, 12 wk	Serum zonulin: Int ↑ 0.2 ng/ml, Pla ↑ 0.2 ng/ml[NS]
Roberts et al. (94)	$n = 20$ RCT	Synbiotic vs. prebiotic	*L. acidophilus* CUL-60 (NCIMB 30157), *L. acidophillus* CUL-21 (NCIMB 30156), *B. bifidum* CUL-20 (NCIMB 30172), *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153, Fructooligosaccharides, 12 wk	Urinary lactulose/mannitol ratio: Int ↑ 0.011, Pla ↑ 0.029[NS]
West et al. (98)	$n = 22$ RCT	Synbiotic vs. prebiotic	*L. paracasei* subs Paracasei (*L. casei* 431®), *B. animalis* ssp. lactis (BB-12®), *L. acidophilus* LA-5, *L. rhamnosus* GG, Raftiline, Raftilose GR, 3 wk	Lactulose/mannitol ratio: [NS]between groups (data not reported)
Endotoxin responses				
Burton et al. (66)	$n = 13$ RXT	Probiotic vs. placebo	*S. thermophilus, L. delbrueckii* spp. *Bulgaricus*, *L. rhamnosus GG*, 2 wk	LPS: Int ↓ 0.3 pg/ml, Pla ↓ 0.05 pg/ml[NS]
Gill et al. (68)	$n = 8$ RXT	Probiotic vs. placebo	*L. casei*, 1.0×10^{11} cells/bottle Commercial supplement, taken twice daily for 7 days	Gram negative endotoxin: Int ↑ 0.1 EU/ml, Pla ↑ 0.3 EU/ml[NS]
Carbuhn et al. (67)	$n = 17$ RCT	Probiotic vs. placebo	*B. longum* 35624, 6 wk	LPS: [NS]between groups (data not reported) LBP: [NS]between groups (data not reported)
Roberts et al. (94)	$n = 20$ RCT	Synbiotic vs. placebo	*L. acidophilus* CUL-60 (NCIMB 30157), *L. acidophillus* CUL-21 (NCIMB 30156), *B. bifidum* CUL-20, *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), Fructooligosaccharides, 12 wk	Endotoxin units: Int ↓ 2.30 pg/ml, Pla ↓ 0.84 pg/ml [NS] IgG endotoxin antibodies (anti-LPS): Int ↑ 42 MU/ml, Pla ↓ 42 MU/ml[NS]
Cytokine responses				
Axelrod et al. (63)	$n = 7$ RXT	Probiotic vs. placebo	*L. salivarius* UCC118, 4 wk	IL-6: (ΔΔ pre to post-exercise, pre to post-intervention) Int ↑ 0.5 pg./ml, Pla: ↑ 1.4pg/ml [NS]
Batatinha et al. (65)	$n = 27$	Probiotic vs. placebo	*B. animalis. Lactis* 10×10^9; *L. Acidophilus* 10×10^9 1 sachet daily for 30 days	IL-10: (baseline to pre-ex Δ) Int: ↓ 5.5 ng/ml, Pla: ↓ 3.2 ng/ml[NS] IL-4: Int: ↓ 3.0 ng/ml, Pla: ↓ 0.9 ng/ml[NS] IL-6: Int: ↔ 0 ng/ml, Pla: ↓ 2.5 ng/ml[NS] IL-2: Int: ↓ 0.4 ng/ml, Pla: ↓ 2.6 ng/ml[NS] IL-15: Int: ↓ 0.4 ng/ml, Pla: ↓ 0.6 ng/ml[NS] IL-8 (ng/ml): Int: ↑ 0.4 ng/ml, Pla: ↓ 3.8 ng/ml[NS] IL-1β: Int: ↓ 0.7 ng/ml, Pla: ↓ 0.8 ng/ml[NS] TNF-α: Int: ↓ 2.2 ng/ml, Pla: ↓ 3.7 ng/ml[NS] IFN-γ : Int: ↓ 2.6 ng/ml, Pla: ↓ 9.0 ng/ml [NS]
Burton et al. (66)	$n = 13$ RXT	Probiotic vs. placebo	*S. thermophilus, L. delbrueckii* spp. *Bulgaricus*, *L. rhamnosus GG*, 2 wk	TNF-α: Int ↑ 0.75 pg/ml, Pla ↑ 0.95 pg/ml[NS] IL-6: Int ↓ 0.45 pg/ml, Pla ↑ 0.65 pg/ml[NS] CCL2: Int ↑ 1.8 pg/ml, Pla ↑ 12.55 pg/ml ($p = 0.01$) CCL5: Int ↓ 12.75 pg/ml, Pla ↓ 7.6 pg/ml [NS]

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Carbuhn et al. (67)	$n = 17$ RCT	Probiotic vs. placebo	*B. longum* 35624, 6 wk	IL-1ra: Int ↓ 107 pg/ml, Pla ↓ 37 pg/ml^{NS} IFN-γ, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-17, IL-17F, and IL-22, TNF-α_ were below detectable levels in assay.
Gill et al. (68)	$n = 8$ RXT	Probiotic vs. placebo	*L. casei*, 1 wk	IL-6: Int ↑ 0.1 pg/ml, Pla ↑ 0.4 pg/ml^{NS} IL-1β: Int ↓ 0.05 pg/ml, Pla ↓ 0.02 pg/ml^{NS} TNF-α: Int ↓ 0.1 pg/ml, Pla ↑ 0.2 pg/ml^{NS} IFN-γ: Int ↑ 0.1 pg/ml, Pla ↑ 0.7 pg/ml^{NS} IL-10: Int ↑ 1.2 pg/ml, Pla ↑ 4.8 pg/ml^{NS} IL-8: Int ↔ 0.0 pg/ml, Pla ↑ 0.3 pg/ml ^{NS}
Hoffman et al. (73)	$n = 15$ RCT	Probiotic vs. placebo	*Inactivated Bacillus coagulans; 2 wk*	IFN-γ : Int: ↓ 0.2 pg/ml, Pla: ↓ 3.6 pg/ml^{NS} IL-10 : Int: ↑ 0.4 pg/ml, Pla: ↓ 1.4 pg/ml^{NS} IL1-B: Int: ↑ 0.3 pg/ml, Pla: ↑ 2.8 pg/ml^{NS} IL-2: Int: ↓ 0.3 pg/ml, Pla: ↓ 0.3 pg/ml^{NS} IL-6: Int: ↓ 0.2 pg/ml, Pla: ↓ 1.0 pg/ml^{NS} IL-8: Int: ↓ 2.4 pg/ml, Pla: ↓ 3.6 pg/ml^{NS} TNF-α: Int: ↓ 1.7 pg/ml, Pla: ↓ 4.5 pg/ml ^{NS}
Lamprecht et al. (76)	$n = 23$ RCT	Probiotic vs. placebo	*B. bifidum W23, B lactis W51, E. faecium W54, L. acidophilus W22, L. brevis W63, L. lactis W58,* 14 wk	TNF-α: Int ↓ 17.1 pg/ml, Pla ↑ 4.7 pg/ml^{NS} IL-6: Int ↓ 1.0 pg/ml, Pla ↑ 0.1 pg/ml ^{NS}
Schreiber et al. (82)	$n = 27$ RCT	Probiotic vs. placebo	*L. helveticus* Lafti L10, *B. animalis* ssp. lactis Lafti B94 *E. faecium* R0026, *B. longum* R0175 *Bacillus subtilis* R0179, 90 days	ANCOVA, (Δ) changes from baseline, adj. for training loads. IL-6 adj: Int: 0.11 ± 0.64, Pla: −0.25 ± 0.6^{NS} TNF-α adj: Int: −0.02 ± 0.23, Pla: 0.06 ± 0.21^{NS} CRP adj: Int: 443.82 ± 238.73, Pla: 231.55 ± 381.28 ^{NS}
Smarkusz-Zarzecka et al. (83)	$n = 66$ RCT	Probiotic vs. placebo	*B. lactis* W52, *L. brevis* W63, *L. casei* W56, *Lactococcus lactis* W19, *Lactococcus lactis* W58, *L. acidophilus* W37, *B. bifidum* W23, *L. salivarius* W24. 3 months	CRP: Male: Δ: Int: ↓0.12 mg/L, Pla: ↓ 0.31 mg/L^{NS} Female Δ: Int: ↓1.3 mg/L, Pla: ↓ 0.6 mg/L^{NS} TNF-α: Male: Δ: Int: ↓ 1.62 mg/L, Pla: ↓ 0.88 mg/L^{NS} Female: Δ: Int: ↓ 1.43 mg/L, Pla: ↓1.72 mg/L ^{NS}
Tavares-Silva et al. (87)	$n = 14$ RCT	Probiotic vs. placebo	Gelatinous capsules: *Lactobacillus acidophilus-LB-G80, Lactobacillus paracasei-LPc-G110, Lactococcus* subp. *lactis-LLL-G25, Bifidobacterium animalis* subp. *lactis-BL-G101, Bifidobacterium bifidum-BB-G90;* 30 days	IL-2 (Baseline to 24 h before marathon) Int: ↓ 0.37 pg/ml ($p < 0.04$), Pla: ↓ 0.2 pg/ml^{NS} IL-4 (Baseline to 24 h before marathon) Int: ↓ 0.73 pg/ml^{NS}, Pla: ↓ 0.89 pg/ml ($p < 0.04$) IL-10 (Baseline to 24 h before marathon) Int: ↓ 0.97 pg/ml ($p < 0.001$), Pla: ↓ 0.05 pg/ml^{NS} TNF-α (Baseline to 24 h before marathon) Int: ↑ 0.09 pg/ml^{NS}, Pla: ↓0.05 pg/ml ^{NS}
Townsend et al. (88)	$n = 25$ RCT	Probiotic vs. placebo	*Bacillis subtilis* DE111, 12 wk	TNF-α: Int: Δ: ↓ 0.25 pg/ml, Pla: Δ: ↑ 0.36 pg/ml Int ↓ Pla, $p = 0.024$ IL-10: Int: Δ: ↑ 0.1 pg/ml, Pla Δ: ↑ 0.15 pg/ml ^{NS}
Vaisberg et al. (89)	$n = 42$ RCT	Probiotic vs. placebo	*L. casei Shirota*, 30 days	IL-1β: Int ↑ 22.7 pg/ml, Pla ↑ 20.9 pg/ml^{NS} IL-1ra: Int ↑ 16.3 pg/ml, Pla ↑ 10.5 pg/ml^{NS} IL-4: Int ↑ 9.4 pg/ml, Pla ↑ 11.3 pg/ml^{NS} IL-5: Int ↑ 7.2 pg/ml, Pla ↑ 6.7 pg/ml^{NS} IL-6: Int ↑ 4.4 pg/ml, Pla ↑ 0.9 pg/ml^{NS} IL-10: Int ↑ 5.7 pg/ml, Pla ↑ 2.6 pg/ml^{NS} IL-12p70: Int ↑ 6.9 pg/ml, Pla ↑ 3.6 pg/ml^{NS} IL-13: Int ↑ 7.3 pg/ml, Pla ↑ 6.9 pg/ml^{NS} TNF-α: Int ↑6.6 pg/ml, Pla ↑ 22.2 pg/mL ^{NS}

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Quero et al. (93)	$n = 27$ RCT	Synbiotic vs. placebo	*B. lactis* CBP-001010, *L. rhamnosus* CNCM I-4036, *B. longum* ES1, Fructooligosaccharides, 30 days	IL-1β: Sedentary: Int: ↑ 0.3 pg/mL ($p < 0.01$), Pla: ↑ 0.1 pg/mL^{NS} Athletes: Int: ↓ 0.2 pg/mL^{NS}, Pla: ↓ 0.2 pg/mL^{NS} IL-10 Sedentary: Int: ↓ 0.3 pg/mL ($p < 0.01$), Pla: ↓ 0.4 pg/mL ($p < 0.05$) Athletes: Int: ↑ 0.05 pg/mL^{NS}, Pla: ↓ 0.05 pg/mL ^{NS}
West et al. (98)	$n = 22$ RCT	Synbiotic vs. prebiotic	*L. paracasei* subs Paracasei (*L. casei* 431®), *B. animalis* ssp. lactis (BB-12®), *L. acidophilus* LA-5, *L. rhamnosus* GG, Raftiline, Raftilose GR, 3 wk	IL-16: 50% greater increase in Pre vs. Syn ($p = 0.02$) IL-18: ^{NS}between pre and syn, no additional data shown IL-12 and IFN-γ: Undetectable in assay
Gastrointestinal function				
Damen et al. (55)	$n = 27$ RXT	Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	Defecation frequency: Int ↓ 0.1/day, Pla ↑ 0.1/day ($p < 0.05$) Bristol stool form scale: Int ↓ 0.1/day, Pla ↑ 0.2/day ^{NS}
Kleessen et al. (59)	$n = 45$ RCT	Prebiotic vs. prebiotic vs. placebo	Chicory inulin (CH) or Jerusalem artichoke inulin (JA), 3 wk	Defecation frequency: CH ↑ 3/wk ($p < 0.05$), JA ↑ 2/wk ($p < 0.05$), Pla ↑ 2/wk ($p < 0.05$) Stool consistency (1–4 scale, hard to soft): CH: ↑ 2 ($p < 0.05$), JA: ↑ 3 ($p < 0.05$), Pla: ↑ 1 ^{NS}
Russo et al. (61)	$n = 20$ RXT	Prebiotic vs. placebo	Inulin-enriched pasta, 5 wk	Ultrasound full gastric emptying time: Int ↑ 30 min ($p < 0.05$), Pla ↔ 0 min^{NS} Electrogastrography (% normal slow waves): Pre-Prandial: Int ↑ 12.5%, Pla ↑ 6.5% ($p = 0.05$) Post-prandial: Int ↑ 5.6%, Pla ↑ 2.0% ($p = 0.03$)
Russo et al. (45)	$n = 20$ RXT	Prebiotic vs. placebo	Inulin-enriched pasta, 5 wk	Ultrasound half gastric emptying time: Int ↑ 8.3 min ($p < 0.05$), Pla ↑ 1.4 min^{NS}
Reimer et al. (60)	$N = 48$ RXT	Prebiotic vs. Prebiotic vs. placebo	Chicory inulin type fructans (ITF), 4 wk	Stools/d (Δ c/f baseline): Int 1: -0.1 ± 0.2, Con 1: 0.3 ± 0.2^{NS} Int 2: -0.1 ± 0.2, Con 2: 0 ± 0.1^{NS} Bristol Stool Rating [(1–7) Δ c/f baseline]: Int 1: -0.1 ± 0.3, Con 1: -0.4 ± 0.3^{NS} Int 2: -0.1 ± 0.3, Con 2: 0 ± 0.3 ^{NS}
Bacterial taxa				
Damen et al. (55)	$n = 27$ RXT	Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	FISH analysis to count number of different bacterial groups. Total bacteria cell counts were determined by 4′-6-diamidino-2-phenylindole. ^{NS}Changes in abundance or diversity between groups or pre-post supplementation in the same group
Finegold et al. (56)	$n = 32$ RCT	Prebiotic vs. Prebiotic vs. placebo	Xylooligosaccharides (XOS), 8 wk	Bacterial diversity (Operational Taxonotic Units, species level and Shannon index): ^{NS}in α-diversity (OTU) or Shannon index. 16S rRNA gene sequencing/$\log_{10}$ scale of bacterial counts (CFU/g) ↑ *Bifidobacterium* count in high dose XOS only ($p < 0.05$) ↑ *Bacteroides fragilis* in high dose XOS only ($p < 0.05$) ↑ total anaerobes count in high dose XOS only ($p < 0.05$) ^{NS}For total aerobes, *Lactobacillus*, *Enterobacteriaceae,* and *Clostridium* counts cf. baseline in all groups ↓ *Enterobacteriaceae* count *cf* placebo after washout ($p < 0.05$)
François et al. (57)	$n = 63$ RXT	Prebiotic vs. Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	FISH analysis to count number of different bacterial groups. Total bacteria cell counts were determined by 4'-6-diamidino-2-phenylindole. Percentage of bifidobacterial calculated as the ratio of the absolute amounts of bifidobacteria to the total bacterial cell count. *Bifidobacteria* ($\log_{10}$ *counts/g dry weight feces):* High: 9.3, Low: 9.0, Pla: 8.9 High vs. Low $p < 0.05$ High vs. Pla $p < 0.001^{NS}$ for *Lactobacilli, Faecalibacterium prausnitzii*, *Clostridium histolyticum–lituseburense* or *Roseburia–Eubacterium rectale*
Kleessen et al. (59)	$n = 45$ RCT	Prebiotic vs. prebiotic vs. placebo	Chicory inulin (CH) or Jerusalem artichoke inulin (JA), 3 wk	Bacterial counts were assessed by fluorescent *in situ* hybridization or colony forming units, as assessed by conventional culture methods.

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Reimer et al. (60)	$n = 48$ RXT	Prebiotic vs. Prebiotic vs. placebo	Chicory inulin type fructans (ITF), 4 wk	*All data expressed in log^{10} counts/g wet weight feces* Total bacteria: CH: ↑ 0.1, JA: ↔0, Pla: ↔0, ^{NS}Clostridium coccoides/Eubacterium rectale cluster : CH: ↓ 0.6 ($p < 0.05$), JA: ↓ 0.6 ($p < 0.05$), Pla: ↓0.3, ^{NS}Bacteroides/Prevotella: CH: ↓ 0.4 ($p < 0.05$), JA: ↓ 0.6 ($p < 0.05$), Pla: ↑ 0.1 CH and JA both > Pla ($p < 0.05$) Faecalibacterium prausnitzii: CH: ↓ 0.2, JA: ↓ 0.2, Pla: ↓ 0.1, ^{NS}Bifidobacterium: CH: ↑ 1.2, JA: ↑ 1.2, Pla: ↑ 0.3 CH and JA both > Pla ($p < 0.05$) Atopobium group: CH:↔ 0, JA: ↓ 0.2, Pla: ↔ 0, ^{NS}Lactobacillus: CH: ↓ 0.9, JA: ↓ 0.5, Pla: ↓ 0.7, ^{NS}Enterococcus: CH: ↓ 0.9, JA: ↓ 0.4, Pla: ↑ 0.4, ^{NS}Enterobacteriaceae: CH: ↓ 0.4, JA: ↓ 0.9, Pla: ↓ 0.7 ^{NS} Results are expressed as relative abundance (%) of *Bifidobacterium* per total bacteria (*Bifidobacterium* 16S rRNA gene copies × 100/total 16S rRNA gene copies). Bacterial diversity ^{NS}in α-diversity. Community Structure ^{NS} in β-diversity Microbial abundance (phylum) *(Con => Int 1/Int 1 => Con)* Actinobacteria: Con: 6.02 ± 5.26 Int 1: 15.23 ± 12.37, ↑ 153% $p < 0.01$(adj) Int: 11.70 ± 8.65 Con: 6.36 ± 3.95 ↓ 83% $p < 0.01$(<adj) Firmicutes Con: 85.91 ± 9.02 Int 1: 78.72 ± 10.96, ↓ 8% $p < 0.01$(adj) Int: 82.52 ± 9.58 Con: 88.13 ± 4.37 ↑ 6% $p < 0.01$(adj) Bacteroidetes Con: 6.51 ± 7.96 Int 1: 3.48 ± 4.26, ↓ 46% $p = 0.05$(adj) *(Con => Int 2/Int 2 => Con)* Actinobacteria Con: 8.07 ± 7.38 Int 2: 13.19 ± 12.37 0.01 ↑ 63% ^{NS}Proteobacteria Con: 0.79 ± 1.29 Int 2: 0.38 ± 0.39 0.04 ↓ 51%^{NS} *Family (Con ≥ Int 1/Int 1 ≥ Con)* Bifidobacteriaceae Con: 2.52 ± 2.90 Int 1: 10.28 ± 9.09, $p < 0.01$(adj) ↑ 308% $p < 0.01$(adj) 7.57 ± 8.08 2.63 ± 1.88 ↓ 65% $p < 0.01$(adj) Actinomycetaceae Con: 0.06 ± 0.09 Int 1: 0.24 ± 0.29 ↑ 300% $p < 0.01$(adj) Int 1: 0.23 ± 0.24 Con: 0.09 ± 0.08 ↑ 60% $p < 0.01$(adj) Microbacteriaceae Con: 0.003 ± 0.01 Int 1: 0.01 ± 0.02 ↑ 233% $p < 0.01$(adj) Int 1: 0.01 ± 0.02 0.0006 ± 0.003 ↓ 94% $p < 0.05$(adj) Cellulomonadaceae Con: 0.0003 ± 0.002 Int 1: 0.01 ± 0.01 ↑ 3,233% $p < 0.01$(adj) Micrococcaceae Con: 0.06 ± 0.10 Int 1: 0.19 ± 0.33 ↑ 216% $p < 0.01$(adj) Brevibacteriaceae Con: 0.01 ± 0.03 Int 1: 0.03 ± 0.05 ↑ 200% $p < 0.01$(adj) *Family (Con ≥ Int 2/Int 2 ≥ Con)* Micrococcaceae Con: 1.18 ± 1.72 Int 2: 2.37 ± 2.67 ↑ 101%^{NS}

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
				Vibrionaceae Con: 0.21 ± 0.44 Int 2: 0.05 ± 0.08 ↓ 76%^{NS} Bifidobacteriaceae Con: 1.17 ± 1.91 Int 2: 2.39 ± 3.63 ↑ 104%^{NS} Enterobacteriaceae Con: 0.42 ± 0.93 Int 2: 0.10 ± 0.17 ↓ 76^{NS} Actinomycetaceae Con: 0.44 ± 0.77 Int 2: 0.88 ± 1.44 ↑ 100%^{NS} *Genus (Con ≥ Int 1/Int 1 ≥ Con)* Bifidobacterium Con: 5.30 ± 5.87 Int 1: 18.73 ± 14.99, ↑ 253% p < 0.01(adj) Int 1: 11.91 ± 12.02 Con: 4.63 ± 3.42 ↓ 61% $p < 0.01$(adj) Actinomyces Con: 0.13 ± 0.18 Int 1: 0.45 ± 0.49, ↑ 246% $p < 0.01$(adj) Int 1: 0.37 ± 0.38 Con: 0.16 ± 0.15 ↓ 56% $p < 0.02$(adj) Cellulomonas Con: 0.0007 ± 0.01 Int 1: 0.01 ± 0.03 ↑ 1,328% $p < 0.02$(adj) Nesterenkonia Con: 0.12 ± 0.21 Int 1: 0.35 ± 0.54 ↑ 191% $p < 0.03$(adj) Lachnospira Con: 2.20 ± 2.70 Int 1: 0.93 ± 1.36 ↓ 57% $p < 0.04$(adj) Oscillospira Con: 1.11 ± 1.01 Int 1: 0.65 ± 0.54 ↓ 41% $p < 0.04$(adj) Brevibacterium Con: 0.03 ± 0.05 Int 1: 0.06 ± 0.08 ↑ 100% $p < 0.04$(adj) *Genus (Con => Int 2)* Nesterenkonia Con: 2.46 ± 3.32 Int 2: 4.86 ± 4.75 ↑ 97% ^{NS} Vibrio Con: 0.50 ± 1.12 Int 2: 0.10 ± 0.16 ↓ 80% ^{NS} Bifidobacterium Con: 2.47 ± 3.83 Int 2: 4.62 ± 6.13 ↑ 87%^{NS} Actinomyces Con: 0.91 ± 1.53 Int 2: 1.67 ± 2.46 ↑ 83%^{NS}
Axelrod et al. (63)	$n = 7$ RXT	Probiotic vs. placebo	*L. salivarius* UCC118, 4 wk	DNA extraction by shotgun metagenomic sequencing. Shannon and Simpson index ^{NS}in α-diversity or richness. *Probiotic data only, no placebo data available* Phyla: *Verrucomicrobia* ↓ 0.144% ($q = 0.001$) Genus: *Prosthecobacter* ↓ 0.141% ($q = 0.004$) Species: *fusiformis* ↓ 0.051% ($q = 0.006$)
Burton et al. (66)	$n = 13$ RXT	Probiotic vs. placebo	*S. thermophilus*, *L. delbrueckii* spp. *Bulgaricus, L. rhamnosus* GG, 2 wk	16S rRNA gene sequencing *Relative abundance compared to baseline* *S. salivarius* spp. *thermophilus*: Int ↑ 0.10%, Pla ↔ 0.0% ($p < 0.05$) *L. delbrueckii* spp. *Bulgaricus*: Int ↑ 0.02%, Pla ↔ 0.0% ($p < 0.05$) *L. rhamnosus* GG: Int ↔ 0.0%, Pla ↔ 0.0% ^{NS} *Bilophila wadsworthia*: Int ↓ 0.07%, Pla ↓ 0.27% ^{NS} *B. kashiwanohense*/*B. pseudocatenulatum*: Int ↓ 0.05%, Pla ↑ 0.05% ($p < 0.05$)

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Huang et al. (74)	$n = 20$ male RCT	Probiotic vs. placebo	*L. plantarum PS128, 4 wk*	16S rRNA gene sequencing **No baseline data reported**. % Relative abundance (Phyla) *Int: Fermicutes 46.6%, Bacteriodetes 47.0%, Proteobacteria 3.8%, Actinobacteria 2.1%, Fusobacteria 0.3%* *Pla: Fermicutes 50.3%, Bacteriodetes 41.6%, Proteobacteria 4.9%, Actinobacteria 1.0%, Fusobacteria 1.5%* ^{NS} between groups. Relative abundance (Genus) *Anaerotruncus ($\times 10^{-4}$) Int 0, Pla 1.0; Caproiciproducens ($\times 10^{-4}$) Int 0.1, Pla 1.0; Coprobacillus ($\times 10^{-5}$) Int 0, Pla 3.3; Desulfovibrio ($\times 10^{-5}$), Int 0, Pla 5.9; Dielma ($\times 10^{-5}$), Int 0, Pla 2.6; Family_XIII_UCG_001 ($\times 10^{-5}$), Int 0.9, Pla 9.2; Holdemania ($\times 10^{-5}$), Int 0.6, Pla 7.2; Oxalobacter ($\times 10^{-5}$), Int 0, Pla 6.1;* ***Int < Pla ($p < 0.05$)*** *Akkermansia ($\times 10^{-3}$), Int 5.0, Pla 1.3; Bifidobacterium ($\times 10^{-2}$), Int 1.5, Pla 0.8; Butyricimonas ($\times 10^{-3}$), Int 4.7, Pla 2.3; Lactobacillus ($\times 10^{-3}$), Int 1.7, Pla 0.7;* ***Int > Pla ($p < 0.05$)***
Klein et al. (75)	$n = 26$ RXT	Probiotic vs. placebo	*B. lactis* 420x, *L. acidophilus* 74-2, 5 wk	Preparation of fecal samples by FISH analysis. Relative abundance compared to baseline *B. lactis*: Int ↑ 1.43%, Pla ↑ 0.39% ($p < 0.05$) *L. acidophilus*: Int ↑ 0.18%, Pla ↑ 0.02% ($p < 0.05$)
Lee et al. (77)	$n = 16$ RCT	Probiotic vs. placebo	*L. paracasei DSM 32785 (LPC12), L. rhamnosus DSM 32786 (LRH10), L. helveticus DSM 32787 (LH43), L. fermentum DSM 32784 (LF26), and S. thermophilus DSM 32788 (ST30)* *28 days*	qPCR method was used for the identification and quantification of gut microbiota. *Pre-post Δ in Log10 cells/g* *Lactobacillus:* *Int: ↑ 0.2, Pla: ↑ 0.5, ^{NS}* *Bifidobacterium:* *Int: ↓ 0.2, Pla: ↑ 0.3 "Decreased in intervention group" ($p < 0.05$)* *Clostridium: Int: ↓ 1.0, Pla:↓ 1.0 ^{NS}* *Bacteroides: Int: ↓ 0.3, Pla: ↑ 0.1 ^{NS}*
Lin et al. (78)	$n = 21$	Probiotic vs. placebo	*Bifidobacterium longum* subsp. *Longum, 5 wk*	16S rRNA gene sequencing *Phylum:* *Int: Actinobacteria and Firmicutes greater abundance post-supplementation, compared with Pla. (p-value not shown).* *Proteobacteria reduced abundance post-supplementation, compared with Pla. (p-value not shown).* *Genus:* *Int: ↑ Bifidobacterium compared with Pla ($p = 0.0027$). 9-fold ↑ in Lactobacillus count.* *Species:* *Bifidobacterium longum subsp. longum relative abundance* *Int: 0.95%; ↑ 8.63-fold ($p = 0.0178$).* *Pla: 0.11%* *^{NS} in amounts of common strains*
Sánchez Macarro et al. (80)	$n = 43$ RCT		*Bifidobacterium longum CECT 7347 Lactobacillus casei CECT 9104, and Lactobacillus rhamnosus CECT 8361 6 wk*	16S rRNA gene sequencing Bacterial diversity *Richness:* Int:↔ 0, Pla: ↓6 ^{NS} *Simpson index:* Int: ↔ 0, Pla: ↑0.03, ^{NS} *Shannon index:* Int: ↑0.01, Pla: ↑0.15 ^{NS} *Family (log relative counts)* *Rhodospirillaceae:* *Int < Pla, log2 fold = 2.71, $p = 0.019$ (adj)* *Streptococcaceae:* *Int < Pla, log2 fold = 2.20, $p = 0.019$(adj)* *Genera (log relative counts)* *Rhodospirillum:* *Pla > Int, $p = 0.007$(adj)* *Streptococcus:* *Pla > Int, $p = 0.007$(adj)* *Within group differences noted in genera.*

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Son et al. (84)	$n = 15$ RCT	Probiotic vs. placebo	*L. acidophilus, L. casei, L. helveticus, B. bifidum, 60 days*	16S rRNA gene sequencing Shannon and Simpson index ^{NS} in α-diversity, pre and post *Species:* ^{NS} changes in the abundance of the four microorganisms present (three Lactobacilli and one Bifidobacterium).
West et al. (90)	$n = 88$ RCT	Probiotic vs. placebo	*Lactobacillus fermentum* VRI-003 PCC®, 11 wk	Microbiome Diversity (16SrRNA) ^{NS} changes in bacterial diversity (data not shown) *All data reported as raw bacterial counts—no statistical testing of between group changes.* Total bacteria: Males: Int ↓ 0.5×10^{10}, Pla Pre: ↓ 0.5×10^{10} Females: Int ↑ 0.7×10^{10}, Pla ↓ 1.0×10^{10} *C. coccoides*: Males: Int ↓ 2.3×10^{8}, Pla ↓ 3.4×10^{8} Females: Int ↔ 0, Pla ↓ 1.54×10^{9} *E. coli*: Males: Int ↑ 6.4×10^{5}, Pla ↑ 6.8×10^{5} Females: Int ↑ 1.36×10^{7}, Pla ↑ 4.3×10^{4} *Bifibacteria*: Males: Int ↓ 0.3×10^{7}, Pla ↓ 5.6×10^{6} Females: Int ↑ 0.7×10^{6}, Pla ↓ 6.1×10^{6} *Bacteroides*: Males: Int ↑ 0.6×10^{6}, Pla ↑ 1.6×10^{6} Females: Int ↑ 1.3×10^{6}, Pla ↓ 4.4×10^{7} *Lactobacillus*: Males: Int ↑ 5.8×10^{4}, Pla ↓ 2.8×10^{6} Females: Int ↑ 7.0×10^{4}, Pla ↑ 6.9×10^{4}
Coman et al. (92)	$n = 10$ RCT	Synbiotic vs. placebo	*L. rhamnosus* IMC 501[R], *L. paracasei* IMC 502[R], plus oat bran fiber, 4 wk	qPCR procedure for quantification of selected bacterial groups Log CFU/g feces *Bacteroides-Prev.-Porphyr.* spp.: Int ↓ 0.18 log CFU/g, Pla ↑ 0.21 log CFU/g ^{NS} *Staphylococcus* spp.: Int ↓ 0.08 log CFU/g, Pla ↑ 0.16 log CFU/g ^{NS} *Cl. coccoides-Eubact. rectale* group: Int: ↓ 0.33 log CFU/g, Pla ↓ 0.01 log CFU/g ^{NS} *Lactobacillus* spp.: Int: ↑ 1.44 log CFU/g ($p < 0.05$), Pla ↓ 0.43 log CFU/g ^{NS} *Bifidobacterium* spp.: Int: ↑ 1.52 log CFU/g ($p < 0.05$), Pla ↑ 0.16 log CFU/g ^{NS} *Enterobacteriaceae:* Int: ↓ 0.14 log CFU/g, Pla ↑ 0.35 log CFU/g ^{NS}
Valle et al. (96)	$n = 65$ RCT	Synbiotic vs. placebo	*Lactobacillus acidophilus LA-5; Bifidobacterium animalis BB-12* *2.3 g of inulin, 30 days*	16S gene sequencing *α–Diversity (Shannon index):* *Int:*↓ 0.125 *Pla:* ↑ *0.027* ^{NS} *α–Diversity (Simpson index): Int:* ↓ *0.017, Pla:* ↑ *0.01* ^{NS} *Microbiome Diversity (16SrRNA)* ^{NS} changes in bacterial diversity (data not shown) *All data reported as raw bacterial counts.* Total bacteria: Syn ↔ 0, ^{NS}, Pre ↑ 2×10^{8} Total *Lactobacillus* (mean): Syn ↔ 0, Pre ↑ 1.5×10^{4} ^{NS} L. *paracasei* (mean): Syn ↑ 8×10^{2}, Pre ↓ 2×10^{2} ("large" 9-fold difference) B. *lactis* (mean): Syn ↑ 2.7×10^{4}, Pre ↑ 4.8×10^{3} ^{NS}
West et al. (98)	$n = 22$ RCT	Synbiotic vs. prebiotic	*L. paracasei* subs *Paracasei* (*L. casei* 431®), *B. animalis* ssp lactis (BB-12®), *L. acidophilus* LA-5, *L. rhamnosus* GG, Raftiline, Raftilose GR, 3 wk	
Short chain fatty acids				
Damen et al. (55)	$n = 27$ RXT	Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	*All data reported in μmol/g wet feces* Total SCFA: Int: ↑ 25.3 ($p < 0.05$), Pla ↑ 9.6 ^{NS} Acetic acid: Int: ↑ 10.2, Pla ↑ 4.8^{NS} Butyric acid: Int: ↑ 7.6 ($p < 0.05$), Pla ↑ 2.3^{NS} Propionic acid: Int: ↑ 3.2, Pla ↑ 1.3 ^{NS}
Finegold et al. (56)	$n = 32$ RCT	Prebiotic vs. Prebiotic vs. placebo	High and Low dose Xylooligosaccharides (XOS), 8 wk	Total SCFA (μmol/g dry feces): High ↓ 0.01, Low ↓ 0.06, Pla ↓ 0.06^{NS}
François et al. (57)	$n = 63$ RXT	Prebiotic vs. Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	*All data reported in μmol/g dry feces* Total SCFA: High vs. Pla: ↑ 53.1 ($p = 0.001$), Low vs. Pla: ↑ 7.8 ^{NS} Acetic acid: High vs. Pla: ↑ 38.5 ($p = 0.003$), Low vs. Pla: ↑ 8.9 ^{NS} Butyric acid: High vs. Pla: ↑ 5.0 ($p = 0.05$), Low vs. Pla: ↓ 3.9 ^{NS} Propionic acid: High vs. Pla: ↑ 9.7 ($p = 0.003$), Low vs. Pla: ↑ 2.9 ^{NS}

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
Kleessen et al. (59)	$n = 45$ RCT	Prebiotic vs. prebiotic vs. placebo	Chicory inulin (CH) or Jerusalem artichoke inulin (JA), 3 wk	Total SCFA post-intervention (μmol/g wet feces): CH: 142.4, JA: 135.2, Pla: 138.8 ^{NS}
Reimer et al. (60)	$n = 48$ RXT	Prebiotic vs. Prebiotic vs. placebo	Chicory inulin type fructans (ITF), 4 wk	Fecal acetate Int 1: ↑ 2.9 umol/g, Con 1: ↑ 6.7 umol/g ^{NS} Int 2: ↑ 6.7 umol/g, Con 2: ↑ 9.2 umol/g ^{NS} Fecal proprionate Int 1: ↑ 3.8 umol/g, Con 1: ↑ 2.4 umol/g ^{NS} Int 2: ↓ 1.3 umol/g, Con 2: ↑ 1.3 umol/g ^{NS} Fecal butyrate Int 1: ↑ 2.5 umol/g, Con 1: ↑ 0.3 umol/g ^{NS} Int 2: ↑ 4.4 umol/g, Con 2: ↑ 6.7 umol/g ^{NS} Fecal Isobutyrate Int 1: ↔ 0 umol/g, Con 1: ↑ 0.6 umol/g ^{NS} Int 2: ↔ 0 umol/g, Con 2: ↔ 0 umol/g ^{NS} Fecal Isovalerate Int 1: ↔ 0 umol/g, Con 1: ↔ 0 umol/g ^{NS} Int 2: ↔ 0 umol/g, Con 2: ↑ 0.4 umol/g ^{NS}
Huang et al. (74)	$n = 20$ male RCT	Probiotic vs. placebo	*L. plantarum PS128, 4 wk*	Acetic acid (mean, post only): Int: 4.7 ng/ml, Pla: 3.8 ng/ml Int > Pla ($p < 0.05$) Proprionic acid (mean, post only): Int: 1.18 ng/ml, Pla: 0.5 ng/ml Int > Pla ($p < 0.05$) Butyric acid (mean, post only): Int: 0.5 ng/ml, Pla: 0.3 ng/ml Int > Pla ($p < 0.05$) Decanoic acid (mean, post only): Int: 0.005 ng/ml, Pla: 0.002 ng/ml ^{NS} Heptanoic acid (mean, post only): Int: 0.6 ug/ml, Pla: 0.4 ug/ml ^{NS} Hexanoic acid (mean, post only): Int: 1.7 ug/ml, Pla: 4.0 ug/ml ^{NS} Isobutyric acid (mean, post only): Int: 0.050 ng/ml, Pla: 0.052 ng/ml ^{NS} Isovaleric acid (mean, post only): Int: 0.03 ng/ml, Pla: 0.04 ng/ml ^{NS} Octanoic acid (mean, post only): Int: 1.1 ug/ml, Pla: 0.7 ug/ml ^{NS} Valeric acid (mean, post only): Int: 0.07 ng/ml, Pla: 0.07 ng/ml ^{NS}
Son et al. (84)	$n = 15$ RCT	Probiotic vs. placebo	*L. acidophilus, L. casei, L. helveticus, B. bifidum, 60 days*	Acetic acid Int: ↓ 40 umol/g, Pla: ↓ 85 umol/g ^{NS} Buytric acid Int: ↓ 142 umol/g, Pla: ↑ 125 umol/g Int > Pla at baseline (*p*-value not shown) Propionic acid Int: ↓ 1.31 umol/g, Pla: ↓ 1.51 umol/g ^{NS}
Klein et al. (75)	$n = 26$ RXT	Probiotic vs. placebo	*B. lactis* 420×, *L. acidophilus* 74-2, 5 wk	*All data post-intervention concentration (μmol/g feces)* Total SCFAs: Int 85.0 μmol/g, Pla 88.5 μmol/g ^{NS} Acetic acid Int: 46.7 μmol/g, Pla 49.5 μmol/g ^{NS} *i*-Butyric acid Int 1.9 μmol/g, Pla 2.0 μmol/g ^{NS} n-Butyric acid Int 14.6 μmol/g, Pla 15.1 μmol/g ^{NS} Propionic acid Int: 16.7 μmol/g, Pla 16.9 μmol/g ^{NS} Valeric acid: Int: 2.1 μmol/g, Pla 2.0 μmol/g ^{NS} Isovaleric acid: Int: 2.3 μmol/g, Pla 2.4 μmol/g ^{NS} Caproic acid: Int: 0.7 μmol/g, Pla 0.6 μmol/g ^{NS}
Valle et al. (96)	$n = 65$	Synbiotic vs. placebo	*Lactobacillus acidophilus LA-5; Bifidobacterium animalis BB-12* *2.3 g of inulin, 30 days*	Fecal acetate (mmol/L): Int: OR 0.34, 95%CI −0.06, 0.74 Pla: OR 0.16, 95%CI −0.25, 0.57 ^{NS} between groups

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
				Fecal proprionate (mmol/L): Int: OR 0.20, 95%CI −0.01, 0.41 Pla: OR 0·31, 95%CI −0.02, 0.63 NS between groups Fecal butyrate (mmol/L): Int: OR 0.39, 95%CI 0.20, 0.59 Pla: OR 0.25, 95%CI −0.03, 0.47 NS between groups Fecal ammonia (mmol/l): Pla: OR 0.09, 95%CI 0.01, 0.17 Int: OR 0.11, 95%CI 0.04, 0.18 NS between groups
West et al. (98)	$n = 22$ RCT	Synbiotic vs. prebiotic	*L. paracasei* subs Paracasei (*L. casei* 431®), *B. animalis* ssp. lactis (BB-12®), *L. acidophilus* LA-5, *L. rhamnosus* GG, Raftiline, Raftilose GR, 3 wk	*All data reported in μmol/g feces* Acetic acid: Syn ↓ 2, Pre ↓ 6 NS Butyric acid: Syn ↓ 2, Pre ↓ 3 NS Propionic acid: Syn ↓ 2, Pre ↓ 1.5 NS
Gastrointestinal symptoms				
Damen et al. (55)	$n = 27$ RXT	Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	GIS: Insufficient incidence to analyze abdominal pain or bloating. Flatulence: Int: NS difference pre-to-post Pla: ↑ 0.53 on 0–4 scale pre-to-post ($P = 0.02$)
Finegold et al. (56)	$n = 32$ RCT	Prebiotic vs. Prebiotic vs. placebo	Xylooligosaccharides (XOS), 8 wk	*Symptoms rated from 0 (no symptoms) to 3 (severe)* Excess flatus: High ↑ 0.27, Low ↑ 0.26, ↑ Pla 0.19 NS Borborygmi: High ↑ 0.26, Low ↑ 0.11, ↑ Pla 0.02 NS Bloating: High ↑ 0.28, Low ↑ 0.22, ↑ Pla 0.06 NS Abdominal pain: High ↓ 0.01, Low ↑ 0.27, ↑ Pla 0.10 NS
François et al. (57)	$n = 63$ RXT	Prebiotic vs. Prebiotic vs. placebo	Arabinoxylan oligosaccharides (AXOS), 3 wk	↑ Occurrence frequency + ↑ Distress severity, flatulence only cf Pla ($P = 0·02$) Flatulence (*mild/moderate/very disturbing symptoms, %*): High 27/7/2, Low 16/7/2, Pla 11/6/2 NS NS all other symptoms.
Kleessen et al. (59)	$n = 45$ RCT	Prebiotic vs. prebiotic vs. placebo	Chicory inulin (CH) or Jerusalem artichoke inulin (JA), 3 wk	*All data reported as incidence (%) post-supplementation* Flatulence: CH 87, JA 93, Pla 47 (CH and JA > Pla, $p < 0.05$) Abdominal bloating: CH: 0, JA: 27, Pla: 27 NS Abdominal pain or cramps: CH: 20, JA: 7, Pla: 7 NS Bowel Rumbling: CH: 13, JA: 13, Pla: 13 NS Bowel Cramps: CH: 20, JA: 20, Pla: 13 NS
Reimer et al. (60)	$n = 48$ RXT	Prebiotic vs. Prebiotic vs. placebo	Chicory inulin type fructans (ITF), 4 wk	Abdominal pain, 0–4 Int 1: 0.3 ± 0.2, Con 1: 0.3 ± 0.2 NS Int 2: 0.4 ± 0.2, Con 2: 0.3 ± 0.2 NS Distension/bloating (0–4) Int 1: 0.5 ± 0.3, Con 1: 0.4 ± 0.2 ($P = 0.025$) Int 2: 0.6 ± 0.2 ($P = 0.023$), Con 2: 0.2 ± 0.1 ($P = 0.048$) NS between groups Flatulence (0–4) Int 1: 0.3 ± 0.2, Con 1: 0.3 ± 0.2 NS Int 2: 0.3 ± 0.2, Con 2: 0.0 ± 0.2 NS Stomach rumbling (0–4) Int 1: 0.1 ± 0.2, Con 1: 0.1 ± 0.2 NS Int 2: 0.2 ± 0.2, Con 2: 0.1 ± 0.1 NS
Russo et al. (45)	$n = 20$ RXT	Prebiotic vs. placebo	Inulin-enriched pasta, 5 wk	NS Differences and no major symptoms (data not reported)
Gleeson et al. (70)	$n = 58$ RCT	Probiotic vs. placebo	L. casei Shirota, 16 wk	GIS Incidence: Int 54%, Pla 57% NS Proportion of days with GIS: Int 2%, Pla 3% ($p = 0.008$)

(Continued)

TABLE 4 (Continued)

References	*N* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Outcome measure/s (Δ in mean/median from pre- to post-supplementation period unless otherwise indicated)
				Severity Score: Int 9, Placebo 12 ^{NS} Symptom duration (days): Int 4.2, Pla 5.9 ^{NS}
Haywood et al. (72)	$n = 30$ RCT	Probiotic vs. placebo	*L. gasseri*, *B. bifidum, B. longum,* 4 wk	GIS Incidence: Int: 13%, Pla: 13% ^{NS}
Pugh et al. (79)	$n = 24$	Probiotic vs. placebo	*L. acidophilus* CUL60, *L. acidophilus* CUL21, *B. bifidum* CUL20, *B. animalis* subsp. *Lactis* CUL34, 4 wk	Number of GI scores ≥ 4: Days 1–14: Int 4, Pla 5^{NS} Days 15–28: Int 2, Pla 11 ^{NS}
Schreiber et al. (82)	$n = 27$ RCT	Probiotic vs. placebo	*L. helveticus* Lafti L10, *B. animalis* ssp. lactis Lafti B94 *E. faecium* R0026, *B. longum* R0175 *Bacillus subtilis* R0179, 90 days	GIS incidence at rest (ΔGI): Int: −30 ± 48%, Pla: −27 ± 47% ^{NS} Categorized as: Nausea incidence at rest (ΔGI): Int −16 ± 43%, Pla: 71 ± 119% Int < Pla, $P = 0.01$, $d = 0.9$ Belching incidence at rest (ΔGI): Int: −14 ± 53%, Pla: 62 ± 115%, Int < Pla, $P = 0.04$, $d = 1$ Vomiting incidence at rest (ΔGI): Int: −7 ± 30%, Pla: 49 ± 114%, Int < Pla, $P = 0.04$, $d = 0.7$ Other sub-categories not reported.
Strasser et al. (85)	$n = 29$ RCT	Probiotic vs. placebo	*Bifidobacterium bifidum* W23 *Bifidobacterium lactis* W51 *Enterococcus faecium* W54 *Lactobacillus acidophilus* W22 *Lactobacillus brevis* W63 *Lactococcus lactis* W58 12 wk	Incidence: "Only one participant in the placebo group experienced GI-discomfort symptoms during the study period."
West et al. (90)	$n = 88$ RCT	Probiotic vs. placebo	*Lactobacillus fermentum* VRI-003 PCC®, 11 wk	No. of GIS episodes: Male: Int 1.01, Pla: 0.49 (Likely ↑) Female: Int 1.44, Pla 0.48, (Likely ↑) Duration of GIS episodes (days): Male: Int 3.3, Pla 1.3, (Likely ↑) Female: Int 3.9, Pla 2.1, (Possible ↑) GIS severity (1–3 scale): Male: Int 1.31, Pla 1.78 (Possible ↓) Female: Int 1.44, Pla 1.75 (Possible ↓) Symptom Load (severity-days): Male: Int 4.4, Pla 2.5 (Possible ↑) Female: Int 5.2, Pla 2.9 (Possible ↑)
Coman et al. (92)	$n = 10$ RCT	Synbiotic vs. placebo	*L. rhamnosus* IMC 501[R], *L. paracasei* IMC 502[R], oat bran fiber, 4 wk	*Data reported as change from baseline, Likert scale (0–5)* Intestinal regularity: Int ↑ 2.6, Pla ↑ 1.8 ($p < 0.05$) Stool volume: Int ↑ 1.8, Pla ↑ 2.2^{NS} Ease at defecation: Int ↑ 2.2, Pla ↑ 0.6 ($p < 0.05$) Bloating: Int ↑ 0.2, Pla ↑ 0.4^{NS} Abdominal pain: Int ↔ 0.0, Pla ↓ 0.2^{NS} Intestinal cramps: Int ↔ 0.0, Pla ↓ 0.4 ^{NS}
Roberts et al. (94)	$n = 20$ RCT	Synbiotic vs. placebo	*L. acidophilus* CUL-60 (NCIMB 30157), *L. acidophillus* CUL-21 (NCIMB 30156), *B. bifidum* CUL-20, *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), Fructooligosaccharides, 12 wk	Mean total GIS score during supplemental period: Int 7.00, Pla 13.9 ($p < 0.001$) Mean GIS severity score during supplemental period: Int 8.00, Pla 16.7 ($p < 0.001$)
Valle et al. (96)	$n = 65$	Synbiotic vs. placebo	*Lactobacillus acidophilus LA-5; Bifidobacterium animalis BB-12* *2·3 g of inulin, 30 days*	Sum of symptoms (nausea, vomiting, diarrhea, abdominal pain, flatulence, loss of appetite, burning and dysphagia) Int: Δ Post-supp.: OR −2·24, 95%CI −3·15, −1·34 Pla: Δ Post-supp: OR −1·16, 95%CI −2·51, 0·18 ^{NS}Between groups.

CFU, colony forming units; CRP, c-reactive protein; FISH, fluorescence *in situ* hybridization; GIS, Gastrointestinal symptoms; IFN, interferon; IL, interleukin; Int, Intervention; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; NS, Not significant; OTU, operational taxonomy units; Pla, Placebo; Pre, Prebiotic intervention; RCT, Randomized control trial; RXT, Randomized crossover trial; Syn, Synbiotic intervention; wk, weeks.

In contrast with this, following 3 weeks of consumption of AXOS fortified bread, flatulence was only increased during the control bread period (55). Probiotic supplementation in all but two studies, did not influence GIS incidence at rest. Supplementation with *L. fermentum* VRI-003 PCC® for 11 weeks increased GIS incidence, and also resulted in a ∼2-day increased duration of symptoms, but a small reduction in symptom severity (90). Whilst overall GIS incidence at rest remained unchanged following 90 days of supplementation with a multistrain probiotic (*Lactobacillus helveticus Lafti L10, Bifidobacterium animalis ssp. lactis Lafti B94 Enterococcus faecium, Bifidobacterium longum R0175* and *Bacillus subtilis R0179*), the subcategories of nausea, belching and vomiting were significantly lower in the probiotic group compared to placebo

(82). The majority of participants in the synbiotic studies reported no GIS incidence at rest (96). However, $n = 1$ study observed GIS severity scores at rest that were approximately half of those reported following placebo supplementation, following 12 weeks of a multi-strain synbiotic with added fructooligosaccharides (94). Increased intestinal regularity and ease of defecation was also reported following 4 weeks supplementation with *L. rhamnosus* IMC 501[R], *L. paracasei* IMC 502[R], plus oat bran fiber (92).

Fecal microbial composition at rest

A total of $n = 17$ studies reported on resting microbial composition of stool samples (i.e., representative of luminal microbial composition) before and after the supplementation intervention period, of which $n = 5$ prebiotic (55–57, 59, 60), $n = 9$ probiotic (63, 66, 74, 75, 77, 78, 80, 84, 90), and $n = 3$ synbiotic interventions (92, 96, 98) (Tables 3, 4). Supplementation with xylooligosaccharides (XOS) (56), high dose (8.0 g/d) AXOS (57), chicory or Jerusalem artichoke inulin (59) or moderate dose (7.0 g/d) chicory inulin type fructans (60) resulted in significant increases in *Bifidobacterium* relative abundance (i.e., proportion of total bacterial counts), with a trend for the same effect in the fourth study (55) as determined by 16S rRNA sequencing (56, 60), and fluorescent *in situ* hybridization (FISH) analysis (57, 59). In contrast, low dose chicory inulin type fructan supplementation showed no increase in *Bifidobacterium* relative abundance (60). AXOS supplementation was also observed to increase *Bacteroides fragilis* group count, and total anaerobes count, compared to baseline (56). Reductions in *Bacteroides/Prevotella* were observed for both chicory and Jerusalem artichoke inulin supplementation, as assessed by grams of wet feces (59). Moderate dose chicory inulin-type fructan supplementation showed increases in microbial abundance at the phylum level in *Actinobacteria*, with reductions in *Firmicutes* and *Bacteroidetes*, with non-significant changes in the low dose group compared with control (60). At the family level, moderate dose chicory inulin-type fructan supplementation resulted in increases in *Bifidobacteriaceae, Actinomycetaceae, Microbacteriaceae, Cellulomonadaceae, Micrococcaceae,* and *Brevibacteriaceae*, but this effect was not observed in low doses of the supplement. At the genus level, increases were observed in *Bifidobacterium, Actinomyces, Cellulomonas,* and *Nesterenkonia*, with reductions in *Lachnospira*, *Oscillospira*, and *Brevibacterium* following moderate dose chicory inulin-type fructan supplementation (60). No effects of prebiotic supplementation were seen for other fecal sample bacterial taxa analyses, including α-diversity (56, 60).

Of the probiotic studies, $n = 4$ demonstrated significant increases in the supplemented species (*S. salivarius* spp. *thermophilus and L. delbrueckii* spp. *Bulgaricus, B. lactis* 420× and *L. acidophilus*, *L. fermentum* VRI-003 PCC®, and *B. longum* subsp. *Longum*) (66, 75, 78, 90) as assessed by FISH analysis (75), and 16S gene sequencing (66, 78, 90). The only other species-level change reported was a reduction in *Fusiformis* following 4 weeks of supplementation with *L. salivarius* UCC118 (63), with no other significant changes in bacterial species reported (66, 75, 78, 84). At the phylum level, a decrease was observed in *Verrucomicrobia*, following 4 weeks of supplementation with *L. salivarius* UCC118 (63). Five weeks of supplementation with *Bifidobacterium longum* subsp. *Longum* showed a greater abundance of *Actinobacteria* and *Firmicutes*; with a reduction in *Proteobacteria,* albeit statistical significance or lack thereof was not reported (78). No other changes were observed at the phylum level following probiotic supplementation (74, 78, 94). Genus level changes observed following probiotic supplementation include a reduction in *Prosthecobacter* following 4 weeks of supplementation with *L. salivarius* UCC118 (63). A significant increase in *Bifidobacterium and Lactobacillus* was observed following 5 weeks of supplementation with *Bifidobacterium longum subsp. Longum* (78). However, 4 weeks of supplementation with a multi-strain probiotic (*L. paracasei, L. rhamnosus, L. helveticus, L. fermentum,* and *S. thermophilus)*, showed no change in *Lactobacillus* and a comparative decrease in *Bifidobacterium* (77). Four weeks of supplementation with *L. plantarum* showed a lower relative abundance in *Anaerotruncus, Caproiciproducens, Coprobacillus, Desulfovibrio, Dielma, Family_XIII_UCG_001, Holdemania,* and *Oxalobacter* compared with placebo. In addition, a greater relative abundance in *Akkermansia, Bifidobacterium, Butyricimonas,* and *Lactobacillus* was observed, however baseline data was not reported (74). Probiotic supplementation did not result in any further changes in other fecal sample bacterial taxa groups, including α-diversity (63, 80, 84, 90). The $n = 2$ synbiotic studies demonstrated increases in the genus of some of the supplemented strains, reported as raw bacterial count (62) or log CFU/g feces (92), but no differences in other determined bacterial groups (62, 92). No change in the α-diversity of the supplementation or placebo groups was detected in any of the synbiotic studies (96, 98).

Fecal short chain fatty acid concentration at rest

Fecal SCFA concentrations were measured before and after supplementation in $n = 9$ included studies (Tables 3, 4), which included $n = 5$ prebiotic (55–57, 59, 60), $n = 2$ probiotic (75, 84), and $n = 2$ synbiotic interventions (96, 98). An additional probiotic study only reported post-intervention values for SCFA (74). $N = 2$ studies that provided 3 weeks AXOS supplementation demonstrated significant increases in

total SCFA and butyric acid concentration (55, 57). Additionally, significant increases in acetic and propionic acid were observed following high (8.0 g/d), but not low dose (2.4 g/d) AXOS supplementation for 3 weeks in one study (57), but this finding was not replicated in another study that administered high dose (7.2 g/d) AXOS (55). No prebiotic intervention using XOS or inulin, resulted in increases in fecal SCFA (56, 59, 60). The figure presented in $n = 1$ multi-strain probiotic study appeared to show a drop in fecal butyric acid following 60 days of supplementation with *L. acidophilus*, *L. casei*, *L. helveticus*, and *B. bifidum*, compared with a rise in the placebo group (84). However, this study only reported a statistical difference at baseline, albeit with no indication of the *p*-value and did not report whether or not the changes over time were statistically significant. Another study reported greater concentrations in fecal acetic acid, propionic acid and butyric acid compared with the placebo group; however, whether these are genuine changes following supplementation cannot be determined as baseline data was not collected (74). No probiotic or synbiotic study observed and reported positive differences in SCFA concentrations at rest, as a result of supplementation.

Studies assessing markers of exercise-induced gastrointestinal syndrome after a period of pre-, pro-, and syn-biotic supplementation

$N = 13$ studies reported at least one of the review outcomes, prior to, during, and/or following an acute exercise bout (Table 5). All except $n = 2$ studies investigated probiotic supplementation, the remaining papers investigated a synbiotic supplement intervention. No study of prebiotics being provided prior to an acute exercise bout was found in the search strategy. Probiotic supplements varied from single to multi-strain (i.e., up to nine different species), given either as capsules, powder sachets, or dairy-based beverage. The synbiotic supplements included two to four probiotic strains, plus additional fructo-oligosaccharides or inulin (94, 96). The acute exercise bouts varied substantially, and included $n = 3$ studies of treadmill running (either 2 h steady state with or without environmental heat exposure (e.g., 35°C), or time to exhaustion test) (63, 68, 100); $n = 4$ studies investigated supplementation prior to an outdoor marathon (65, 79, 87, 89); $n = 3$ studies on a cycle ergometer (either an incremental exercise test, time to fatigue tests or 2 h steady state cycle ergometer followed by 1 h time trial) (63, 90, 100); and, in one study participants completed an ultra-distance triathlon event (94). One study observed participant outcomes in response to 5 days of continuous intense military training, day and night including marching 8–30 km whilst carrying a pack up to 30 kg, sleep deprivation, and a range of environmental conditions (96). Another used online questionnaires to assess included measures (82). Outcome measures were taken prior to the exercise bout, but the timing of post-exercise measures varied from immediately to 6 days post-exercise. In all studies participants mean or median age was ≤42 years, and in all studies participants were from an endurance sport background (i.e., mean or median VO_{2max} range 47–64 ml/kg/min) or military training background.

Intestinal epithelial injury in response to acute exercise

$N = 3$ studies (all probiotics) assessed the effect of the intervention on markers of intestinal injury, none of which observed differences in plasma or serum I-FABP concentration (79, 100), or urinary claudin-3 concentration (102) (Tables 5, 6).

Intestinal permeability in response to acute exercise

$N = 4$ probiotic (63, 79, 100, 102) and $n = 1$ synbiotic study (94) assessed the effects of supplementation on intestinal permeability in response to an acute exercise bout (Tables 5, 6). None of the studies observed differences between intervention and placebo for urinary lactulose:mannitol or lactulose:rhamnose ratio, indicative of small intestinal permeability. $N = 1$ study assessed sucrose permeability, indicative of gastroduodenal permeability, at baseline and immediately post-exercise, reporting a 38% significantly lower incremental area under the curve from baseline in the intervention trial, and a 169% increase from baseline in the placebo trial, after 4 weeks *Lactobacillus salivarius* UCC118 (2×10^8 CFU daily) (63). The effect of a synbiotic supplement (four probiotic strains plus fructooligosaccharides) was investigated on urinary lactulose:mannitol ratio, before and 6 days after a long course triathlon event, with no effect of trial observed (94).

Systemic bacterial endotoxin profile in response to acute exercise

$N = 2$ probiotic studies (68, 100) and $n = 1$ synbiotic study (94) investigated changes in circulating bacterial endotoxin concentration in response to acute exercise (Tables 5, 6). Biomarkers included overall endotoxin units, gram-negative endotoxin concentration, serum LPS, anti-endotoxin antibodies (i.e., IgM and IgG), and neutrophil elastase (*in vitro E. coli*

TABLE 5 Systematic review search results, included studies that investigated the impact of prebiotic, probiotic and synbiotic supplementation on markers of EIGS and associated GIS in response to an acute exercise bout.

References	Population and study design	Sample size determination	Supplementation protocol	Physical activity/dietary control	Exercise protocol	Outcome/s reported
Probiotic studies						
Axelrod et al. (63)	$n = 7$ endurance runners, VO_{2max} 57.9 mL/kg/min RXT	A priori power analysis based on a previous investigation from healthy runners (64) estimated ~6 needed to obtain statistical power at the recommended 0.80 level based upon mean, between-groups comparison effect size ($d = 1.2$).	*L. salivarius* UCC118, 2×10^8 CFU/cap 1 capsule daily for 4 wk	Normal lifestyle (not monitored)	Treadmill running: 2 h at 60% $VO_{2max.}$ T_{amb}: 25°C, RH: 31%	Intestinal permeability Cytokine responses
Batatinha et al. (65)	$n = 27$ male marathon runners, age: Int: 35.96 ± 5.81; Pla: 40.46 ± 7.79 Fitness status not stated RCT	Not specified	*B. animalis. Lactis* 10×10^9; *L. Acidophilus* 10×10^9 1 sachet daily for 30 days	Training volume monitored, NS between groups. Dietary control not stated.	Marathon race: Race time was 4.08 ± 0.55 h T_{abm} not stated	Cytokine responses
Gill et al. (68)	$n = 8$ male runners and triathletes Age: 26 yrs VO_{2max} 59 ml/kg/min RXT	Based on the typical standard deviation of 0.7 EU/ml for circulatory endotoxin responses to exertional-stress (16, 69), and using standard alpha (0.05) and beta values (0.8) (www.dssresearch.com), a sample size of $n = 8$ is estimated to provide adequate statistical precision to detect a >10% difference in circulatory endotoxin concentration in response to EHS in the target population.	*L. casei*, 1.0×10^{11} cells/bottle Commercial supplement, taken twice daily for 7 days	Dietary recall. Activity control not stated.	Treadmill running: 2 h at 60% $VO_{2max.}$ T_{amb}: 34°C, RH: 32%	Endotoxaemia Cytokine responses
Kekkonen et al. (99)	$n = 119$ (125M/16F recruited) marathon competitors, Best marathon time Int: 3:10 (2:35–3:42) Pla: 3:11 (2:23–3:40) Age 40 yrs. RCT	Not specified	Milk-based fruit drink or capsules (participant choice) containing *L. rhamnosus* GG, 4.0×10^{10} CFU 2 × 65 mL bottles or capsules daily for 3 months	Diary questionnaire with ready-made questions Training diaries, NS for running sessions or weekly distance.	Helsinki Marathon, 2003: Int: 3 h 32 min Pla: 3 h 30 min	GIS
Lamprecht et al. (76)	$n = 23$ male triathletes, runners and cyclists Age, Int: 37.6 yrs, Pla: 38.2 yrs VO_{2max}: Int: 51.2 mL/kg/min Pla: 50.3 mL/kg/min RCT	Sample size calculation based on oxidation markers CP and MDA. Between 7 and 9 subjects estimated per group—depending on parameter, SD and effect size—to reach probability of error (alpha/2) of 5 and 80% power.	Ecologic® Performance: *B. bifidum W23, B lactis W51, E. faecium W54, L. acidophilus W22, L. brevis W63, L. lactis W58.* 2 × 2 g sachets daily for 14 wk, providing (10^{10} CFU/day)	Habitual diet, food diary and repeated for 7 days prior to each exercise trial. Habitual training, no exercise 3 days prior to each exercise test.	3 × incremental "step" tests on cycling ergometer to exhaustion, with 15 min active recovery between each test. Total test time ~80–90 min. T_{amb}: 20°C, RH: 60% RH	Cytokine responses
Pugh et al. (79)	$n = 24$ (20M/4F) marathon runners, age: Int: 34.8 ± 6.9 yrs Pla: 36.1 ± 7.5 yrs VO_{2max}: Int: 57.6 ± 8.0 mL/kg/min Pla: 56.4 ± 8.6 mL/kg/min RCT	Not specified	Proven Probiotics Ltd., Port Talbot, Wales: *L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34 (). >25 billion CFU/cap 1 capsule daily for 4 wk	Dietary control not stated outside of acute exercise. Training not stated.	Non-sanctioned marathon (outdoor running track). Finish time: Int: 234 ± 38 min, Pla: 247 ± 47 NS % LT: Int: 90.2 ± 9.1, Pla: 91.3 ± 8.7 NS	Intestinal permeability Intestinal injury Cytokine responses GIS

(Continued)

10.3389/fnut.2022.1003620

TABLE 5 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol	Physical activity/dietary control	Exercise protocol	Outcome/s reported
Pugh et al. (100)	$n = 7$ male cyclists Age: 23 ± 4 yrs VO_{2peak} 64.0 ± 2.2 mL/kg/min RXT	To detect a meaningful increase in exogenous CHO oxidation of 0.1 g/min with SD = 0.05 g/min (101) at 80% power, a minimum $n = 5$ required.	Proven Probiotics Ltd., Port Talbot, Wales: *L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34 (). >25 billion CFU/cap 1 capsule daily for 4 wk	Not stated	Cycle ergometer: 2 h at 55% W_{max} followed by 100 kJ time trial. Ambient conditions not stated	Intestinal permeability Intestinal injury Cytokine responses GIS
Schreiber et al. (82)	$n = 27$ male cyclists Age: Int: 25.9 ± 4.6 Pla: 29.5 ± 6.2 $VO2_{max}$ (mL/kg/min) Int: 66.9 ± 6.4 Pla: 63.2 ± 5.0 RCT	Not specified	1 capsule containing: *L. helveticus* Lafti L10 (28.6%), *B. animalis* ssp. lactis Lafti B94 (28.6%), *E. faecium* R0026 (25.7%), *Bifidobacterium longum* R0175 (14.3%) *B. subtilis* R0179 (2.8%) 15×10^9 CFU/cap 1 capsule daily for 90 days	Diet not controlled. "Difference in training hours during the study period" (Data not shown)	Evaluation (online survey) at training, competition, and during the first 2 h recovery from training or competition.	GIS
Shing et al. (102)	$n = 10$ male runners Age: 27 ± 2 yrs VO_{2max}: 62.6 ± 2.1 mL/kg/min RXT	Sample size was determined to detect a treatment difference at a two-sided 5% significance level with a probability of 80% from primary outcome variables of lactulose/rhamnose and LPS. The lactulose:rhamnose ratio following exercise is reported to be 0.0625 (SD 0.0125) (103). Assuming that probiotics reduced the lactulose:rhamnose by 20% (similar to reduction following bovine colostrum supplementation) (104), a total of eight runners were required. Recent literature has shown a 20% increase in LPS concentration following running in the heat with an increase in training load (105). Assuming that probiotics reduced LPS concentration by 20% (post-exercise LPS concentration of 27 pg mL^{-1} with a within-subject standard deviation of 5 pg mL^{-1}), a total of nine runners were required. Based on these calculations, 10 runners were recruited for the present study.	UltraBiotic45, BioCeuticals™: *L. acidophilus*, 7.4×10^9 CFU/cap *L. rhamnosus*, 15.55×10^9 CFU/cap *L. casei*, 9.45×10^9 CFU/cap *L. plantarum*, 3.15×10^9 CFU/cap *L. fermentum*, 1.35×10^9 CFU/cap *B. lactis*, 4.05×10^9 CFU/cap *B. breve*, 1.35×10^9 CFU/cap *B. bifidum*, 0.45×10^9 CFU/cap *S. thermophilus*, 2.25×10^9 CFU/cap 1 capsule daily for 4 wk	Food diary ^{NS} between trials. Required to avoid strenuous exercise for 24 h prior to each testing session.	Treadmill running: time to fatigue at 80% ventilatory threshold. T_{amb}: 35°C, RH: 40%	Intestinal permeability Intestinal injury Endotoxaemia Cytokine responses GIS
Tavares-Silva et al. (87)	$N = 14$ male marathon runners Age: Pla: 38.28 ± 3.09 Int: 41.57 ± 3.20 VO_{2Peak}: Pla: 54.53 ± 6.88 kg/mL/min Int: 56.92 ± 8.35 kg/mL/min RCT	Not specified	*L. acidophilus-LB-G80, L. paracasei-LPc-G110, Lactococcus* subp. *lactis-LLL-G25,* *B. animalis* subp. *lactis-BL-G101, B. bifidum-BB-G90* Capsules containing 5×10^9 CFU / day	Physical activity control not stated. Questionnaire 2×/wk + once on weekends: Energy intake (kcal) Pla: 1,994.46 ± 365.73 Int: 2,434.69 ± 505.53 ^{NS} difference between groups	Marathon race Race Time (min) Pla: 243.0 ± 33.73 Int: 252.87 ± 39.77 ^{NS} Difference Tamb: 21.5°C, RH: 67%.	Cytokine responses

(Continued)

10.3389/fnut.2022.1003620

TABLE 5 (Continued)

References	Population and study design	Sample size determination	Supplementation protocol	Physical activity/dietary control	Exercise protocol	Outcome/s reported
Vaisberg et al. (89)	$n = 42$ male marathon runners Age: Int: 39.6 ± 8.8 yrs, Pla: 40.1 ± 10.3 yrs VO_{2max}: Int: 57.64 ± 6.89 mL/kg/min, Pla: 57.86 ± 6.85 mL/kg/min RCT	Not specified	*Lactobacillus casei Shirota*, 40 × 10^9 live cells/bottle 1 × 80 g bottle daily for 30 days	Dietary control not stated. Instructed to keep usual training/physical exercise schedules—not reported	Marathon race Ambient conditions not stated	Cytokine responses
West et al. (90)	$n = 88$ cyclists and triathletes Age: Int: M: 35.2 yrs, F: 36.5 yrs, Pla: M: 36.4 yrs, F 35.6 VO_{2max} (mL/kg/min): Int: M: 56.5, F: 57.6 Pla: M: 55.8, F: 51.6 RCT	A sample size of 80 subjects was required for identifying substantial changes in the incidence of illness, assuming a rate of URTI symptoms of 60% in the placebo group, with sufficient power (86% at an alpha-level of 0.05) to detect a 50% reduction in symptoms.	*Lactobacillus fermentum* VRI-003 PCC®, 10^9 CFU/cap 1 capsule daily for 11 wk	4 d food diary. Usual diet, without probiotic foods. Training log kept	Incremental cycling ergometer test (VO_{2max} protocol). Ambient conditions not stated	Cytokine responses
Synbiotic studies						
Roberts et al. (94)	$n = 20$ (9M/1F) long course triathletes VO_{2max}: Int: 47.6 mL/kg/min Pla: 50.5 mL/kg/min RCT	Power calculation assessment for sample size (G*power3, Dusseldorf (95); using $\alpha = 0.05$; $1 - \beta = 0.80$; based on observed data.	Bio-Acidophilus Forte, Biocare Ltd., Birmingham, UK): *L. acidophilus* CUL-60 (NCIMB 30157), 10^{10} CFU/cap *L. acidophillus* CUL-21 (NCIMB 30156), 10^{10} CFU/cap *B. bifidum* CUL-20 (NCIMB 30172), 9.5^{10} CFU/cap *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), 0.5^{10} CFU/cap. Fructooligosaccharides, 55.8 mg per cap 1 capsule daily for 12 wk	Habitual diet, food diary first and last wk of each month. NS between groups or over intervention time period. Prescribed triathlon training program, individualized. NS between groups for training load throughout intervention period.	Ironman triathlon. Mean finish time: Int: 12 h 47 min, Pla: 14 h 12 min NS	Endotoxin responses Intestinal permeability
Valle et al. (96)	$n = 65$ (39M/26F) military personnel Age: Pla: 19.5 ± 1.22, Int: 19.69 ± 1.25 Fitness status not stated RCT	Sample calculation in G * Power 3.1.9.2 software was based on the following data: 5% sample error, 95% CI and 0.72 effect size considering pre and post-intervention IgA values. The effect size was estimated based on the study by Olivares et al. (97).	*Lactobacillus acidophilus LA-5, 10·3 log CFU* *Bifidobacterium animalis BB-12, 11·0 log CFU* *Inulin, 2.3 g* *60g serve of ice cream, daily for 30 days*	Participants recommended not to consume any foods containing prebiotics and probiotics 15 d before the beginning of the research period, particularly over the weekend, when they are released to go home. During the week all food was provided. Not reported, however participants were undergoing military training.	5 d military training, including physical and psychological exhaustion, marching 8–20 km, carrying ∼ 30 kg equipment, sleep deprivation. Subject to various weather extremes.	Bacterial taxa Fecal SCFA GIS

RCT, Randomized control trial; RXT, Randomized crossover trial; Cap, capsule; T_{amb}, Ambient temperature; RH, Relative humidity; Int, Intervention; Pla, Placebo; NS, Not significant; wk, week.

10.3389/fnut.2022.1003620

TABLE 6 Systematic review study outcomes for included studies that investigated the impact of prebiotic, probiotic and synbiotic supplementation on markers of EIGS in response to an acute exercise bout.

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
Intestinal permeability					
Axelrod et al. (63)	$n = 7$ RXT	Probiotic vs. placebo	*L. salivarius* UCC118, 4 wk	Treadmill running: 2 h at 60% $VO_{2max.}$ Tamb: 25°C, RH: 31%	Sucrose permeability (Δ in iAUC from baseline): Int ↓ 38%, Pla ↑ 169% ($p = 0.029$) Rhamnose permeability (Δ in iAUC from baseline): Int ↓ 0.1-fold, Pla ↑ 0.5-fold ^{NS} Lactulose permeability (Δ in iAUC from baseline): Int ↓ 0.1-fold, Pla ↑ 0.4-fold ^{NS} L/R Ratio (Δ in iAUC from baseline): ^{NS} (data not reported)
Pugh et al. (79)	$n = 24$ RCT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Non-sanctioned marathon (outdoor running track). Finish time: Int: 234 ± 38 min, Pla: 247 ± 47 ^{NS} % LT: Int: 90.2 ± 9.1, Pla: 91.3 ± 8.7 ^{NS} T_{amb}: 16–17°C; Wind: 8–16 km/h	Serum L/R ratio (Δ from baseline test): Int ↑ 0.04, Pla ↑ 0.02 ^{NS}
Pugh et al. (100)	$n = 7$ RXT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Cycle ergometer: 2 h at 55% W_{max} followed by 100 kJ time trial. Ambient conditions not stated.	Serum L/R ratio (Δ from baseline test): Int ↑ 0.045, Pla ↑ 0.052 ^{NS}
Shing et al. (102)	$n = 10$ RXT	Probiotic vs. placebo	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, S. thermophilus*, 4 wk	Treadmill running: time to fatigue at 80% ventilatory threshold. Tamb: 35°C, RH: 40%	Urinary L/R ratio: 8% lower following Int cf Pla ^{NS}–other data not reported
Roberts et al. (94)	$n = 20$ RCT	Synbiotic vs. placebo	*L. acidophilus* CUL-60 (NCIMB 30157), *L. acidophillus* CUL-21 (NCIMB 30156), *B. bifidum* CUL-20 (NCIMB 30172), *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), Fructooligosaccharides, 12 wk	Ironman triathlon. Mean finish time: Int: 12 h 47 min, Pla: 14 h 12 min ^{NS}	Urinary lactulose/mannitol ratio (Δ from pre- to 6 d post-race): Int ↑ 0.005, Pla ↑ 0.020 ^{NS}
Intestinal injury					
Pugh et al. (79)	$n = 24$ RCT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Non-sanctioned marathon (outdoor running track). Finish time: Int: 234 ± 38 min, Pla: 247 ± 47 ^{NS} % LT: Int: 90.2 ± 9.1, Pla: 91.3 ± 8.7 ^{NS} T_{amb}: 16–17°C; Wind: 8–16 km/h	Serum I-FABP: Int ↑ 1,359 pg/mL, Pla ↑ 932 pg/mL ^{NS}
Pugh et al. (100)	$n = 7$ RXT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Cycle ergometer: 2 h at 55% W_{max} followed by 100 kJ time trial. Ambient conditions not stated.	Plasma I-FABP: Post-exercise: Int ↓ 207 pg/mL, Pla ↓ 295 pg/mL ^{NS} 1 h post-exercise: Int ↓ 182 pg/mL, Pla ↓ 263 pg/mL ^{NS}
Shing et al. (102)	$n = 10$ RXT	Probiotic vs. placebo	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, S. thermophilus*, 4 wk	Treadmill running: time to fatigue at 80% ventilatory threshold. Tamb: 35°C, RH: 40%	Urinary Claudin 3 (absolute values): Int 6.1 ± 3.3 ng/mmol creatinine, Pla 8.1 ± 5.1 ng/mmol creatinine ^{NS}
Bacterial endotoxin responses					
Gill et al. (68)	$n = 8$ RXT	Probiotic vs. placebo	*L. casei*, 7 days	Treadmill running: 2 h at 60% $VO_{2max.}$ Tamb: 34°C, RH: 32%	Gram-negative bacterial endotoxin (ΔPre-ex - 1-h Post-ex): Int ↑ 0.5 EU/mL (23%), Pla ↓ 0.2 EU/mL (8%) ($p = 0.05$) Serum LPS: Int ↑ 0.03 EU, Pla ↑ 0.05 EU ^{NS} Anti-LPS IgM: Int ↓ 1.0 MU/mL, Pla ↑ 0.3 MU/mL ^{NS}
Shing et al. (102)	$n = 10$ RXT	Probiotic vs. placebo	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, S. thermophilus*, 4 wk	Treadmill running: time to fatigue at 80% ventilatory threshold. Tamb: 35°C, RH: 40%	

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
Roberts et al. (94)	$n = 20$ RCT	Synbiotic vs. placebo	*L. acidophilus* CUL-60 (NCIMB 30157), *L. acidophillus* CUL-21 (NCIMB 30156), *B. bifidum* CUL-20 (NCIMB 30172), *B. animalis* subspecies *lactis* CUL-34 (NCIMB 30153), Fructooligosaccharides, 12 wk	Ironman triathlon. Mean finish time: Int: 12 h 47 min, Pla: 14 h 12 min [NS]	Endotoxin units (Δ from pre- to 6 d post-exercise): Int ↓ 1.6 pg/mL ($p = 0.047$), Pla ↓ 0.44 pg/mL [NS] Anti-LPS IgG (Δ from pre- to 6 d post-exercise): Int ↓ 90 MU/mL, Pla ↑ 27 MU/mL [NS]
Cytokine responses					
Axelrod et al. (63)	$n = 7$ RXT	Probiotic vs. placebo	*L. salivarius* UCC118, 4 wk	Treadmill running: 2 h at 60% $VO_{2max.}$ Tamb: 25°C, RH: 31%	IL-6: (ΔΔ pre to post-exercise, pre to post-intervention) Int ↑ 0.5 pg/mL, Pla ↑ 1.4 pg/mL [NS]
Batatinha et al. (65)	$n = 27$ RCT	Probiotic vs. placebo	*B. animalis. Lactis* 10×10^9; *L. Acidophilus* 10×10^9 1 sachet daily for 30 days	Marathon race: Race time was 4.08 ± 0.55 h T_{abm} not stated	IL-10: Int: ↑ 254 ng/ml, Pla: ↑ 219 ng/ml [NS] IL-4: Int: ↑ 6.9 ng/ml, Pla:↑ 2.2 ng/ml [NS] IL-6: Int: ↑ 14.0 ng/ml, Pla: ↑ 14.1 ng/ml [NS] IL-2: Int: ↓ 2.6 ng/ml, Pla: ↓ 0.3ng/ml [NS] IL-15: Int: ↓ 0.7 ng/ml, Pla: ↓ 0.5 ng/ml [NS] IL-8: Int: ↑ 7.1 ng/ml, Pla: ↑ 10.4 ng/ml [NS] IL-1β: Int: ↓ 1.1 ng/ml, Pla: ↑ 0.1 ng/ml [NS] TNF-α: Int: ↑ 3.9 ng/ml, Pla: ↑ 3.8 ng/ml [NS] IFN-γ: Int: ↓ 5.3 ng/ml, Pla: ↓ 2.8 ng/ml [NS]
Gill et al. (68)	$n = 8$ RXT	Probiotic vs. placebo	*L. casei*, 7 days	Treadmill running: 2 h at 60% $VO_{2max.}$ Tamb: 34°C, RH: 32%	IL-6: Post-exercise: Int ↑ 3.6 pg/mL, Pla ↑ 3.1 pg/mL [NS] 1 h post-exercise: Int ↑ 2.1 pg/mL, Pla ↑ 1.2 pg/mL [NS] 2 h post-exercise: Int ↑ 1.1 pg/mL, Pla ↑ 0.4 pg/mL [NS] 4 h post-exercise: Int ↑ 0.4 pg/mL, Pla ↔ 0.0 pg/mL [NS] 24 h post-exercise: Int ↔ 0.0 pg/mL, Pla ↓ 0.3 pg/mL [NS] IL-1β: Post-exercise: Int ↑ 0.09 pg/mL, Pla ↑ 0.02 pg/mL [NS] 1 h post-exercise: Int ↑ 0.03 pg/mL, Pla ↓ 0.03 pg/mL [NS] 2 h post-exercise: Int ↓ 0.01 pg/mL, Pla ↓ 0.01 pg/mL [NS] 4 h post-exercise: Int ↓ 0.01 pg/mL, Pla ↓ 0.01 pg/mL [NS] 24 h post-exercise: Int ↑ 0.02 pg/mL, Pla ↔ 0.0 pg/mL [NS] TNF-a: Post-exercise: Int ↑ 0.5 pg/mL, Pla ↑ 0.3 pg/mL [NS] 1 h post-exercise: Int ↑ 0.3 pg/mL, Pla ↓ 0.3 pg/mL [NS] 2 h post-exercise: Int ↔ 0.0 pg/mL, Pla ↓ 0.4 pg/mL [NS] 4 h post-exercise: Int ↑ 0.1 pg/mL, Pla ↓ 0.3 pg/mL [NS] 24 h post-exercise: Int ↑ 0.3 pg/mL, Pla ↑ 0.1 pg/mL [NS]

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
					IFN-γ: Post-exercise: Int ↔ 0.0 pg/mL, Pla ↑ 0.1 pg/mL ^{NS} 1 h post-exercise: Int ↓ 0.1 pg/mL, Pla ↓ 0.2 pg/mL ^{NS} 2 h post-exercise: Int ↓ 0.3 pg/mL, Pla ↓ 0.4 pg/mL ^{NS} 4 h post-exercise: Int ↑ 0.3 pg/mL, Pla ↓ 0.4 pg/mL ^{NS} 24 h post-exercise: Int ↑ 0.2 pg/mL, Pla ↔ 0.0 pg/mL ^{NS} IL-10: Post-exercise: Int ↑ 10.8 pg/mL, Pla ↑ 7.2 pg/mL ^{NS} 1 h post-exercise: Int ↑ 10.6 pg/mL, Pla ↑ 9.0 pg/mL ^{NS} 2 h post-exercise: Int ↑ 2.8 pg/mL, Pla ↑ 2.7 pg/mL ^{NS} 4 h post-exercise: Int ↓ 0.1 pg/mL, Pla ↔ 0.0 pg/mL ^{NS} 24 h post-exercise: Int ↔ 0.0 pg/mL, Pla ↑ 0.2 pg/mL ^{NS} IL-8: Post-exercise: Int ↑ 1.8 pg/mL, Pla ↑ 1.2 pg/mL ^{NS} 1 h post-exercise: Int ↑ 1.1 pg/mL, Pla ↑ 0.6 pg/mL ^{NS} 2 h post-exercise: Int ↑ 0.1 pg/mL, Pla ↑ 0.1 pg/mL ^{NS} 4 h post-exercise: Int ↔ 0.0 pg/mL, Pla ↓ 0.1 pg/mL ^{NS} 24 h post-exercise: Int ↓ 0.1 pg/mL, Pla ↓ 0.4 pg/mL ^{NS}
Lamprecht et al.	$n = 23$ RCT	Probiotic vs. placebo	*B. bifidum W23, B lactis W51, E. faecium W54, L. acidophilus W22, L. brevis W63, L. lactis W58*, 14 wk	3 x incremental "step" tests on cycling ergometer to exhaustion, with 15 min active recovery between each test. Total test time ~80–90 min. T_{amb}: 20°C, RH: 60% RH	IL-6: Int ↑ 3.3 pg/mL, Pla ↑ 1.9 pg/mL ^{NS} TNF-α: Int ↑ 2.3 pg/mL, Pla ↑ 0.24 pg/mL ^{NS}
Pugh et al. (79)	$n = 24$ RCT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Non-sanctioned marathon (outdoor running track). Finish time: Int: 234 ± 38 min, Pla: 247 ± 47 ^{NS} % LT: Int: 90.2 ± 9.1, Pla: 91.3 ± 8.7 ^{NS} T_{amb}: 16–17°C; Wind: 8–16 km/h	Serum CD14: Int ↑ 5.9 μg/mL, Pla ↑ 5.4 μg/mL ^{NS} IL-6: Int ↑ 9.95 μg/mL, Pla ↑ 12.76 μg/mL ^{NS} IL-8: Int ↑ 11.21 μg/mL, Pla ↑ 9.98 μg/mL ^{NS} IL-10: Int ↑ 4.36 μg/mL, Pla ↑ 5.05 μg/mL ^{NS}
Pugh et al. (100)	$n = 7$ RXT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Cycle ergometer: 2 h at 55% W_{max} followed by 100 kJ time trial. Ambient conditions not stated.	IL-1α: Int ↑ 1.15 μg/mL, Pla ↑ 0.45 μg/mL ^{NS} IL-6: Int ↑ 1.05 μg/mL, Pla ↑ 1.37 μg/mL ^{NS} IL-8: Int ↑ 1.96 μg/mL, Pla ↑ 2.21 μg/mL ^{NS} IL-10: Int ↑ 2.11 μg/mL, Pla ↑ 1.18 μg/mL ^{NS}
Shing et al. (102)	$n = 10$ RXT	Probiotic vs. placebo	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, S. thermophilus*, 4 wk	Treadmill running: time to fatigue at 80% ventilatory threshold. Tamb: 35°C, RH: 40%	IL-1ra: Post-exercise: Int ↑ 74 pg/mL, Pla ↑ 79 pg/mL ^{NS} 1 h post-exercise: Int ↑ 183 pg/mL, Pla ↑ 188 pg/mL ^{NS} TNF-α: Post-exercise: Int ↑ 0.62 pg/mL, Pla ↑ 1.65 pg/mL ^{NS} 1 h post-exercise: Int ↓ 0.33 pg/mL, Pla ↑ 1.47 pg/mL ^{NS}

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
Tavares-Silva et al. (87)	$n = 14$ RCT	Probiotic vs. placebo	*Lactobacillus acidophilus-LB-G80, Lactobacillus paracasei-LPc-G110, Lactococcus subp. lactis-LLL-G25, Bifidobacterium animalis subp. lactis-BL-G101, Bifidobacterium bifidum-BB-G90 5× 10^9 CFU 2.0 g/day for 30 days*	Marathon race Race Time (min) Pla: 243.0 ± 33.73 Int: 252.87 ± 39.77 ^{NS} Difference Tamb: 21.5°C, RH: 67%.	IL-6: Post-exercise: Int ↑ 0.91 pg/mL, Pla ↑ 1.32 pg/mL ^{NS} 1 h post-exercise: Int ↑ 1.04 pg/mL, Pla ↑ 1.45 pg/mL ^{NS} IL-10: Post-exercise: Int ↑ 1.96 pg/mL, Pla ↓ 0.22 pg/mL ^{NS} 1 h post-exercise: Int ↑ 7.61 pg/mL, Pla ↑ 9.89 pg/mL ^{NS} Neutrophil elastase: Post-exercise: Int ↓ 269 fg/cell, Pla ↑ 74 fg/cell ^{NS} 1 h post-exercise: Int ↓ 259 fg/cell, Pla ↓ 12 fg/cell ^{NS} All changes relative to 24 h pre resting value IL-2 Post-ex: Pla: ↑ 0.01 pg/ml ^{NS}, Int: ↑0.06 pg/ml ^{NS} 1 h post-ex.: Pla: ↓ 0.05 pg/ml ^{NS}, Int: ↑ 0.11 pg/ml ^{NS} IL-4 Post-ex: Pla: ↑0.59 pg/ml ^{NS}, Int: ↑0.32 pg/ml ^{NS} 1 h post-ex.: Pla: ↓ 0.05 pg/ml ^{NS}, Int: ↑0.21 pg/ml ^{NS} IL-10 Post-ex : Pla: ↑ 1.05 pg/ml ($p < 0.05$), Int: ↑1.31 pg/ml^{NS} 1 h post-ex.: Pla: ↑ 0.7 pg/ml ^{NS}, Int: ↑ 1.3 pg/ml ($p < 0.05$) TNF-α Post-ex: Pla: ↑0.99 pg/ml ($p < 0.05$), Int: ↑1.2 pg/ml ($p < 0.05$) 1 h post-ex: Pla: ↑ 1.1 pg/ml ($p < 0.05$), Int: ↑ 0.2 pg/ml ^{NS}
Vaisberg et al. (89)	$n = 42$ RCT	Probiotic vs. placebo	*Lactobacillus casei Shirota*, 30 days	Marathon race Ambient conditions not stated	IL-1β: Int ↑ ↓1.8 pg/mL^{NS}, Pla ↑13.5 pg/mL ^{NS} IL-1ra: Int ↑18.7 pg/mL^{NS}, Pla ↑ 24.7 pg/mL $p < 0.01$ IL-4: Int ↑1.4 pg/mL^{NS}, Pla ↑ 6.6 pg/mL ^{NS} IL-5: Int ↑3.1 pg/mL^{NS}, Pla ↓ 34.5 ↑ 31.8 pg/mL ^{NS} IL-6: Int ↑ 34.8 pg/mL$p < 0.01$, Pla ↑ 36.5 pg/mL $p < 0.001$ IL-10: Int ↑ 15.0 pg/mL $p < 0.01$, Pla ↑ 19.0 pg/mL $p < 0.01$ IL-12p70: Int ↑ 19.2 pg/mL $p < 0.05$, Pla ↑ 23.0 pg/mL $p < 0.01$ IL-13: Int ↑ 2.1 pg/mL^{NS}, Pla ↑ 1.2 pg/mL ^{NS} TNF-α: Int ↑ 20.0 pg/mL^{NS}, Pla ↑ 123.4 pg/mL$p < 0.05$ Pla > Int, $p < 0.01$ between groups
West et al. (90)	$n = 88$ RCT	Probiotic vs. placebo	*Lactobacillus fermentum* VRI-003 PCC®, 11 wk	Incremental cycling ergometer test (VO_{2max} protocol). Ambient conditions not stated	*Factor changes in acute post-exercise cytokine responses:* IL-1ra: Male: Int 0.84, Pla 1.39 (very likely ↓) Female: Int 0.80, Pla 1.88 (very likely ↓)

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
					IL-10: Male: Int 0.95, Pla 1.16 (possible ↓) Female: Int 0.89, Pla 1.45 (possible ↓) IL-6: Male: Int 0.92, Pla 1.22 (likely ↓) Female: Int 0.71, Pla 2.29 (likely ↓) IL-8: Male: Int 0.80, Pla 0.87 (unclear) Female: Int 0.71, Pla 1.15 (probably ↓) GM-CSF: Male: Int 0.78, Pla 1.75 (very likely ↓) Female: Int 0.85, Pla 3.3 (very likely ↓) IFN-γ: Male: Int 1.2, Pla 1.49 (likely ↓) Female: Int 1.07, Pla 1.56 (likely ↓) TNF-α: Male: Int 1.27, Pla 1.66 (likely ↓) Female: Int 1.15, Pla 1.72 (likely ↓)
Short chain fatty acids					
Valle et al. (96)	$n = 65$ RCT	Synbiotic vs. Placebo	*Lactobacillus acidophilus LA-5, Bifidobacterium animalis BB-12, Inulin 2.3 g* 60 g serve of ice cream, daily for 30 days	5 d military training, including physical and psychological exhaustion, marching 8–20 km, carrying ∼ 30 kg equipment, sleep deprivation. Subject to various weather extremes.	Fecal acetate (mmol/L): Pla: Pre: 3·07 ± 1·64, Post: OR 0·16, 95%CI −0·25, 0·57 Post-training: OR −0·71, 95%CI−1·08, −0·34 Int: Pre: 2·82 ± 1·78 Post: OR 0·34, 95%CI −0·06, 0·74 Post-training: OR −0·80, 95%CI −1·14, −0·46 Main effect of time, $P < 0·001$ ^{NS} between groups Fecal proprionate (mmol/L): Pla: Pre: 0·97 ± 0·61, Post: OR 0·31, 95%CI −0·02, 0·63 Post-training: OR−0·08, 95%CI −0·24, 0·08 Int: Pre: 0·83 ± 0·50 Post: OR 0·20, 95%CI −0·01, 0·41 Post-training: OR −0·12, 95%CI −0·26, 0·02 Main effect of time, $P = 0·004$ ^{NS} between groups Fecal butyrate (mmol/L): Pla: Pre: 1·18 ± 0·85 Post: OR 0·25, 95%CI −0·03, 0·47 Post-training: OR −0·09, 95%CI −0·28, 0·10 Int: Pre: 1·04 ± 0·73 Post: OR 0·39, 95%CI 0·20, 0·59 Post-training: OR −0·17, 95%CI −0·33 −0·01 Main effect of time, $P = 0·002$ ^{NS} between groups

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
Bacterial taxa					
Valle et al. (96)	*n* = 65 RCT	Synbiotic vs. Placebo	*Lactobacillus acidophilus LA-5, Bifidobacterium animalis BB-12,* Inulin 2.3 g 60 g serve of ice cream, daily for 30 days	5 d military training, including physical and psychological exhaustion, marching 8–20 km, carrying ∼ 30 kg equipment, sleep deprivation. Subject to various weather extremes.	16S gene sequencing *α - Diversity (Shannon index):* *Int:*↑ 0.119 *Pla:* ↓ 0.095 ^{NS} *α - Diversity (Simpson index):* *Int:* ↑ 0.015, *Pla:* ↓ 0.021 ^{NS} ^{NS} group by time effect ^{NS} α-diversity between groups or periods
Gastrointestinal symptoms					
Kekkonen et al. (99)	*n* = 119 RCT	Probiotic vs. placebo	*L. rhamnosus* GG, 3 months	Helsinki Marathon Finish time: Int: 3 h 32 min (range 2 h 24 min to 4 h 35 min) Pla: 3 h 30 min (range 2 h 52 min to 4 h 19 min) ^{NS} between groups Ambient conditions not specified.	During training period: Subjects with GIS episodes: Int 27%, Pla 30% ^{NS} No. of GIS episodes/subject: Int 0.4, Pla 0.6 ^{NS} GIS episode duration: Int 2.9 days, Pla 4.2 days ^{NS} During 2 wk after marathon: Subjects with GIS episodes: Int: 6%, Pla: 6% ^{NS} No. of GIS episodes/subject: Int 0.1, Pla 0.1 ^{NS} GIS episode duration: Int 1.0 days, Pla 2.3 days ($p < 0.05$)
Pugh et al. (79)	*n* = 24 RCT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Non-sanctioned marathon (outdoor running track). Finish time: Int: 234 ± 38 min, Pla: 247 ± 47 ^{NS} % LT: Int: 90.2 ± 9.1, Pla: 91.3 ± 8.7 ^{NS} T_{amb}: 16–17°C; Wind: 8–16 km/h	Global GIS score during marathon (median): 1st third: Int 1.3, Pla 1.6 ^{NS} 2nd third: Int 3.0, Pla 3.2 ^{NS} 3rd third: Int 3.5, Pla 6.1 ($p = 0.01$) GIS Score Post-Race (median): Total GIS: Int: 13, Pla 15 ^{NS} Lower GIS: Int 10, Pla 7 ^{NS} Upper GIS: Int 6, Pla 5 ^{NS} GIS Score 24 h Post-Race (median): Total GIS: Int 16, Pla 12 ^{NS} Lower GIS: Int 7, Pla 5 ^{NS} Upper GIS: Int 6, Pla 4 ^{NS} ^{NS} between. trials, data not reported
Pugh et al. (100)	*n* = 7 RXT	Probiotic vs. placebo	*L. acidophilus* CUL60 *L. acidophilus* CUL21 *B. bifidum* CUL20 *B. animalis* subsp. *Lactis* CUL34, 4 wk	Cycle ergometer: 2 h at 55% W_{max} followed by 100 kJ time trial. Ambient conditions not stated.	
Schreiber et al. (82)	*n* = 27 RCT	Probiotic vs. placebo	l. helveticus Lafti L10, b. animalis ssp. lactis Lafti B94, e. faecium R0026, b. longum R0175, Bacillus subtilis R0179, 90 days	Evaluation (online survey) at training, competition, and during the first 2 h recovery from training or competition.	GIS incidence by slider questionnaire: GIS incidence during training (ΔGI): Int: −27 ± 47%, Pla: 8 ± 29%, Int < Pla, $P = 0.04$, $d = 0.9$ GIS incidence during competition (ΔGI): Int: 0 ± 47%, Pla: 9 ± 30%, ^{NS} GIS incidence after training (ΔGI): Int: −10 ± 32%, Pla: 9 ± 54%, ^{NS} GIS incidence after competition (ΔGI): Int: −20 ± 42%, Pla: 9 ± 54%, ^{NS}
Shing et al. (102)	*n* = 10 RXT	Probiotic vs. placebo	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, S. thermophilus*, 4 wk	Treadmill running: time to fatigue at 80% ventilatory threshold. Tamb: 35°C, RH: 40%	GIS Symptom Severity Score: Int: 1.4, Pla 1.6 ^{NS}

(Continued)

10.3389/fnut.2022.1003620

TABLE 6 (Continued)

References	*n* and study design	Supplement/ comparator	Intervention ingredient/s and supplement duration	Exercise protocol	Outcome measure/s (Δ in mean/median from pre-exercise to immediately post-exercise unless otherwise indicated)
Valle et al. (96)	$n = 65$ RCT	Synbiotic vs. Placebo	*Lactobacillus acidophilus LA-5, Bifidobacterium animalis BB-12*, Inulin, 2.3 g, 30 days	5 d military training, including physical and psychological exhaustion, marching 8–20 km, carrying ~ 30 kg equipment, sleep deprivation. Subject to various weather extremes.	GIS Sum of different symptoms: Int: pre: 8.06 ± 5.65 Δ Post-supp.: OR −2.24, 95%CI −3.15, −1.34 Δ Post-military training: OR −4.31, 95%CI −5.31, −3.30 $P < 0.05$, Δ post-supplementation v. Δ post-military training. Pla: pre: 8.48 ± 5.09 Δ Post-supp.: OR −1.16, 95%CI −2.51, 0.18 Δ Post-military training: OR −3.91, 95%CI −5.01, −2.82 $P < 0.05$, Δ post-supplementation v. Δ post-military training ^{NS} Group × time effect "Both groups showed a decreased number of gastrointestinal symptoms at post-military training ($P < 0.001$; main effect of time) with no differences between them ($P = 0.37$; group × time effect)"

Int, Intervention; Pla, Placebo; RCT, Randomized control trial; RXT, Randomized crossover trial; I-FABP, Intestinal fatty acid binding protein; NS, No significant difference between groups/trials; LPS, Lipopolysaccharide; wk, week.

LPS stimulation). No supplement intervention reduced markers of endotoxemia compared with the study's respective placebo, whilst one intervention (7 days *Lactobacillus casei*) reported an increased gram-negative bacterial endotoxin concentration in response to 2 h steady-state treadmill running (60% VO_{2max}) in hot ambient conditions (34.0°C, 32% RH), compared to a modest reduction in the placebo group (68). $N = 1$ study reported a significant reduction in endotoxin units compared to pre-supplementation (94); however this data was compared to the pre-exercise and not the pre-supplementation time point (i.e., a sample taken the day before an ultra-distance triathlon event), then assessed 6 days post-race.

Systemic inflammatory cytokine profile in response to acute exercise

$N = 10$ studies assessed systemic inflammatory cytokine responses to acute exercise, all of which utilized probiotic supplementation interventions (63, 65, 68, 76, 79, 87, 89, 90, 100, 102) (Tables 5, 6). Only $n = 3$ studies observed differences in the cytokine response to exercise between probiotic and placebo trials (87, 89, 90). One study reported statistical analysis using magnitude-based inferences, suggesting probiotic supplementation (11 weeks *Lactobacillus fermentum* VRI-003 PCC®, 10^9 CFU/day) resulted in possible and very likely reductions in IL-10 and IL-1ra, respectively, a likely reduction in IL-6, and likely or very likely reductions in GM-CSF, IFN-γ, and TNF-α, respectively (90). In contrast with this, a significant increase in IL-10 was observed only in the probiotic group at 1 h post-exercise, and TNF-α increased significantly only in the placebo group at 1 h post-exercise compared with 24 h pre-exercise levels, following 30 days of supplementing with *Lactobacillus acidophilus-LB-G80, Lactobacillus paracasei-LPc-G110, Lactococcus subp. lactis-LLL-G25, Bifidobacterium animalis subp. lactis-BL-G101* and *Bifidobacterium bifidum-BB-G90* (87). Following 30 days of supplementation with *L. casei Shirota*, a significant rise in IL-1ra was observed on the intervention group only (within group difference) but the only significant between group difference was observed in TNF-α, whereby a significant increase in TNF-α only occurred in the placebo group and not the intervention group (89).

Gastrointestinal functional markers in response to acute exercise

None of the included studies assessed the effect of pre-, pro-, syn-biotic supplementation on aspects of gastrointestinal function (e.g., gastric emptying, gastrointestinal motility,

intestinal transit, intestinal nutrient absorption, and/or malabsorption) in response to acute exercise.

Gastrointestinal symptoms in response to acute exercise

$N = 5$ studies, of which $n = 4$ were probiotic and $n = 1$ synbiotic supplementation interventions, included an assessment of GIS during the acute exercise bout (79, 82, 96, 100, 102). Of the probiotic interventions, $n = 2$ reported no effect of supplementation on GIS during exercise (100, 102). $N = 1$ study reported no difference in median global GIS score (GIS severity) during the first two-thirds of a simulated marathon. However, the authors emphasized a greater score in the placebo group during the final third of the simulated marathon, although this discrepancy appears likely due to lack of experimental control to confounding factors (refer to risk of bias assessment) (79). Additional data presented shows slightly higher overall GIS incidence in the probiotic group (90%) compared with the placebo group (88%) in response to the simulated marathon, and median GIS score immediately post-race was not different between groups. $N = 1$ study using online surveys to report GIS incidence by participants during training, reported a greater improvement in symptoms in the probiotic group, although GIS incidence after training, and during and after competition, showed no changes following probiotic supplementation (82). Similarly, 3 months of supplementation with a probiotic showed no significant effects on GIS incidence or duration in training, however during 2 weeks after the marathon, the category of GIS episode duration was more than double the number of days in the placebo group than the probiotic group (99). The $n = 1$ synbiotic study reporting GIS during an exercise bout (5 days continuous military training exercise) following supplementation showed no difference between groups, but an effect of time was observed, whereby symptoms reduced in both groups following the military training exercise bout (96).

Fecal bacterial taxa changes and short chain fatty acid concentration in response to acute exercise

Only $n = 1$ included studies assessed changes on bacterial taxa and fecal SCFA concentration in response to acute exercise (96) (Tables 5, 6). Following 30 days of supplementation with a synbiotic containing *L. acidophilus LA-5, B. animalis BB-12*, and 2.3 g of inulin, military recruits participated in 5 days of continuous combat simulation. No difference in fecal acetate, propionate or butyrate was observed. Some changes in bacterial taxa were noted in text, however due to the method of presentation in the manuscript (i.e., heat map), logical conclusions could not be drawn. Measures of bacterial diversity (Shannon and Simpson Index) before and after the exercise bout showed no difference in α-diversity following synbiotic supplementation compared with placebo, and no group by time effect was observed.

Risk of bias assessment

Results of the risk of bias assessment appear in Table 7. $N = 18$ out of the $n = 39$ included studies were judged as high risk of bias in at least one criterion. This included $n = 2$ due to a sequenced allocation as part of a counterbalanced randomization (59, 88), $n = 10$ due to inadequate reporting of outcome assessor blinding (72–74, 77, 78, 80, 82, 84, 87, 93), $n = 5$ due to incomplete outcome reporting (74, 78, 87, 100, 102), and $n = 4$ due to selective data reporting (78, 79, 84, 98). Other potential sources of bias were identified in $n = 5$ studies (79, 84, 88, 94, 102). $N = 1$ study reported increased GIS in the placebo group during the final third of a non-sanctioned marathon, however closer inspection of the data suggested that relative incidence (i.e., 91 vs. 89% of total group, respectively) and severity (i.e., 63 vs. 44% of total group above the mean of the assigned global GIS score, respectively) of GIS on the 4-weeks probiotic supplementation group was greater than the placebo group throughout the simulated marathon. Furthermore, the severity of GIS findings may have also been confounded due to large differences in total fluid volume intake between groups (e.g., varied completion times and total intake volumes (i.e., carbohydrate gel and water) that were not systematically assessed, discrepancies in reported plasma volume changes between groups, the absence of validated hydration status or change markers and body mass data), rather than any effect of the intervention itself (79), as highlighted in Costa et al. (19), Costa et al. (46), and Hoffman et al. (106). The remaining studies failed to provide evidence of correction of blood-based biomarkers for changes in plasma volume (107) as would be expected to occur in the exercise and/or heat stress models used (63, 65, 87, 89, 90, 94, 102). More than half of the included studies were either directly funded by, had intervention supplementation and/or placebo substances supplied by, or were authored by employees or paid consultants of, the manufacturer of the pre-, pro-, or syn-biotic product studied (56, 57, 62, 63, 70, 76, 77, 79, 82, 85, 88–90, 92–94, 99, 100, 102).

Discussion

The aim of this systematic literature review was to determine the beneficial, detrimental, or neutral effects of differing supplementation periods and dosages of pre-, pro- and syn-biotic supplementation, taken by healthy active adults, on gastrointestinal outcomes at rest and in response to exercise, with a specific focus on markers characteristic of EIGS and associated GIS. At rest, positive outcomes have been

10.3389/fnut.2022.1003620

TABLE 7 Risk of bias assessment.

References	Sequence generation	Allocation concealment	Participant/ personnel	Outcome assessment blinding	Incomplete outcome data	Selective reporting	Other potential sources of bias
Axelrod et al. (63)	+	+	?	?	?	?	?
Batatinha et al. (65)	?	−	+	?	+	?	?
Burton et al. (66)	+	+	?	?	+	+	+
Carbuhn et al. (67)	?	?	?	?	+	+	+
Coman et al. (92)	?	?	+	+	+	+	?
Damen et al. (55)	+	+	?	?	+	+	+
Finegold et al. (56)	?	+	+	?	+	+	?
François et al. (57)	+	+	+	+	+	+	?
Gill et al. (68)	+	+	+	?	+	+	?
Gleeson et al. (70)	?	?	?	?	+	+	?
Haywood et al. (72)	+	+	−	−	+	+	+
Huang et al. (74)	−	−	−	−	−	?	+
Hoffman et al. (73)	?	?	+	−	+	+	+
Kekkonen et al. (99)	?	+	?	?	+	+	?
Kleessen et al. (59)	?	?	?	?	+	+	+
Klein et al. (75)	+	+	?	?	+	+	+
Lamprecht et al. (76)	+	+	+	+	+	+	?
Lee et al. (77)	−	−	−	−	+	+	?
Lin et al. (78)	−	?	−	−	−	−	+
Pugh et al. (79)	+	+	+	+	?	−	−
Pugh et al. (100)	+	+	+	+	−	+	?
Quero et al. (93)	−	−	−	−	+	+	?
Reimer et al. (60)	+	?	+	+	+	+	+
Roberts et al. (94)	+	?	?	?	?	+	−
Russo et al. (61)	+	+	?	?	+	+	+
Russo et al. (45)	+	+	?	?	?	+	+
Russo et al. (62)	+	+	?	?	?	+	+
Sánchez Macarro et al. (80)	+	−	−	−	+	+	+
Schreiber et al. (82)	−	−	+	−	+	+	?
Shing et al. (102)	+	+	+	+	−	+	−
Smarkusz-Zarzecka et al. (83)	−	?	−	+	+	+	?
Son et al. (84)	−	−	?	−	?	−	−
Strasser et al. (85)	+	+	+	+	+	+	?
Tavares-Silva et al. (87)	−	−	?	−	−	+	+
Townsend et al. (88)	−	−	+	+	?	+	−
Vaisberg et al. (89)	?	?	+	+	+	+	?
Valle et al. (96)	+	+	+	+	?	+	+
West et al. (90)	+	+	+	+	?	+	?
West et al. (98)	?	+	?	?	+	−	?

+ Low risk of bias, − High risk of bias, ? Unclear risk of bias.

reported on measures of reduced intestinal permeability in 2/6 studies ($n = 1$ pre- and $n = 1$ pro-biotic interventions), improvements in functional measures in 2/4 prebiotic studies, improvements in gastrointestinal symptoms in 2/7 probiotic studies, and improvements in resting systemic cytokines in 3/15 studies. No changes were detected in all other studies assessing

these measures at rest. In response to exercise, where the gastrointestinal tract is acutely perturbed (2, 3, 108), the effects were even more modest with 0/3 studies showing a reduction in intestinal injury following probiotic supplementation. Only 1/5 studies showing a significant reduction in measures of intestinal permeability, 1/3 studies suggested an increase in systemic bacterial endotoxin profile, and only 3/10 studies suggested an effect of supplementation on systemic inflammatory cytokine profile in response to exercise. Improvements in selectively reported measures of GIS in response to exercise following probiotic supplementation were reported in 2/5 studies. It is important to highlight the magnitude of exercise-associated gastrointestinal disturbances and differences between intervention and placebo groups in studies reporting positive effects of supplementation interventions, are modest in nature and study conclusions suggesting beneficial effects of supplementation (i.e., lower intestinal permeabiluty, endotoxaemia, cytokine responses) are to be interpreted with caution given methodological issues and concerns identified, as recently discussed in Costa et al. (44). Only a limited number of studies have assessed GIS during exercise, with either minimal or no effect of probiotic supplementation observed, and with likely distorted outcomes associated with a lack of control of established confounding factors (79). The effect of prebiotics on gastrointestinal outcomes during exercise have not yet been studied, preventing any conclusions being drawn. The data synthesized in this review suggest pre-, pro-, and syn-biotic supplementation exerts inconsistent effects on gastrointestinal integrity, function, symptoms and resultant systemic response, at rest. In response to exertional or exertional heat stress, no consistent and substantial beneficial effects are seen with probiotics or synbiotics on gastrointestinal status.

Pre-, pro-, and syn-biotics and markers of gastrointestinal integrity

The role of intestinal barrier integrity, in both adequate nutrient absorption and in preventing unwanted translocation of bacterial endotoxins into circulation, is seen as one key factor influencing the likelihood of EIGS (2). There is now substantial evidence that exercise-associated epithelial enterocyte injury, measured through the surrogate marker I-FABP, is accompanied by an increase in systemic bacterial endotoxin from luminal origin, and subsequent systemic inflammatory responses (109, 110), similar to those values observed in clinical populations (e.g., medical complications of the gastrointestinal tract) (2, 19, 34, 111–118). These gastrointestinal integrity outcomes are relatively asymptomatic during exercise, but may instigate GIS in the post-exercise recovery period (e.g., abdominal pain, osmotic diarrhea, urge to regurgitate, regurgitation, and/or fecal blood loss), as a result of acute reversible colitis (2, 3). Such perturbations to gastrointestinal integrity (e.g., plasma I-FABP concentration: Δ pre- to post-exercise ≥1,000 pg/ml) are consistently seen with exercise stress loads ≥2 h of endurance exercise at 60% VO_{2max} in hot ambient conditions (≥35.0°C) where peak core temperature reaches ≥39.0°C, irrespective of relative humidity (33, 35, 109, 110, 119), or with ≥3 h of endurance exercise at 60% VO_{2max} in temperate conditions (∼20°C) with minimal rise in core body temperature (19, 34). Any lesser exertional or exertional-heat stress appears to result in no or minimal perturbations to gastrointestinal integrity, or perturbations of no clinical relevance. It is therefore unsurprising that the studies included in this review, almost universally failed to substantially influence aspects of intestinal integrity, endotoxemia or cytokinemia at rest, given that these mechanisms are unlikely to occur to any significant extent in the absence of a medical gastrointestinal condition, or a bout of substantial exercise stress. However, this may be in part due to the insufficient exercise or heat stress required to significantly perturb the intestinal barrier in most studies. In addition, one study reported no pre- to post-exercise increase in plasma I-FABP concentration in the probiotic or placebo groups as a result 2 h cycling at 55% W_{max} followed by a time trial in which a carbohydrate beverage was provided throughout the exercise bout (100). The authors purported this outcome was due to insufficient exercise stress load, but it is also likely that carbohydrate consumption during the exercise protocol was able to completely ameliorate exercise-associated epithelial injury [i.e., abolished plasma I-FABP response, as reported by Snipe et al. (38)], as observed during other exercise carbohydrate feeding studies (103, 120–123). This effect has been attributed to carbohydrate absorption-associated, nitric oxide-induced, villi microvascular dilation and perfusion (37, 103, 123). Together the data presented in this systematic review provides no evidence that probiotics exert an effect on gastrointestinal integrity, and to date no studies of synbiotics or prebiotics have investigated this aspect of EIGS.

Pre-, pro-, and syn-biotics and markers of gastrointestinal permeability

Exercise-associated modulation to intestinal epithelial injury and intestinal epithelial permeability, and their respective biomarkers (i.e., direct or indirect surrogate biomarkers) are not the same, and outcome data for these cannot be used interchangeably, as discussed in a recent study by Gaskell et al. (34). Several recent studies have observed a mismatch between exercise-associated changes to plasma I-FABP concentration (i.e., epithelial enterocyte injury) and lactulose:mannitol or lactulose:rhamnose dual sugar test (i.e., intestinal epithelial tight-junction permeability), and/or plasma or fecal claudin-3 concentration that is a proposed surrogate marker for epithelial tight-junction damage (35, 109, 124, 125). Indeed, the magnitude of intestinal epithelial injury and permeability

differs in response to the same exertional or exertional-heat stress (38, 109, 124, 125), with permeability measures not increasing in proportion to exercise stress, and not leading to post-permeability outcomes (i.e., increased plasma endotoxin, anti-endotoxin, and inflammatory cytokine concentrations) (2, 3). Considering increased intestinal permeability in response to exercise stress does not correlate with epithelial injury, systemic endotoxin and inflammatory cytokine profiles, and GIS; in studies that have included a global gastrointestinal assessment (35, 38, 109, 125), it appears increases in intestinal permeability is a habitual response to exercise stress, with a set threshold, and of little relevance to the key health outcomes of EIGS (e.g., aggressive acute or repetitive strain epithelial injury, systemic endotoxemia and inflammatory cytokinemia, and/or gastroparesis with or without paralytic ileus). Regardless, the studies included in this systematic literature review did not provide any substantial evidence, at rest or in response to exercise, that pre-, pro- or syn-biotic supplementation could reduce intestinal permeability. $N = 1$ study that reported improvements in permeability at rest following 14 weeks multi-strain probiotic supplementation (63) should be interpreted with caution given known limitations, including analysis procedures now identified as poor indicators of intestinal permeability (e.g., fecal or plasma zonulin concentration determination) (126, 127). Only $n = 1$ prebiotic study assessed permeability at rest, concluding an improvement in urinary lactulose:mannitol ratio following 5 weeks of consumption of inulin enriched pasta, compared with placebo (62). In response to exercise, $n = 1$ study assessed intestinal permeability 6-days after completion of the exercise stress (i.e., long course triathlon event) (94). Given the transient nature of exercise-induced changes in gastrointestinal permeability, it is not surprising that this study did not observe any substantial differences from pre-exercise values (i.e., sample time point was 1 day prior to the event). Despite one included study measuring claudin-3 in response to exercise, fecal sampling for biomarker determination was the included method (102). Considering gastrointestinal integrity perturbations of EIGS are transient in nature, as opposed to the consistent perturbation seen in inflammatory diseases of the gastrointestinal tract (e.g., Crohn's disease and ulcerative colitis), it is now well established that measuring fecal biomarkers to determine the extent of gastrointestinal permeability, with or without adjoining injury and inflammation biomarkers, risks erroneous interpretations due to issues surrounding sample collection timing, volume, and processing methods (44).

Pre-, pro-, and syn-biotics and systemic endotoxin response

Since intestinal integrity is not compromised at rest in otherwise healthy individuals, endotoxemia is unlikely to occur to any significant extent (2, 3). Consistent with this, none of the $n = 5$ studies reporting resting endotoxin concentration showed any changes following supplementation with a pro- or syn-biotic. In response to an acute exercise bout, since intestinal injury was not significantly impacted, subsequent systemic endotoxin remained unaffected to any substantial degree by the supplementation with the pro- or syn-biotics studied (94, 102). In contrast, $n = 1$ study showed an increase in gram-negative bacterial endotoxin concentration during the recovery period of exertional-heat stress, as a result of *L. casei* supplementation (68). Thus, the evidence to date suggests that supplementation with pro- or syn-biotics show no benefit to endotoxin response at rest or following exercise stress.

Pre-, pro-, and syn-biotics and cytokine response

Cytokine responses, which are consistently reported as the key pathophysiological endpoint for clinical significance (i.e., negative health affects), were largely unaffected by the majority of studied supplements. Where positive effects on cytokines following probiotic supplementation compared with placebo were observed, no consistent pattern was seen across the cytokine profile studied, but rather isolated changes were observed, such as; an attenuation in the rise in inflammatory C-C Motif Chemokine Ligand 2 (CCL2) following 2 weeks supplementation with *S. thermophilus*, *L. delbrueckii* spp. *Bulgaricus*, and *L. rhamnosus* GG, compared with placebo (66) but no change in other inflammatory cytokines observed (TNF-α, CCL5, IL-6); and an attenuated rise in tumor necrosis factor alpha (TNF-α) following 12 weeks supplementation with *B. subtilis*, compared with placebo, but no change in anti-inflammatory interleukin IL-10 (88). Furthermore, inconsistent results were shown following 30 days of supplementation with a multi-strain probiotic, whereby a greater reduction in pro-inflammatory interleukin IL-2, an attenuated reduction in anti-inflammatory IL-4, and a greater reduction in anti-inflammatory IL-10 was observed, compared with placebo (87). Multi-strain synbiotic supplementation also showed inconsistent results, namely a 50% lower circulating IL-16 concentration, compared to a prebiotic control (i.e., acacia gum) with no difference observed in Il-18, while IL-12 and IFN-γ were undetectable in assay (62). Another study with a multi-strain synbiotic for 30 days reported greater reduction in circulating IL-10 concentration in the placebo group than those on the intervention (93). In response to exercise, only two studies showed improvements and, in some cases, contradictory findings in cytokine response following exercise. Eleven weeks of supplementation with *Lactobacillus fermentum* VRI-003 PCC®, resulted in lower pre- to post-exercise increases in plasma IL-1ra, IL-6, IL-8, IL-10, GM-CSF, IFN-γ, and TNF-α concentrations

(90) using magnitude based inferences, whereas a significant increase in IL-10 was observed 1 h post-exercise, following 30 days of supplementing with a multi-strain probiotic, and TNF-α increased only in the placebo group at 1 h post-exercise compared with 24 h pre-exercise levels (87). However, most important to note is that the magnitude of systemic cytokine responses in these studies were minimal in comparison to more aggressive exercise models and ultra-endurance field events (16, 69, 109), and are unlikely to be of clinical relevance. It therefore appears that there is no compelling evidence that probiotics or synbiotics exert any clinically relevant effect on resting cytokines and perhaps less so on cytokine responses to exercise at the intensities and exercise volumes observed. Whether these biotic interventions could show an attenuated systemic inflammatory effect at exercise interventions causing more activation of the immune system, as reported in Costa et al. (44), remains unknown.

Pre-, pro-, and syn-biotics and markers of gastrointestinal function

Gastrointestinal functional responses are an important component of EIGS, and give rise to many of the unpleasant GIS experienced by active adults, both at rest and during exercise (128). Functional responses include measures of gastrointestinal motility and transit, such as gastric emptying rate, EGG, OCTT, defecation frequency, and stool consistency (45, 55, 59, 61). Other functional responses include magnitude of malabsorption to a nutrient challenge and subsequent bacterial fermentation of intestinal residue, typically assessed through breath hydrogen and/or methane responses (6, 38, 46, 129). It was somewhat surprising that only 4/39 included studies reported data pertaining to functional responses, and none in response to exercise. Of the measures included, only gastric emptying rate was reduced by the consumption of prebiotic inulin enriched pasta, compared with a placebo meal (61), overall gastrointestinal motility and function appears minimally affected, and only $n = 1$ study observed a beneficial reduction in GIS, with other studies suggesting a possible increase, which is consistent with bacterial fermentation of poorly absorbed nutrient/s increasing luminal content and pressure. Whilst this data was captured at rest, it appears consistent with a recent study, not included in this review's inclusion criteria, showing that a 24 h high FODMAP diet (46.9 g/day) and high FODMAP pre-exercise meal (26.2 g), both which contained a substantial fructan component (10.1 and 1.4 g, respectively) that is consistent with prebiotic supplementation doses, increased upper-GIS severity at rest and in response to exercise in a healthy active population compared to a 24 h low FODMAP diet (<5 g/day) (35). The authors speculated that reduced gastric motility was the likely mechanistic cause of such findings, as FODMAPs pass through the small intestine as residue, are readily fermentable by commensal bacteria, and the residue and fermentation contribute to increase intestinal lumen content and pressure. These outcomes are likely to activate the gastrointestinal braking mechanism that reduces gastric emptying rate and intestinal transit (6–10).

Pre-, pro-, and syn-biotics and gastrointestinal symptoms

Of all the outcomes presented in this systematic review, the one of most interest and relevance to active adults is the experience of GIS, given this is likely one of the main reasons consumers would choose to consume a pre-, pro-, or syn-biotic product, and the factor that has performance implications (i.e., GIS directly linked to reduced distance test performance; and workload reduction, cessation or withdrawal from exercise activity) (19, 130). At rest, the majority of studies saw minimal GIS incidence and severity, and therefore minimal differences between supplement intervention and placebo. Of those that did show statistically significant differences in GIS, most were of low incidence and/or severity, or categories of symptoms were selectively reported, to the exclusion of others where no change in overall symptoms were observed (83); and in some cases, GIS were greater during consumption of the supplement intervention compared to placebo. Due to the potential health and performance debilitating effect of EIGS and associated GIS in active populations, there is substantial interest in manipulating factors that may reduce the incidence and/or severity of EIGS and associated symptoms. Using the EIGS model (Figure 1), it can be seen that interventions designed to reduce either the effect of primary causal mechanisms (i.e., splanchnic blood flow, and the neuroendocrine stress response to exercise), or the secondary outcomes (i.e., intestinal barrier integrity, nutrient absorption capability, and the presence or absence of undigested and/or fermentable residue in the gastrointestinal tract), should theoretically contribute to a reduction in unwanted outcomes. In the case of pre-, pro- and syn-biotics, such interventions are mostly aimed at targeting the secondary mechanisms of the gastrointestinal-circulatory pathway of EIGS, by potentially enhancing the stability and function of individual epithelial cells, and their bonded relationship with adjacent cells within the gastrointestinal epithelial layer. Only $n = 5$ studies were identified that have assessed GIS in response to exercise, none of which provided compelling evidence that probiotics could improve GIS incidence or severity. Moreover, it is important to note that the majority of studies did not use a validated or reliability-checked GIS assessment tool, instead using in-house or Likert-type rating scales, or online questionnaires with unclear origins and symptom types, possibly because GIS was a secondary outcome

in many study designs. Validated and reliability-checked GIS assessment tools like the visual analog scales and ROME III criteria for symptom type were not consistently applied (131–133). The only study investigating GIS following synbiotic supplementation, in military recruits engaged in a 5-day continuous military training exercise following supplementation regime, showed no difference between groups, pre- to post-supplementation, or following the multi-day training exercise, but an effect of time was observed, whereby symptoms reduced in both groups following the military training exercise bout (96). This suggests that military training is more effective at reducing symptoms than the synbiotic supplement provided at rest. Currently there are no published studies that have assessed the impact of prebiotic supplementation on GIS during exercise, warranting further research.

Pre-, pro-, and syn-biotics and gut microbial composition and short chain fatty acids

The interaction between the "*gut microbiota*" and human biological systems has gained much research interest and translational application traction. The role of commensal and pathogenic bacteria, and their metabolic by-products (e.g., SCFA) and structural residues (e.g., endotoxins) are increasingly being recognized as contributing to the attenuation or exacerbation of pathophysiologic pathways in numerous clinical conditions (e.g., cardiometabolic, mental health, gastrointestinal disease and disorders, and systemic inflammatory conditions) (43). Whilst most of the gastrointestinal mechanistic research has been conducted with *in vitro* or animal models, translation to interventions targeting human gut microbiota are growing rapidly (40–43, 134–136). From the current literature it appears that the beneficial role of the gut microbiota is associated with intestinal commensal bacteria producing SCFA (i.e., butyrate, acetate, and propionate) and other metabolic by-products (e.g., anti-inflammatory factors). The family groups *Lachnospiraceae* and *Ruminoccoccaceae*, and genus *Akkamensia*, *Bacteroides*, *Bifidobacterium*, *Clostridium* (e.g., species *leptum*), *Faecalibacterium, Lactobacillus*, and *Rosburia* are reported to stimulate luminal host immunity *via* intestinal secretion of anti-microbial proteins and activation of innate immune responses, enhance the intestinal epithelial structural barrier (i.e., mucus production, enterocyte cell proliferation, and tight-junction protein expression), reduce pathogenic adhesion to intestinal epithelial apical surface, and improved gastrointestinal motility, including facilitating peristalsis. Conversely, pathogenic bacteria including *Escherichia coli*, *Salmonella*, *Shigella*, and (or) *Campylobacter* and their structural residues (e.g., endotoxins- LPS, peptidoglycan, flagellin, lipoteichoic acid, and muramyldipeptide) are potent stimulators of local epithelial and systemic immune responses (*via* Nfκβ and phagocytic immune cell activation), through the TLR-4 activation pathway identifying PAMP on pathogenic bacterial surfaces. Therefore, it appears increased bacterial α-diversity, increased relative abundance of SCFA producing commensal bacteria, and decreased relative abundance of endotoxin-presenting pathogenic bacteria, meets the criteria for optimal "*gut health*" in respect to gut microbiota composition.

Changes in intestinal microbial composition, as determined by fecal bacterial counts as CFU/g feces *via* fluorescence *in situ* hybridization (FISH), traditional cell cultures, or quantitative polymerase chain reaction (qPCR), or determined by fecal bacterial taxa as relative abundance and α-diversity of operational taxonomic units (OTU) *via* more modern sequencing techniques (e.g., 16S or shotgun sequencing), were highly variable in the included studies. The direct comparison between studies is difficult to establish due to differences in the methods of reporting data at different levels of taxonomy, the diverse or limited bacterial types reported in various studies, and the differing reporting units used (i.e., absolute vs. relative values, and reporting bacterial counts per wet vs. dry mass of feces). For example, determination of bacterial composition using FISH and/or bacteria specific qPCR methods, as predominaly used in the older dated studies, provides a value for bacterial counts relative to the total identifiable bacterial counts as per weight of sampled feces (e.g., CFU/g). Whereas, the more recent studies used gene sequencing techniques, which are limited to the relative abundance of the total bacterial count detected (e.g., %). Thus, caution is warranted in interpreting the outcomes obtained in regards to the biotic interventions when comparing studies using different bacterial determination techniques. Furthermore, despite attempts to establish a "*healthy gut microbiota profile*"—or normative composition, as discussed by Bennett et al. (33), there is currently no well-established gut microbiota profile considered as a "healthy athlete" profile. This is likely due to the large individual variability within and between individuals, and from an experimental perspective the heterogeneous experimental designs and lack of confounding factor control (e.g., dietary, exercise, circadian, ambient conditions, etc.) within and between studies (33, 44). Within the current review, and taking these limitations into consideration, the most consistent changes in gut microbial composition came from prebiotic supplementation interventions, with increases in the relative abundance of *Bifidobacterium* reported in all included studies, except one, and no change in the abundance of *Lactobacillus* in any prebiotic study (Table 4). Probiotic and synbiotic supplement interventions appeared to significantly increase the relative abundance of the supplemented strains where measured. However, the effect on the abundance of other microbiota appeared inconsistent and mostly negligible, with the exception of a nine-fold increase in *Lactobacillus* following supplementation with *Bifidobacterium longum subsp. Longum* (78). In all included studies, any changes in selected bacterial

taxa did not result in improvements in any measure of bacterial diversity reported (e.g., α-diversity (Shannon index), richness, Simpson index or 16SrRNA gene sequencing).

SCFA have previously been proposed as key by-products of bacteria metabolism that support intestinal epithelial integrity (137, 138). The presence of greater concentrations of SCFA in fecal samples following pre-, pro- or syn-biotic supplementation at rest may indicate a successful increase in the absolute or relative abundance of SCFA-producing bacteria. A clear delineation was made between pre- and pro-biotics with respect to SCFA concentration. AXOS prebiotics are produced as a by-product of the bread-making process by enzymatic reaction with naturally occurring arabinoxylans in grains, allowing bread manufacturers to manipulate prebiotic content of the baked product, without fortification (139). Higher dose AXOS based prebiotic supplemental protocols demonstrated an increase in fecal total SCFA, acetic and butyric acids (7.2 g/day AXOS for 3 weeks) (55) and in one study these changes also included an increase in propionic acid concentrations (8.0 g/day AXOS for 3 weeks) (57). These increases in SCFA were not seen in lower dose prebiotic supplemental protocols (2.4 g/day AXOS) (57), 2.8 g/day XOS (56) or any dose of inulin-based prebiotic. No positive effects in resultant fecal SCFA due to probiotic supplementation were observed. Only one of the included studies investigated fecal SCFA concentrations following synbiotic supplementation, that included an acute exercise component (5 days continuous intense military training exercise) but no change was reported between groups at rest or following the prolonged exercise bout (96). Further research targeting increases in SCFA producing bacteria, utilizing prebiotic ingredients shown to have such an effect, and including an exertional-heat stress component, is required to demonstrate if such supplements can consequently improve intestinal integrity and reduce EIGS outcomes in athletes. It has been noted in one of the included studies that a lack of change in fecal SCFA concentration may not necessarily reflect a lack of change in production, but instead be caused by increased metabolism of SCFA by the host (56), suggesting that any such study should also include measurement of changes in the abundance of SCFA producing bacteria. Indeed, changes in SCFA are best measured in blood, as increases in circulatory SCFA have been noted in the absence of fecal SCFA changes, indicating higher luminal absorption (140).

Study limitations

The major limitation of this systematic review is the very small number of studies identified that took a comprehensive approach of well-validated biomarkers, in such a way that readers can establish the cause-and-effect relationship between the supplement intervention, and both the mechanisms and outcomes of EIGS in a systematic manner. Indeed, a significant number of included studies consisted of small sample sizes, with $n = 10$ studies consisting of $n \leq 15$ participants and only $n = 12$ studies consisting of $n \geq 30$ participants. Sample size determination was either not specified, or reported as underpowdered in $n = 20/39$ included papers. Additionally, the complete absence of studies that have provided prebiotic supplements and investigated the subsequent response to acute exercise, prevents us from drawing conclusions in this area. Whilst the possibility exists that we failed to identify all previous studies related to the research question, this risk was minimized through the use of six academic databases in the literature search. In addition, all recent review papers found during the search were scanned for additional papers, however no further records were identified. The lack of effective dietary control in the vast majority of studies included is a significant limitation to study interpretation, with no dietary control other than instruction of what foods or beverages to avoid in $n = 15$ studies, and habitual diet with the request that participants keep a food-fluid log (e.g., 1–3 days before exercise trial) or record pre-trial intake and attempt to duplicate intake on any subsequent trial/s in a further $n = 15$ studies, many of which only stated non-significant difference without reporting the actual data for energy and macronutrients, including fiber. Only $n = 8$ included studies provided food to participants to control dietary intake prior to measures being taken; one of which dietary control was inherent to the research setting (military barracks food service), with no indication of energy or macronutrient content controlled for. None of the included studies controlled for FODMAP content of the diet, which is a known prebiotic food constituent that is broadly represented in the western diet (141). It is now well established that dietary FODMAP intake leading into exercise (e.g., experimental trials) influences gastrointestinal integrity and functional outcomes, systemic responses, and GIS (35, 44). The absence of a meta-analysis may also be considered a limitation of this review, highlighting the heterogeneity in reporting findings of key gastrointestinal markers. Therefore, in accordance with the data presented in this systematic literature review the impact of pre-, pro- and syn-biotics on gastrointestinal outcomes in healthy and active adults at rest and in response to exercise remains largely negligible, with no substantial effect on markers of gastrointestinal integrity and systemic responses, and minimal and inconsistent effects on function and symptoms.

Implications for research and practice

As already discussed, the data captured by this review does not provide any convincing evidence for beneficial effects, and/or the methodological issues acknowledged and raised in the included studies does not allow many definitive conclusions

to be drawn regarding the impact of pre-, pro-, and syn-biotic supplementation on markers of gastrointestinal status at rest and in response to exercise. Future research would benefit from taking a bottom-up approach, utilizing existing findings in academic literature to build a pathway from supplementation to changes in gut microbiota, to mechanistic changes in the host, and finally to beneficial outcomes (e.g., barrier integrity, function, systemic responses, and symptoms). Given that symptoms are likely a main reason consumers would choose to consume a pre-, pro- or syn-biotic product, future studies should use exercise protocols of sufficient intensity, duration and ambient conditions to adequately provoke GIS, and purposefully recruit athletes with a history of GIS, thus making it more likely to observe improvements following a period of supplementation. Considering the acute and rapid plasticity of the gastrointestinal tract and emerging evidence that pre-exercise dietary intake can influence the magnitude of EIGS and exercise-associated GIS (35, 46), future research in this area should provide participants, and report on, all food and fluid consumed at least 24 h before experimental procedures and throughout the experimental period. Laboratory-based research targeting EIGS management strategies, application and reporting of at least a 24 h low FODMAP, and matching for fiber intake, to meet energy needs that is macronutrient balanced is recommended (34, 35, 38, 109, 110, 125); this may not necessarily apply to exploratory field-based research. Prospective food-fluid intake logs are best used to assess compliance with the control diet provided. Few studies have investigated the effect of synbiotic supplementation on EIGS mechanisms or outcomes, but those that have tended to produce results more closely resembling probiotics than prebiotics. This probably reflects the very small quantities of the included prebiotic, as many synbiotic supplements are consumed in capsule form, preventing larger quantities of prebiotic ingredients from being consumed. Mechanistically it appears that AXOS prebiotics exert beneficial effects on SCFA production and bacterial taxa, warranting further exploration. Well controlled studies using appropriate exercise stress models and a range of well validated EIGS markers would determine whether these changes indeed confer a benefit to gastrointestinal integrity and resultant systemic effects.

Conclusion

The effect of pre-, pro- and syn-biotic supplementation, taken by healthy and active adults, on gastrointestinal outcomes at rest and in response to exercise are highly varied, however the following can be concluded: (i) Supplementation with prebiotic ingredients appears to alter the gut bacterial microbiota, particularly increasing the relative abundance of total *Bifidobacterium;* (ii) supplementation with probiotics usually results in an increase in the relative abundance of the supplemented species and/or strain, however the effect on other bacterial types is inconsistent and may be specific to the supplement chosen; (iii) Pre-, pro- and syn-biotic supplements do not significantly change bacterial α-diversity, as determined by Shannon index, Simpson index or 16S gene sequencing; (iv) supplementation with AXOS prebiotic ingredients, appears to increase fecal SCFA content at rest; (v) both pre- and pro-biotic supplements do not appear to significantly influence intestinal injury and permeability, systemic endotoxin and inflammatory cytokine responses, or GIS at rest, and have minimal impact on gastrointestinal motility and function at rest in otherwise, healthy, active adults, with the exception of gastric emptying which may be delayed (i.e., slower) with inulin supplementation; (vi) probiotic supplementation with the species studied to date do not substantially influence intestinal injury and permeability, and subsequent systemic endotoxin or inflammatory cytokine responses, or GIS in response to exercise, although many studies lack adequate exertional stress or heat stress, or appropriate biomarkers, to definitively make this conclusion; (vii) currently no studies have investigated the effect of prebiotic supplements on gastrointestinal responses to exercise; (viii) synbiotic supplements appear to more closely resemble the effects of probiotic than prebiotic supplements, due to the generally very small quantity of prebiotic ingredients included in them; (ix) the choice of supplements studied to date appears to lack a logical, evidence-based approach to finding the ideal prebiotic ingredient and/or probiotic strain/s, based on existing mechanistic or observational studies of gut microbiota and EIGS outcomes. Therefore, the above conclusions may reflect poor choice of supplement ingredients rather than a failure of pre-, pro- or syn-biotic products in general. In addition to a more evidence-based approach to ingredient selection, research methodologies, including biomarker choice, timing of biological sampling in relation to exercise, the chosen exercise protocol and ambient conditions, may all contribute to the success or failure to find suitable pre-, pro- and syn-biotic products that improve EIGS outcomes in active adults. Future research should be designed to maximize the likelihood of exercise-associated gastrointestinal disturbance, taking biological samples immediately before and after exercise, as well as in the hours following, and utilize a complete, well-validated suite of EIGS biomarkers to ensure data is correctly interpreted.

Data availability statement

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

Author contributions

CR, ASM, and ZH undertook the systematic review (search, screening, eligibility, and data extraction as primary or secondary reviewer) and cross-checked by AJM. CR and RC contributed to the final draft preparation of the manuscript. All authors contributed to the manuscript review, read, and approved the final manuscript.

Funding

The authors declare that this study received funding from True Protein Pty Ltd. Australia as part of the Innovation Connection, Australian Government Grant Scheme and Australian Government, Department of Industry Science Energy and Resources, Innovation Connections Grant—ICG001159. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

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

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Aziz I, Simrén M. The overlap between irritable bowel syndrome and organic gastrointestinal diseases. *Lancet Gastroenterol Hepatol.* (2021) 6:139–48. doi: 10.1016/S2468-1253(20)30212-0

2. Costa RJS, Snipe RMJ, Kitic CM, Gibson PR. Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease. *Aliment Pharmacol Ther.* (2017) 46:246–65. doi: 10.1111/apt.14157

3. Costa RJS, Gaskell SK, Mccubbin AJ, Snipe RMJ. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. *Temperature.* (2020) 7:58–88. doi: 10.1080/23328940.2019.1597676

4. Barrett KE. Epithelial biology in the gastrointestinal system: insights into normal physiology and disease pathogenesis. *J Physiol.* (2012) 590:419–20. doi: 10.1113/jphysiol.2011.227058

5. Holzer P, Farzi A, Hassan AM, Zenz G, Jačan A, Reichmann F. Visceral inflammation and immune activation stress the brain. *Front Immunol.* (2017) 8:1613. doi: 10.3389/fimmu.2017.01613

6. Miall A, Khoo A, Rauch C, Snipe RMJ, Camões-Costa VL, Gibson PR, et al. Two weeks of repetitive gut-challenge reduce exercise-associated gastrointestinal symptoms and malabsorption. *Scand J Med Sci Sports.* (2018) 28:630–40. doi: 10.1111/sms.12912

7. Layer P, Peschel S, Schlesinger T, Goebell H. Human pancreatic secretion and intestinal motility: effects of ileal nutrient perfusion. *Am J Physiol.* (1990) 258:G196–201. doi: 10.1152/ajpgi.1990.258.2.G196

8. Van Citters GW, Lin HC. Ileal brake: neuropeptidergic control of intestinal transit. *Curr Gastroenterol Rep.* (2006) 8:367–73. doi: 10.1007/s11894-006-0021-9

9. Shin HS, Ingram JR, Mcgill AT, Poppitt SD. Lipids, CHOs, proteins: can all macronutrients put a 'brake' on eating? *Physiol Behav.* (2013) 120:114–23. doi: 10.1016/j.physbeh.2013.07.008

10. Van Avesaat M, Troost FJ, Ripken D, Hendriks HF, Masclee AA. Ileal brake activation: macronutrient-specific effects on eating behavior? *Int J Obes.* (2015) 39:235–43. doi: 10.1038/ijo.2014.112

11. Lin YM, Li F, Shi XZ. Mechanical stress is a pro-inflammatory stimulus in the gut: *in vitro*, *in vivo* and *ex vivo* evidence. *PLoS ONE.* (2014) 9:e106242. doi: 10.1371/journal.pone.0106242

12. Fleshner M, Crane CR. Exosomes, DAMPs and miRNA: features of stress physiology and immune homeostasis. *Trends Immunol.* (2017) 38:768–76. doi: 10.1016/j.it.2017.08.002

13. Gaskell SK, Lis DM, Costa RJS. Exercise-induced gastrointestinal syndrome (Chapter 21, pages 551-575). In: Burke L, Deakin V, Minehan M. *Clinical Sports Nutrition*, 6th ed. Sydney, NSW: McGraw-Hill Education (2021).

14. Perko MJ, Nielsen HB, Skak C, Clemmesen JO, Schroeder TV, Secher NH. Mesenteric, coeliac and splanchnic blood flow in humans during exercise. *J Physiol.* (1998) 513:907–13. doi: 10.1111/j.1469-7793.1998.907ba.x

15. Rehrer NJ, Smets A, Reynaert H, Goes E, De Meirleir K. Effect of exercise on portal vein blood flow in man. *Med Sci Sports Exerc.* (2001) 33:1533–7. doi: 10.1097/00005768-200109000-00017

16. Gill SK, Teixeira A, Rama L, Prestes J, Rosado F, Hankey J, et al. Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. *Exerc Immunol Rev.* (2015) 21:114–28. doi: 10.1111/1747

17. Grootjans J, Lenaerts K, Buurman WA, Dejong CH, Derikx JP. Life and death at the mucosal-luminal interface: new perspectives on human intestinal ischemia-reperfusion. *World J Gastroenterol.* (2016) 22:2760–70. doi: 10.3748/wjg.v22.i9.2760

18. Van Wijck K, Lenaerts K, Van Loon LJ, Peters WH, Buurman WA, Dejong CH. Exercise-induced splanchnic hypoperfusion results in gut dysfunction in healthy men. *PLoS ONE.* (2011) 6:e22366. doi: 10.1371/journal.pone.0022366

19. Costa RJS, Miall A, Khoo A, Rauch C, Snipe R, Camões-Costa V, et al. Gut-training: the impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. *Appl Physiol Nutr Metab.* (2017) 42:547–57. doi: 10.1139/apnm-2016-0453

20. Gaskell SK, Rauch CE, Parr A, Costa RJS. Diurnal versus nocturnal exercise-effect on the gastrointestinal tract. *Med Sci Sports Exerc.* (2021) 53:1056–67. doi: 10.1249/MSS.0000000000002546

21. Gaskell SK, Burgell R, Wiklendt L, Dinning P, Costa RJS. Does exertional heat stress impact gastrointestinal function and symptoms. *J Sci Med Sport.* (2022). doi: 10.1016/j.jsams.2022.10.008. In press.

22. Bermon S, Castell LM, Calder PC, Bishop NC, Blomstrand E, Mooren FC, et al. Consensus statement immunonutrition and exercise. *Exerc Immunol Rev.* (2017) 23:8–50.

23. Francavilla VC, Bongiovanni T, Todaro L, Di Pietro V, Francavilla G. Probiotic supplements and athletic performance: a review of the literature. *Medicina Dello Sport.* (2017) 70, 247–59. doi: 10.23736/S0025-7826.17.03037-X

24. Jäger R, Mohr AE, Carpenter KC, Kerksick CM, Purpura M, Moussa A, et al. international society of sports nutrition position stand: probiotics. *J Int Soc Sports Nutr.* (2019) 16:62. doi: 10.1186/s12970-019-0329-0

25. Leite GSF, Resende Master Student AS, West NP, Lancha AH, Jr. Probiotics and sports: a new magic bullet? *Nutrition.* (2019) 60:152–60. doi: 10.1016/j.nut.2018.09.023

26. Mach N, Fuster-Botella D. Endurance exercise and gut microbiota: a review. *J Sport Health Sci.* (2017) 6:179–97. doi: 10.1016/j.jshs.2016.05.001

27. Pyne DB, West NP, Cox AJ, Cripps AW. Probiotics supplementation for athletes - clinical and physiological effects. *Eur J Sport Sci.* (2015) 15:63–72. doi: 10.1080/17461391.2014.971879

28. Sivamaruthi BS, Kesika P, Chaiyasut C. Effect of probiotics supplementations on health status of athletes. *Int J Environ Res Public Health.* (2019) 16:4469. doi: 10.3390/ijerph16224469

29. West NP, Pyne DB, Peake JM, Cripps AW. Probiotics, immunity and exercise: a review. *Exerc Immunol Rev.* (2009) 15:107–26.

30. Guo Y-T, Peng Y-C, Yen H-Y, Wu J-C, Hou W-H. Effects of probiotic supplementation on immune and inflammatory markers in athletes: a meta-analysis of randomized clinical trials. *Medicina.* (2022) 58:1188. doi: 10.3390/medicina58091188

31. Heimer M, Teschler M, Schmitz B, Mooren FC. Health benefits of probiotics in spot and exercise- Non-existent or a matter of heterogeneity? A systematic review. *Front Nutr.* (2022) 9:804046. doi: 10.3389/fnut.2022.804046

32. Möller GB, Da Cunha Goulart MJV, Nicoletto BB, Alves FD, Schneider CD. Supplementation of probiotics and its effects on physically active individuals and athletes: systematic review. *Int J Sport Nutr Exerc Metab.* (2019) 29:481–92. doi: 10.1123/ijsnem.2018-0227

33. Bennett CJ, Henry R, Snipe RMJ, Costa RJS. Is the gut microbiota bacterial abundance and composition associated with intestinal epithelial injury, systemic inflammatory profile, and gastrointestinal symptoms in response to exertional-heat stress? *J Sci Med Sport.* (2020) 23:1141–53. doi: 10.1016/j.jsams.2020.06.002

34. Gaskell SK, Gill P, Muir J, Henry R, Costa RJS. The impact of a 24h low and high FODMAP diet on faecal and plasma short chain fatty acid concentration, and its influence on markers of exercise-induced gastrointestinal syndrome in response to exertionalheat stress. *Nutr Diet.* (2021) 78:8. doi: 10.1111/1747-0080.12709

35. Gaskell SK, Taylor B, Muir J, Costa RJS. Impact of 24-h high and low fermentable oligo-, di-, monosaccharide, and polyol diets on markers of exercise-induced gastrointestinal syndrome in response to exertional heat stress. *Appl Physiol Nutr Metab.* (2020) 45:569–80. doi: 10.1139/apnm-2019-0187

36. Young P, Russo I, Gill P, Muir J, Henry R, Davidson Z, et al. The impact of pre-exercise faecal and plasma SCFA concentration on markers of gastrointestinal integrity in response to 2h of high-intensity interval training. *Nutr Diet.* (2021) 78:9.

37. Matheson PJ, Wilson MA, Garrison RN. Regulation of intestinal blood flow. *J Surg Res.* (2000) 93:182–96. doi: 10.1006/jsre.2000.5862

38. Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. *Appl Physiol Nutr Metab.* (2017) 42:1283–92. doi: 10.1139/apnm-2017-0361

39. Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, et al. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum *in vivo* and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol.* (2010) 298:G851–859. doi: 10.1152/ajpgi.00327.2009

40. Mujagic Z, De Vos P, Boekschoten MV, Govers C, Pieters HH, De Wit NJ, et al. The effects of *Lactobacillus plantarum* on small intestinal barrier function and mucosal gene transcription; a randomized double-blind placebo controlled trial. *Sci Rep.* (2017) 7:40128. doi: 10.1038/srep40128

41. Cani PD. Human gut microbiome: hopes, threats and promises. *Gut.* (2018) 67:1716–25. doi: 10.1136/gutjnl-2018-316723

42. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med.* (2018) 24:392–400. doi: 10.1038/nm.4517

43. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev.* (2010) 90:859–904. doi: 10.1152/physrev.00045.2009

44. Costa RJS, Young P, Gill SK, Snipe RMJ, Gaskell SK, Russo I, et al. Assessment of exercise-associated gastrointestinal perturbations in research and practical settings: methodological concerns and recommendations for best practice. *Int J Sports Nutr Exerc Metab.* (2022) 32:387–418. doi: 10.1123/ijsnem.2022-0048

45. Russo F, Clemente C, Linsalata M, Chiloiro M, Orlando A, Marconi E, et al. Effects of a diet with inulin-enriched pasta on gut peptides and gastric emptying rates in healthy young volunteers. *Eur J Nutr.* (2011) 50:271–7. doi: 10.1007/s00394-010-0135-6

46. Costa RJS, Camões-Costa V, Snipe RMJ, Dixon D, Russo I, Huschtscha Z. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. *J Appl Physiol (1985).* (2019) 126:1281–91. doi: 10.1152/japplphysiol.01032.2018

47. Russo I, Della Gatta PA, Garnham A, Porter J, Burke LM, Costa RJS. Assessing overall exercise recovery processes using carbohydrate and carbohydrate-protein containing recovery beverages. *Front Physiol.* (2021) 12:628863. doi: 10.3389/fphys.2021.628863

48. Russo I, Della Gatta PA, Garnham A, Porter J, Burke LM, Costa RJS. Does the nutritional composition of a dairy-based recovery beverage influence post-exercise gastrointestinal and immune status, and subsequent markers of recovery optimisation in response to high intensity interval exercise? *Front Nutri.* (2021) 7:622270. doi: 10.3389/fnut.2020.622270

49. Russo I, Della Gatta PA, Garnham A, Porter J, Burke LM, Costa RJS. The effects of an acute "train-low" nutritional protocol on markers of recovery optimization in endurance-trained male athletes. *Int J Sports Physiol Perf.* (2021) 16:1764–76. doi: 10.1123/ijspp.2020-0847

50. Hamad A, Fragkos KC, Forbes A. A systematic review and meta-analysis of probiotics for the management of radiatic induced bowel disease. *Clin Nutr.* (2013) 32:353–60. doi: 10.1016/j.clnu.2013.02.004

51. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *System Rev.* (2021) 10:1–11. doi: 10.31222/osf.io/v7gm2

52. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* (2009) 6:e1000100. doi: 10.1371/journal.pmed.1000100

53. Rohatgi A. WebPlotDigitizer. *Web Based Tool to Extract Data from Plots, Images, and Maps.* (2022). Available online at: https://automeris.io/WebPlotDigitizer/ (accessed November 14, 2022).

54. Higgins J. *Cochrane Handbook for Systematic Reviews of Interventions*. Version 5.1. 0 [updated March 2011]. The Cochrane Collaboration (2011). Available online at: www.cochrane-handbook.org (accessed March 31, 2022).

55. Damen B, Cloetens L, Broekaert WF, François I, Lescroart O, Trogh I, et al. Consumption of breads containing *in situ*-produced arabinoxylan oligosaccharides alters gastrointestinal effects in healthy volunteers. *J Nutr.* (2012) 142:470–7. doi: 10.3945/jn.111.146464

56. Finegold SM, Li Z, Summanen PH, Downes J, Thames G, Corbett K, et al. Xylooligosaccharide increases bifidobacteria but not lactobacilli in human gut microbiota. *Food Funct.* (2014) 5:436–45. doi: 10.1039/c3fo60348b

57. François IE, Lescroart O, Veraverbeke WS, Marzorati M, Possemiers S, Evenepoel P, et al. Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: a double-blind, randomised, placebo-controlled, cross-over trial. *Br J Nutr.* (2012) 108:2229–42. doi: 10.1017/S0007114512000372

58. Cloetens L, Broekaert WF, Delaedt Y, Ollevier F, Courtin CM, Delcour JA, et al. Tolerance of arabinoxylan-oligosaccharides and their prebiotic activity in healthy subjects: a randomised, placebo-controlled cross-over study. *Br J Nutr.* (2010) 103:703–13. doi: 10.1017/S0007114509992248

59. Kleessen B, Schwarz S, Boehm A, Fuhrmann H, Richter A, Henle T, et al. Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. *Br J Nutr.* (2007) 98:540–9. doi: 10.1017/S0007114507730751

60. Reimer RA, Soto-Vaca A, Nicolucci AC, Mayengbam S, Park H, Madsen KL, et al. Effect of chicory inulin-type fructan-containing snack bars on the human gut microbiota in low dietary fiber consumers in a randomized crossover trial. *Am J Clin Nutr.* (2020) 111:1286–96. doi: 10.1093/ajcn/nqaa074

61. Russo F, Riezzo G, Chiloiro M, De Michele G, Chimienti G, Marconi E, et al. Metabolic effects of a diet with inulin-enriched pasta in healthy young volunteers. *Curr Pharm Des.* (2010) 16:825–31. doi: 10.2174/138161210790883570

62. Russo F, Linsalata M, Clemente C, Chiloiro M, Orlando A, Marconi E, et al. Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutr Res.* (2012) 32:940–6. doi: 10.1016/j.nutres.2012.09.010

63. Axelrod CL, Brennan CJ, Cresci G, Paul D, Hull M, Fealy CE, et al. UCC118 supplementation reduces exercise-induced gastrointestinal permeability and remodels the gut microbiome in healthy humans. *Physiol Rep.* (2019) 7:e14276. doi: 10.14814/phy2.14276

64. Karhu ERA, Forsgard L, Alanko H, Alfthan P, Pussinen E, et al. Exercise and gastrointestinal symptoms: running-induced changes in intestinal permeability and markers of gastrointestinal function in asymptomatic and symptomatic runners. *Eur J Appl Physiol.* (2017) 117:2519–26. doi: 10.1007/s00421-017-3739-1

65. Batatinha H, Tavares-Silva E, Leite GSF, Resende AS, Albuquerque JAT, Arslanian S, et al. Probiotic supplementation in marathonists and its impact on lymphocyte population and function after a marathon: a randomized placebo-controlled double-blind study. *Sci Rep.* (2020) 10:18777. doi: 10.1038/s41598-020-75464-0

66. Burton KJ, Rosikiewicz M, Pimentel G, Bütikofer U, Von Ah U, Voirol MJ, et al. Probiotic yogurt and acidified milk similarly reduce postprandial inflammation and both alter the gut microbiota of healthy, young men. *Br J Nutr.* (2017) 117:1312–22. doi: 10.1017/S0007114517000885

67. Carbuhn AF, Reynolds SM, Campbell CW, Bradford LA, Deckert JA, Kreutzer A, et al. Effects of probiotic (*Bifidobacterium longum* 35624) supplementation on exercise performance, immune modulation, and cognitive outlook in division I female swimmers. *Sports.* (2018) 6:116. doi: 10.3390/sports6040116

68. Gill SK, Allerton DM, Ansley-Robson P, Hemmings K, Cox M, Costa RJ. Does short-term high dose probiotic supplementation containing lactobacillus casei attenuate exertional-heat stress induced endotoxaemia and cytokinaemia? *Int J Sport Nutr Exerc Metab.* (2016) 26:268–75. doi: 10.1123/ijsnem.2015-0186

69. Gill SK, Hankey J, Wright A, Marczak S, Hemming K, Allerton DM, et al. The impact of a 24-h ultra-marathon on circulatory endotoxin and cytokine profile. *Int J Sports Med.* (2015) 36:688–95. doi: 10.1055/s-0034-1398535

70. Gleeson M, Bishop NC, Oliveira M, Tauler P. Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. *Int J Sport Nutr Exerc Metab.* (2011) 21:55–64. doi: 10.1123/ijsnem.21.1.55

71. Neville V, Gleeson M, Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc.* (2008) 40:1228–36. doi: 10.1249/MSS.0b013e31816be9c3

72. Haywood BA, Black KE, Baker D, Mcgarvey J, Healey P, Brown RC. Probiotic supplementation reduces the duration and incidence of infections but not severity in elite rugby union players. *J Sci Med Sport.* (2014) 17:356–60. doi: 10.1016/j.jsams.2013.08.004

73. Hoffman JR, Hoffman MW, Zelicha H, Gepner Y, Willoughby DS, Feinstein U, et al. The effect of 2 weeks of inactivated probiotic bacillus coagulans on endocrine, inflammatory, and performance responses during self-defense training in soldiers. *J Strength Cond Res.* (2019) 33:2330–7. doi: 10.1519/JSC.0000000000003265

74. Huang WC, Pan CH, Wei CC, Huang HY. *Lactobacillus plantarum* PS128 improves physiological adaptation and performance in triathletes through gut microbiota modulation. *Nutrients.* (2020) 12:2315. doi: 10.3390/nu12082315

75. Klein A, Friedrich U, Vogelsang H, Jahreis G. *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp lactis DGCC 420 modulate unspecific cellular immune response in healthy adults. *Eur J Clin Nutr.* (2008) 62:584–93. doi: 10.1038/sj.ejcn.1602761

76. Lamprecht M, Bogner S, Schippinger G, Steinbauer K, Fankhauser F, Hallstroem S, et al. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *J Int Soc Sports Nutr.* (2012) 9:45. doi: 10.1186/1550-2783-9-45

77. Lee MC, Jhang WL, Lee CC, Kan NW, Hsu YJ, Ho CS, et al. The effect of kefir supplementation on improving human endurance exercise performance and antifatigue. *Metabolites.* (2021) 11:136. doi: 10.3390/metabo11030136

78. Lin CL, Hsu YJ, Ho HH, Chang YC, Kuo YW, Yeh YT, et al. *Bifidobacterium longum* subsp. longum OLP-01 supplementation during endurance running training improves exercise performance in middle- and long-distance runners: a double-blind controlled trial. *Nutrients.* (2020) 12:1972. doi: 10.3390/nu12071972

79. Pugh JN, Sparks AS, Doran DA, Fleming SC, Langan-Evans C, Kirk B, et al. Four weeks of probiotic supplementation reduces GI symptoms during a marathon race. *Eur J Appl Physiol.* (2019) 119:1491–501. doi: 10.1007/s00421-019-04136-3

80. Sánchez Macarro M, Ávila-Gandía V, Pérez-Piñero S, Cánovas F, García-Muñoz AM, Abellán-Ruiz MS, et al. Antioxidant effect of a probiotic product on a model of oxidative stress induced by high-intensity and duration physical exercise. *Antioxidants.* (2021) 10:323. doi: 10.3390/antiox10020323

81. Krotkiewski M, Brzezinska Z. Lipid peroxides production after strenuous exercise and in relation to muscle morphology and capillarization. *Muscle Nerve.* (1996) 19:1530.

82. Schreiber C, Tamir S, Golan R, Weinstein A, Weinstein Y. The effect of probiotic supplementation on performance, inflammatory markers and gastro-intestinal symptoms in elite road cyclists. *J Int Soc Sports Nutr.* (2021) 18:36. doi: 10.1186/s12970-021-00432-6

83. Smarkusz-Zarzecka J, Ostrowska L, Leszczyńska J, Orywal K, Cwalina U, Pogodziński D. Analysis of the impact of a multi-strain probiotic on body composition and cardiorespiratory fitness in long-distance runners. *Nutrients.* (2020) 12:3758. doi: 10.3390/nu12123758

84. Son J, Jang LG, Kim BY, Lee S, Park H. The effect of athletes' probiotic intake may depend on protein and dietary fiber intake. *Nutrients.* (2020) 12:2947. doi: 10.3390/nu12102947

85. Strasser B, Geiger D, Schauer M, Gostner JM, Gatterer H, Burtscher M, et al. Probiotic supplements beneficially affect tryptophan-kynurenine metabolism and reduce the incidence of upper respiratory tract infections in trained athletes: a randomized, double-blinded, placebo-controlled Trial. *Nutrients.* (2016) 8:752. doi: 10.3390/nu8110752

86. Areces F, González-Millán C, Salinero JJ, Abian-Vicen J, Lara B, Gallo-Salazar C, et al. Changes in serum free amino acids and muscle fatigue experienced during a half-ironman triathlon. *PLoS ONE.* (2015) 10:e0138376. doi: 10.1371/journal.pone.0138376

87. Tavares-Silva E, Caris AV, Santos SA, Ravacci GR, Thomatieli-Santos RV. Effect of multi-strain probiotic supplementation on URTI symptoms and cytokine production by monocytes after a marathon race: a randomized, double-blind, placebo study. *Nutrients.* (2021) 13:1478. doi: 10.3390/nu13051478

88. Townsend JR, Bender D, Vantrease WC, Sapp PA, Toy AM, Woods CA, et al. Effects of probiotic (*Bacillus subtilis* DE111) supplementation on immune function, hormonal status, and physical performance in division I baseball players. *Sports.* (2018) 6:70. doi: 10.3390/sports6030070

89. Vaisberg M, Paixão V, Almeida EB, Santos JMB, Foster R, Rossi M, et al. Daily intake of fermented milk containing *Lactobacillus casei* Shirota (Lcs) modulates systemic and upper airways immune/inflammatory responses in marathon runners. *Nutrients.* (2019) 11:1678. doi: 10.3390/nu11071678

90. West NP, Pyne DB, Cripps AW, Hopkins WG, Eskesen DC, Jairath A, et al. *Lactobacillus fermentum* (PCC®) supplementation and gastrointestinal and respiratory-tract illness symptoms: a randomised control trial in athletes. *Nutr J.* (2011) 10:30. doi: 10.1186/1475-2891-10-30

91. Fricker PA, Pyne DB, Saunders PU, Cox AJ, Gleeson M, Telford RD. Influence of training loads on patterns of illness in elite distance runners. *Clin J Sport Med.* (2005) 15:246–52. doi: 10.1097/01.jsm.0000168075.66874.3e

92. Coman M, Verdenelli M, Silvi S, Cecchini C, Gabbianelli R, Amadio E, et al. Knowledge and acceptance of functional foods: a preliminary study on influence of a synbiotic fermented milk on athlete health. *Int J Probiot Prebiot.* (2017) 12:33–42.

93. Quero CD, Manonelles P, Fernández M, Abellán-Aynés O, López-Plaza D, Andreu-Caravaca L, et al. Differential health effects on inflammatory, immunological and stress parameters in professional soccer players and sedentary individuals after consuming a synbiotic. A triple-blinded, randomized, placebo-controlled pilot study. *Nutrients.* (2021) 13:1321. doi: 10.3390/nu13041321

94. Roberts JD, Suckling CA, Peedle GY, Murphy JA, Dawkins TG, Roberts MG. An exploratory investigation of endotoxin levels in novice long distance triathletes, and the effects of a multi-strain probiotic/prebiotic, antioxidant intervention. *Nutrients.* (2016) 8:733. doi: 10.3390/nu8110733

95. Faul F, Erdfelder E, Lang AG, Buchner A. G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* (2007) 39:175–91. doi: 10.3758/BF03193146

96. Valle MCPR, Vieira IA, Fino LC, Gallina DA, Esteves AM, Da Cunha DT, et al. Immune status, well-being and gut microbiota in military supplemented with synbiotic ice cream and submitted to field training: a randomised clinical trial. *Br J Nutr.* (2021) 126:1794–808. doi: 10.1017/S0007114521000568

97. Olivares M, Díaz-Ropero MP, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, et al. The consumption of two new probiotic strains, *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711, boosts the immune system of healthy humans. *Int Microbiol.* (2006) 9:47–52. doi: 10.1016/j.ijfoodmicro.2005.08.019

98. West NP, Pyne DB, Cripps AW, Christophersen CT, Conlon MA, Fricker PA. Gut Balance, a synbiotic supplement, increases fecal Lactobacillus paracasei but has little effect on immunity in healthy physically active individuals. *Gut Microbes.* (2012) 3:221–7. doi: 10.4161/gmic.19579

99. Kekkonen RA, Vasankari TJ, Vuorimaa T, Haahtela T, Julkunen I, Korpela R. The effect of probiotics on respiratory infections and gastrointestinal symptoms during training in marathon runners. *Int J Sport Nutr Exerc Metab.* (2007) 17:352–63. doi: 10.1123/ijsnem.17.4.352

100. Pugh JN, Wagenmakers AJM, Doran DA, Fleming SC, Fielding BA, Morton JP, et al. Probiotic supplementation increases carbohydrate metabolism in trained male cyclists: a randomized, double-blind, placebo-controlled crossover trial. *Am J Physiol Endocrinol Metab.* (2020) 318:E504–e513. doi: 10.1152/ajpendo.00452.2019

101. Yeo SE, Jentjens RL, Wallis GA, Jeukendrup AE. Caffeine increases exogenous carbohydrate oxidation during exercise. *J Appl Physiol.* (2005) 99:844–50. doi: 10.1152/japplphysiol.00170.2005

102. Shing CM, Peake JM, Lim CL, Briskey D, Walsh NP, Fortes MB, et al. Effects of probiotics supplementation on gastrointestinal permeability, inflammation

and exercise performance in the heat. *Eur J Appl Physiol.* (2014) 114:93–103. doi: 10.1007/s00421-013-2748-y

103. Lambert GP, Broussard LJ, Mason BL, Mauermann WJ, Gisolfi CV. Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages. *J Appl Physiol (1985).* (2001) 90:2075–80. doi: 10.1152/jappl.2001.90.6.2075

104. Marchbank T, Davison G, Oakes JR, Ghatei MA, Patterson M, Moyer MP, et al. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. *Am J Physiol-Gastrointest Liver Physiol.* (2011) 300:G477–84. doi: 10.1152/ajpgi.00281.2010

105. Lim CL, Pyne D, Horn P, Kalz A, Saunders P, Peake J, et al. The effects of increased endurance training load on biomarkers of heat intolerance during intense exercise in the heat. *Appl Physiol Nutr Metab.* (2009) 34:616–24. doi: 10.1139/H09-021

106. Hoffman MD, Snipe RMJ, Costa RJS. Ad libitum drinking adequately supports hydration during 2 h of running in different ambient temperatures. *Euro J Appl Physiol.* (2018) 118:2687–97. doi: 10.1007/s00421-018-3996-7

107. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol.* (1974) 37:247–8. doi: 10.1152/jappl.1974.37.2.247

108. Grames C, Berry-Cabán CS. Ischemic colitis in an endurance runner. *Case Rep Gastrointest Med.* (2012) 2012:356895. doi: 10.1155/2012/356895

109. Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. *Eur J Appl Physiol.* (2018) 118:389–400. doi: 10.1007/s00421-017-3781-z

110. Snipe RMJ, Costa RJS. Does biological sex impact intestinal epithelial injury, small intestine permeability, gastrointestinal symptoms and systemic cytokine profile in response to exertional-heat stress? *J Sports Sci.* (2018) 36:2827–35. doi: 10.1080/02640414.2018.1478612

111. Al-Saffar AK, Meijer CH, Gannavarapu VR, Hall G, Li Y, Diaz Tartera HO, et al. Parallel changes in harvey-bradshaw index, TNFα, and intestinal fatty acid binding protein in response to infliximab in Crohn's disease. *Gastroenterol Res Pract.* (2017) 2017:1745918. doi: 10.1155/2017/1745918

112. Haas V, Büning C, Buhner S, Von Heymann C, Valentini L, Lochs H. Clinical relevance of measuring colonic permeability. *Eur J Clin Invest.* (2009) 39:139–44. doi: 10.1111/j.1365-2362.2008.02075.x

113. Jekarl DW, Kim JY, Ha JH, Lee S, Yoo J, Kim M, et al. Diagnosis and prognosis of sepsis based on use of cytokines, chemokines, and growth factors. *Dis Markers.* (2019) 2019:1089107. doi: 10.1155/2019/1089107

114. Linsalata M, Riezzo G, D'attoma B, Clemente C, Orlando A, Russo F. Noninvasive biomarkers of gut barrier function identify two subtypes of patients suffering from diarrhoea predominant-IBS: a case-control study. *BMC Gastroenterol.* (2018) 18:167. doi: 10.1186/s12876-018-0888-6

115. Martinez-Fierro ML, Garza-Veloz I, Rocha-Pizaña MR, Cardenas-Vargas E, Cid-Baez MA, Trejo-Vazquez F, et al. Serum cytokine, chemokine, and growth factor profiles and their modulation in inflammatory bowel disease. *Medicine.* (2019) 98:e17208. doi: 10.1097/MD.0000000000017208

116. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin Chim Acta.* (2005) 352:15–35. doi: 10.1016/j.cccn.2004.09.001

117. Power N, Turpin W, Espin-Garcia O, Smith MI, Croitoru K. Serum zonulin measured by commercial kit fails to correlate with physiologic measures of altered gut permeability in first degree relatives of Crohn's disease patients. *Front Physiol.* (2021) 12:645303. doi: 10.3389/fphys.2021.645303

118. Surbatovic M, Veljovic M, Jevdjic J, Popovic N, Djordjevic D, Radakovic S. Immunoinflammatory response in critically ill patients: severe sepsis and/or trauma. *Mediators Inflamm.* (2013) 2013:362793. doi: 10.1155/2013/362793

119. Snipe RMJ, Costa RJS. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile? *J Sci Med Sport.* (2018) 21:771–6. doi: 10.1016/j.jsams.2017.12.014

120. Alcock R, Mccubbin A, Camões-Costa V, Costa RJS. Case study: providing nutritional support to an ultraendurance runner in preparation for a self-sufficient multistage ultramarathon: rationed versus full energy provisions. *Wilderness Environ Med.* (2018) 29:508–20. doi: 10.1016/j.wem.2018.06.004

121. Flood TR, Montanari S, Wicks M, Blanchard J, Sharp H, Taylor L, et al. Addition of pectin-alginate to a carbohydrate beverage does not maintain gastrointestinal barrier function during exercise in hot-humid conditions better than carbohydrate ingestion alone. *Appl Physiol Nutr Metab.* (2020) 45:1145–55. doi: 10.1139/apnm-2020-0118

122. Jonvik KL, Lenaerts K, Smeets JSJ, Kolkman JJ, VAN Loon LJC, et al. Sucrose but Not Nitrate Ingestion Reduces Strenuous Cycling-induced Intestinal Injury. *Med Sci Sports Exerc.* (2019) 51:436–44. doi: 10.1249/MSS.0000000000001800

123. Rehrer NJ, Goes E, Dugardeyn C, Reynaert H, Demeirleir K. Effect of carbohydrate on portal vein blood flow during exercise. *Int J Sports Med.* (2005) 26:171–6. doi: 10.1055/s-2004-820957

124. March DS, Marchbank T, Playford RJ, Jones AW, Thatcher R, Davison G. Intestinal fatty acid-binding protein and gut permeability responses to exercise. *Eur J Appl Physiol.* (2017) 117:931–41. doi: 10.1007/s00421-017-3582-4

125. Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profiles. *Int J Sports Med.* (2018) 39:255–63. doi: 10.1055/s-0043-122742

126. Scheffler L, Crane A, Heyne H, Tönjes A, Schleinitz D, Ihling CH, et al. Widely used commercial ELISA does not detect precursor of haptoglobin2, but recognizes properdin as a potential second member of the zonulin family. *Front Endocrinol.* (2018) 9:22. doi: 10.3389/fendo.2018.00022

127. Ajamian M, Steer D, Rosella G, Gibson PR. Serum zonulin as a marker of intestinal mucosal barrier function: may not be what it seems. *PLoS ONE.* (2019) 14:e0210728. doi: 10.1371/journal.pone.0210728

128. Horner KM, Schubert MM, Desbrow B, Byrne NM, King NA. Acute exercise and gastric emptying: a meta-analysis and implications for appetite control. *Sports Med.* (2015) 45:659–78. doi: 10.1007/s40279-014-0285-4

129. Bate JP, Irving PM, Barrett JS, Gibson PR. Benefits of breath hydrogen testing after lactulose administration in analysing carbohydrate malabsorption. *Eur J Gastroenterol Hepatol.* (2010) 22:318–26. doi: 10.1097/MEG.0b013e32832b20e8

130. Costa RJ, Snipe R, Camões-Costa V, Scheer V, Murray A. The impact of gastrointestinal symptoms and dermatological injuries on nutritional intake and hydration status during ultramarathon events. *Sports Med Open.* (2016) 2:16. doi: 10.1186/s40798-015-0041-9

131. Bengtsson M, Persson J, Sjölund K, Ohlsson B. Further validation of the visual analogue scale for irritable bowel syndrome after use in clinical practice. *Gastroenterol Nurs.* (2013) 36:188–98. doi: 10.1097/SGA.0b013e3182945881

132. Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterol.* (2006) 130:1377–90. doi: 10.1053/j.gastro.2006.03.008

133. Gaskell SK, Snipe RMJ, Costa RJS. Test-retest reliability of a modified visual analog scale assessment tool for determining incidence and severity of gastrointestinal symptoms in response to exercise stress. *Int J Sport Nutr Exerc Metab.* (2019) 29:411–9. doi: 10.1123/ijsnem.2018-0215

134. Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism and the immune system. *Nat Immunol.* (2013) 14:676–84. doi: 10.1038/ni.2640

135. Gnauck A, Lentle RG, Kruger MC. The characteristics and function of bacterial lipopolysaccharides and their endotoxic potential in humans. *Int Rev Immunol.* (2016) 35:189–218. doi: 10.3109/08830185.2015.1087518

136. Imhann F, Vich Vila A, Bonder MJ, Fu J, Gevers D, Visschedijk MC, et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut.* (2018) 67:108–19. doi: 10.1136/gutjnl-2016-312135

137. Van Der Beek CM, Dejong CHC, Troost FJ, Masclee AM, Lenaerts K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutr Rev.* (2017) 75:286–305. doi: 10.1093/nutrit/nuw067

138. Wong JM, De Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol.* (2006) 40:235–43. doi: 10.1097/00004836-200603000-00015

139. Sanchez JI, Marzorati M, Grootaert C, Baran M, Van Craeyveld V, Courtin CM, et al. Arabinoxylan-oligosaccharides (AXOS) affect the protein/carbohydrate fermentation balance and microbial population dynamics of the Simulator of Human Intestinal Microbial Ecosystem. *Microb Biotechnol.* (2009) 2:101–13. doi: 10.1111/j.1751-7915.2008.00064.x

140. Dalile B, Vervliet B, Bergonzelli G, Verbeke K, Van Oudenhove L. Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: a randomized, placebo-controlled trial. *Neuropsychopharmacol.* (2020) 45:2257–66. doi: 10.1038/s41386-020-0732-x

141. Gibson PR, Halmos EP, Muir JG. Review article: FODMAPS, prebiotics and gut health-the FODMAP hypothesis revisited. *Aliment Pharmacol Ther.* (2020) 52:233–46. doi: 10.1111/apt.15818

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 09 January 2023
DOI 10.3389/fnut.2022.1007725

OPEN ACCESS

EDITED BY
Mihnea-Alexandru Găman,
Carol Davila University of Medicine and Pharmacy, Romania

REVIEWED BY
Mohammad Amin Khazeei Tabari,
Mazandaran University of Medical Sciences, Iran
Victor Daniel Miron,
Carol Davila University of Medicine and Pharmacy, Romania
Veronica Mocanu,
Grigore T. Popa University of Medicine and Pharmacy, Romania

*CORRESPONDENCE
Aliakbar Sayyari
drsayyari@hotmail.com
Amir Hossein Hosseini
amir1981hosseini@gmail.com

SPECIALTY SECTION
This article was submitted to Sport and Exercise Nutrition, a section of the journal Frontiers in Nutrition

RECEIVED 30 July 2022
ACCEPTED 10 November 2022
PUBLISHED 09 January 2023

CITATION
Fatahi S, Alyahyawi N, Albadawi N, Mardali F, Dara N, Sohouli MH, Prabahar K, Rohani P, Koushki N, Sayyari A, Hosseini AH and Abu-Zaid A (2023) The association between vitamin D status and inflammatory bowel disease among children and adolescents: A systematic review and meta-analysis. *Front. Nutr.* 9:1007725. doi: 10.3389/fnut.2022.1007725

The association between vitamin D status and inflammatory bowel disease among children and adolescents: A systematic review and meta-analysis

Somaye Fatahi[1,2], Naseem Alyahyawi[3], Naryman Albadawi[4], Farzaneh Mardali[2], Naghi Dara[1], Mohammad Hassan Sohouli[5], Kousalya Prabahar[6], Pejman Rohani[7], Nazanin Koushki[2], Aliakbar Sayyari[1]*, Amir Hossein Hosseini[1]* and Ahmed Abu-Zaid[8]

[1]Pediatric Gastroenterology, Hepatology, and Nutrition Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2]Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Tehran, Iran, [3]Department of Pediatrics, King Abdulaziz University Hospital, Jeddah, Saudi Arabia, [4]Department of Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [5]Student Research Committee, Department of Clinical Nutrition and Dietetics, Faculty of Nutrition and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [6]Department of Pharmacy Practice, Faculty of Pharmacy, University of Tabuk, Tabuk, Saudi Arabia, [7]Pediatric Gastroenterology and Hepatology Research Center, Pediatrics Centre of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran, [8]College of Medicine, Alfaisal University, Riyadh, Saudi Arabia

Aim: Vitamin D deficiency is very common among children with IBD. Since there are conflicting results regarding the association of vitamin D with IBD, we conducted this systematic review to confirm the association of vitamin D with IBD.

Methods: We conducted a systematic search in Scopus, Cochrane Library, Web of Science, PubMed, and Google Scholar to find relevant studies. Articles with cross-sectional and case-control designs that reported the association between vitamin D and IBD among children were included.

Results: Eventually, 9 studies (with 16 effect sizes) reported the mean and SD or the median and the interquartile range of serum vitamin D levels in both subjects with IBD and control subjects. The random effects meta-analysis revealed that subjects with IBD had −1.159 ng/ml (95% CI: −2.783, 0.464) lower serum vitamin D concentrations compared with their healthy counterparts, but this difference was not significant. A total of 14 studies (with 18 effect sizes) with 2,602 participants provided information for the prevalence of vitamin D deficiency or insufficiency in patients with IBD as 44% (95% CI: 0.34–0.54) with significant heterogeneity noted among studies ($p < 0.001$; $I^2 = 97.31\%$).

Conclusion: This systematic and meta-analysis study revealed that vitamin D deficiency was associated with IBD. Longitudinal studies should be conducted

in the future to confirm our findings. Large randomized controlled trials assessing the doses of supplementation of vitamin D would provide a better understanding of the association between vitamin D and IBD.

KEYWORDS

vitamin D, inflammatory bowel disease (IBD), children, systematic review, supplement

Introduction

Inflammatory bowel disease (IBD) includes both ulcerative colitis (UC) and Crohn's disease (CD). IBD is a systemic, chronic disease. UC is confined to the rectum and/or the colon, whereas CD involves the entire gastrointestinal tract, with the most common occurrence in the ileum and the colon (1). Overall, the pediatric prevalence of IBD increased by 133% from 2007 to 2016 in the United States, and the subgroup of children aged 10–17 years was the major contributor to the rising pediatric IBD prevalence (2). IBD presents differently in adults and children. For example, CD is more prevalent in pediatrics when compared to UC. This is completely different from that of adult IBD (3). Even among the two age groups, the disease characteristics differ. The complications are more in pediatric IBD compared to adults (4).

The exact etiology of IBD may be attributed to changes in the intestinal flora, residing in urban areas, and diets having high amounts of fats and carbohydrates (5). Various studies reported the role of vitamin D in IBD (6, 7). Vitamin D deficiency may lead to a reduction in bacterial clearance in the colon (8). This vitamin changes the immune responses by influencing macrophages and T lymphocytes, hence avoiding excessive immune responses, and also repairs the intestinal mucosal barrier (9, 10).

Low vitamin D levels are more common in patients with IBD (11). However, it is not specific whether low vitamin D levels are related to malabsorption due to damage in the intestinal mucosa (12). Some observational studies reported low vitamin levels in patients with IBD (13, 14), and some studies reported a lack of decrease in vitamin D levels (15, 16). Vitamin D deficiency is very common among children with CD. Even osteoporosis and growth retardation are commonly noticed in CD when compared to UC (17). Similar to our study, a study was conducted previously by Del Pinto et al. (18) in 2015, considering all age groups. However, focusing on the age group of children and adolescents is necessary due to their critical age for growth and development. In addition, no significant results were obtained in that subgroup analysis due to the limitations of studies related to children and adolescents. Since there is no clear evidence of a direct association between vitamin D and IBD among children and adolescents, we conducted this systematic review to confirm the association of vitamin D with IBD.

Materials and methods

In this systematic review and meta-analysis, we investigated the data extracted based on components of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (19) and MOOSE (Meta-Analyses of Observational Studies in Epidemiology) checklist (20) on the relationship between serum vitamin D and inflammatory bowel disease in pediatric patients.

Search strategy and selection process

A structural and comprehensive literature was accomplished based on articles published up to 20 February (year) in the following electronic databases: Scopus, Cochrane Library, Web of Science, PubMed, and Google Scholar. The search used the terms "vitamin D," "ergocalciferol," "inflammatory bowel disease," "Crohn's disease," and "ulcerative colitis" in several combinations. The full electronic search strategy is reported in Supplementary Table 1. There were no date or language restrictions on imported articles. Moreover, all clinical trial and review article references were checked to find any relevant studies. After deleting duplicate articles, the title and abstract screening processes were performed. Subsequently, two researchers studied the full-text papers independently to find all the obligatory data for meta-analysis. The third researcher was called to resolve any discrepancies. Only articles that met the following criteria were included in this meta-analysis:

a) Observational studies—either case-control, cross-sectional, or cohort designs,
b) Studies with adolescents or children (age <18 years),
c) Articles that showed mean and standard deviation (SD) or prevalence of insufficiency or deficiency of serum vitamin D levels in patients with IBD (Crohn's disease or ulcerative colitis).

Papers with (1) randomized clinical trial designs, (2) animal-based studies, (3) gray literature (chapters of books, abstracts in conferences, or review articles), (4) adult patients, (5) athletics, and (6) assessment of IBD in the acute phase were excluded from the analysis.

Data extraction and syntheses

Required information of the eligible papers was extracted by two observers, such as the author's last name, publication date, the study location, study design, mean age of cases and controls, the number of cases and controls, type of study population (UC or CD), mean and SD of vitamin D concentration in patients and healthy participants or prevalence of vitamin D deficiency or insufficiency status, seasonally matching or adjusting, and method of vitamin D assessment.

Quality assessments

The quality of the chosen papers in this meta-analysis was assessed using the Newcastle-Ottawa scale (21), which included 9 questions in three main sections such as selection of participants (0–4 points), comparability between groups (0–3 points), and outcome assessment (0–3 points). The quality evaluation process was reviewed by two investigators, and any differences in scoring were resolved by consensus.

Statistical analyses

All data analyses were performed using the Stata 13 software (Stata Corp., College Station, Texas, USA). Data on vitamin D levels in patients with IBD in case-control studies were reported as weighted mean differences and SD or prevalence of deficiency or insufficiency in cross-sectional articles. The studies were conducted in different populations and countries, so the random-effects analysis was used to control variation. To determine the heterogeneity, the I-square (I^2) index was assessed by random-effects analysis, and high heterogeneity was described if I^2 was more than 50%. A sensitivity analysis was conducted to find out which study had the highest proportion in the pool effect size. Afterward, Egger's regression model was used to examine the publication bias in the funnel plots. Due to the high heterogeneity between records, we decided to carry out the subgroup analysis to distinguish the possible reasons for high heterogeneity and the effect of variant agents on the relationship between serum levels of vitamin D and IBD. These subgroups were based on assessing vitamin D methods such as HPLC (high-performance liquid chromatography), RIA (radioimmunoassay), and chemiluminescence-based competitive protein-binding assay and evaluating matching the season to vitamin D concentration (yes, matched; NR, not reported).

Literature review

The main characteristics of case-control and cross-sectional studies are presented in Table 1 and Figure 1. Approximately 3,564 articles were searched electronically; 1,579 duplicate items were removed in the screening process, and 1,936 articles were eliminated as they had no relation to the purpose of the study. In addition, 14 articles did not meet the qualitative and quantitative criteria given in this study. Notably, a related cohort study was identified, which was omitted due to the format of the analysis, so we described it in the discussion part. Eventually, 9 articles (16 effect sizes) with a case-control design declared the concentration of vitamin D in children with a mean (SD), and 14 studies (with 18 effect sizes) declared the prevalence of vitamin D deficiency or insufficiency in pediatrics. These studies were published between 2003 and 2019, and most of the records ($n = 11$) were conducted in the United States (22, 25, 29–31, 33–36, 38, 39, 42, 43) and the rest in Canada (23, 40), Australia (32), Finland (24), Denmark (26), Italy (28), South Korea (27, 41), and Israel (37). In general, we entered data of 4,803 children participants with an age range of 2–18 years. Based on the methodological assay, the quality score was at least 6 points for the articles, and the majority gained high-quality points. Notably, the vitamin D measurement seasons were different in the studies, and some did not report any data, and some case-control studies presented season matching or adjustment. In addition, various measurement methods have been applied; some studies used HPLC (high-performance liquid chromatography) (24, 43) or RIA (radioimmunoassay) (29) and others applied chemiluminescence-based competitive protein-binding assays (23, 28, 30, 32, 35, 36, 39, 44) to measure the concentration of vitamin D.

Meta-analysis

Eventually, 16 studies reported the mean and SD or median and interquartile range of serum vitamin D levels in both IBD and control subjects. The random effects meta-analysis revealed that subjects with IBD had −1.159 ng/ml (95% CI: −2.783, 0.464) lower serum vitamin D concentrations compared with their healthy counterparts, but this difference was not significant (Figure 2). However, the tests also showed that there was significant heterogeneity among the studies ($I^2 = 74.0\%$, $P < 0.001$). Heterogeneity in meta-analysis refers to the variation in study results between studies. To find out the source of heterogeneity, we conducted studies based on the following subgroups: type of patients (IBD, UC, CD, or IC), assessment method of 25OHD, and matching of the season for the control group. In the subgroup analysis, results remained non-significant, while the types of patients (IBD, UC, and CD) and assessment method of 25OHD were considered as possible sources of heterogeneity (Supplementary Figures 1–3).

The prevalence of vitamin D deficiency or insufficiency in patients with IBD was found as 44% (95% CI: 0.34–0.54) by eighteen studies with a total of 2,602 participants,

TABLE 1 Characteristics of studies.

No	References	Country	Study Design	Population of study	Mean age, y	Total N	N case	Mean/ sdcase (ng/ml)	N control	Mean/sd control (ng/ml)	Deficiency or insufficiency status	Season adjust/ matching	Season of measurement	Q	Method of vitamin D assessing
1	Nwosu (22)	USA	Case-control	CD	8.5		25	28.24+10.28	49	26.16 + 10.44		NR/yes	Winter + spring,14/25 (56)		DiaSorin Liaison
2	El-Matary et al., (23)	Canada	Case-control	CD	12.2		39	26.68 + 10.2	56	32.68 + 6.16		NR/yes	NR		Protein binding assay after column chromatography (Esoterix
3	El-Matary et al. (23)	Canada	Case-control	UC	12.2		21	22.76 + 8.8	56	32.68 + 6.16		NR/yes	NR		
4	Laakso et al. (24)	Finland	Case-control	IBD	14.9		80	21.6 + 25.48	80	18.5 + 19.25		NR/ NR	NR		(HPLC)
5	Alkhouri et al. (15)	USA	Case-control	CD	12.3		46	29.9 + 12.7	61	26.7 + 9.4		NR/ NR	Is less vitamin D deficiency in the summer months as compared with		(HPLC)
6	Alkhouri et al. (15)	USA	Case-control	UC	12.3		12	32 + 25.8	61	26.7 + 9.4		NR/ NR	Spring (P¼0.08), autumn (P¼0.15), and winter (P¼0.08).		
7	Alkhouri et al. (15)	USA	Case-control	ID	12.3		3	21.7 + 7.5	61	26.7 + 9.4		NR/ NR	Spring (P¼0.08), autumn (P¼0.15), and winter (P¼0.08).		
8	Prosnitz (Black participants) (25)	USA	Case-control	CD	13.5		8	10.5 + 4.6	62	15.8 + 7.9					Radioimmunoassay (RIA) with I125-labeled tracer
9	Prosnitz (Non-black participants) (25)	USA	Case-control	CD	13.5		70	23.5 + 0.2	159	25.3 + 8.7		Yes/NR	Winter		
10	Veit et al. (16)	USA	Case-control	IBD	16.4 Control (14.6)		58	24 + 10.24	116	24 + 10.88		Yes/yes	Summer–fall, %:57.6		DiaSorin Liaison

(Continued)

10.3389/fnut.2022.1007725

TABLE 1 (Continued)

No	References	Country	Study Design	Population of study	Mean age, y	Total N	N case	Mean/ sdcase (ng/ml)	N control	Mean/sd control (ng/ml)	Deficiency or insufficiency status	Season adjust/ matching	Season of measurement	Q	Method of vitamin D assessing
11	Thorsen et al. (26)	Denmark	Case-control	CD	14		155	11.2 + 6.54	384	10.68 + 6.9		NR/Yes	All		Liquid chromatography mass spectrometry (LC-MS)
12	Thorsen et al. (26)	Denmark	Case-control	UC	15		210	10.92 + 7.08	384	10.68 + 6.9		NR/Yes	All		
13	Thorsen et al. (26)	Denmark	Case-control	IC	11		19	12.72 + 8.68	384	10.68 + 6.9		NR/Yes	All		
14	Sohn et al. (27)	Korea	Case-control	CD	14.4		43	16.3 + 9.3	45	16.5 + 5.3		NR/NR	NR		Liquid chromatography mass spectrometry (LC-MS)
15	Sohn et al. (27)	Korea	Case-control	UC	14.4		17	19.9 + 7.2	45	16.5 + 5.3		NR/NR	NR		
16	Strisciuglio et al. (28)	Italy	Case-control	IBD	11 (Control: 9.4)		33	19.2 + 9.7	18	28.2 + 12.1		NR/Yes	Winter-spring		Enzyme-linked immunosorbent
17	Sentongo et al. (29)	Philadelphia	Cross-sectional	CD	15.76	113					16%<20 ng/ml Hypovitaminosis< 38 nmol/L.				Radioimmunoassay with a radioiodinated tracer
18	Pappa et al. (30)	USA	Cross-sectional	IBD	15.76	130					34.6%<15 ng/ml 10.8%: 25OHD ≤8				Nichols Advantage chemiluminescence-based competitive protein-binding assay (Nichols Institute Diagnostics,San Clemente, CA

(Continued)

10.3389/fnut.2022.1007725

TABLE 1 (Continued)

No	References	Country	Study Design	Population of study	Mean age, y	Total N	N case	Mean/ sdcase (ng/ml)	N control	Mean/sd control (ng/ml)	Deficiency or insufficiency status	Season adjust/ matching	Season of measurement	Q	Method of vitamin D assessing
19	Pappa et al. (30)	USA	Cross-sectional	CD	15.76	94					38.3%<15 ng/ml 12.8%: 25OHD ≤8				Nichols Advantage chemiluminescence-based competitive protein-binding assay (Nichols Institute Diagnostics,San Clemente, CA
20	Pappa et al. (30)	USA	Cross-sectional	UC	15.76	36					25%<15 ng/ml 5.6 %: 25OHD ≤8				Nichols Advantage chemiluminescence-based competitive protein-binding assay (Nichols Institute Diagnostics,San Clemente, CA
21	Salehi et al. (31)	USA	Cross-sectional	IBD	NR	110					63.6% < 20 ng/ml < 50 nmol/l				NR
22	Levin et al. (32)	Australia	Cross-sectional	IBD	12.6	263					19: 20–30 ng/ml 51–75 nmol/l				Antibody-based chemiluminescence assay
23	Levin et al. (32)	Australia	Cross-sectional	IBD	12.6	263					38 < 20 ng/ml Defficiency<51 nmol/l				Antibody-based chemiluminescence assay
24	Goldberg et al. (33)	USA	Cross-sectional	UC	2-21	24					80 < 20 ng/ml Insufficiency 20–30 nmol/L				NR

(Continued)

10.3389/fnut.2022.1007725

TABLE 1 (Continued)

No	References	Country	Study Design	Population of study	Mean age, y	Total N	N case	Mean/ sdcase (ng/ml)	N control	Mean/sd control (ng/ml)	Deficiency or insufficiency status	Season adjust/ matching	Season of measurement	Q	Method of vitamin D assessing
25	Goldberg et al. (33)	USA	Cross-sectional	CD	2-21	52					60 < 20 ng/ml Insufficiency 20–30 nmol/L				NR
26	Schaefer et al. (34)	USA	Cross-sectional	IBD	NR	92					<30 ng/mL <30 ng/mL				NR
27	Syed et al. (35)	USA	Cross-sectional	IBD	5-18.9	69					77 20–30 ng/ml Insufficiency< 30 ng/mL				Automated chemiluminescent technique (Automated IDS-iSYS System, Immunodiagnostic Systems, Fountain Hills, AZ)
28	Syed et al. (36)	USA	Cross-sectional	IBD	5-18.9	69					38 < 20 ng/ml Deficiency< 20 ng/ml				Automated chemiluminescent technique (Automated IDS-iSYS System, Immunodiagnostic Systems, Fountain Hills, AZ)
29	Brandvayman et al. (37)	Israeil	Cross-sectional	IBD	14	623					21 < 20 ng/ml defficiency				NR
30	Winter et al. (38)	USA	Cross-sectional	IBD		203					31 < 20 ng/ml LOW < 9 ng/ml				NR
31	Sauer et al. (39)	USA	Cross-sectional	UC	12.7	388					57 20–30 ng/ml insufficient < 30 ng/Ml				IDS-iSYS 25-Hydroxyvitamin D automated chemiluminescense immunoassay (Gaithersburg, MD)

(Continued)

10.3389/fnut.2022.1007725

TABLE 1 (Continued)

No	References	Country	Study Design	Population of study	Mean age, y	Total N	N case	Mean/ sdcase (ng/ml)	N control	Mean/sd control (ng/ml)	Deficiency or insufficiency status	Season adjust/ matching	Season of measurement	Q	Method of vitamin D assessing
32	Mager et al. (40)	Canada	Cross-sectional	CD	NR	102					56.80% 20–30 ng/ml insufficiency< 50 nmol/l				Liquid chromatogrpahy-mass spectroscopy (LC-MS) technology
33	Mager et al. (40)	Canada	Cross-sectional	UC	NR	63					43.20% 20–30 ng/ml insufficiency< 50 nmol/l				Liquid chromatogrpahy-mass spectroscopy (LC-MS) technology
34	Kim et al. (41)	Korea	Cross-sectional	IBD	13.1	96					56.80% 20–30 ng/ml insufficiency<50 nmol/l				Liquid chromatogrpahy-mass spectroscopy (LC-MS) technology

10.3389/fnut.2022.1007725

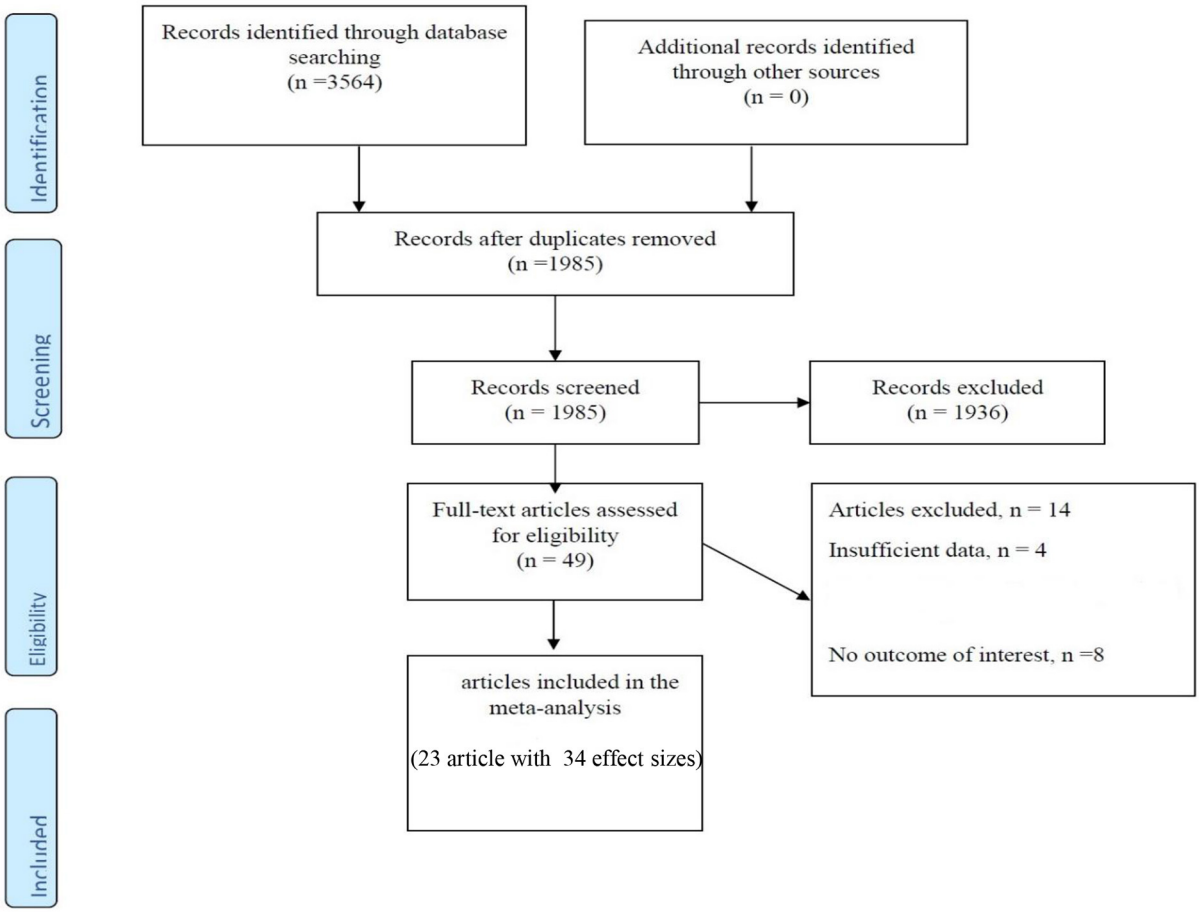


FIGURE 1
Flow chart of the included studies, including identification, screening, eligibility and the final sample included.

with significant heterogeneity noted among studies ($p < 0.001$; $I^2 = 97.31\%$). The pooled prevalence of vitamin D deficiency or insufficiency in patients with IBD was 42% (95% CI: 0.29–0.56), UC was 51% (95% CI: 0.33–0.68), and CD was 42% (95% CI: 0.25–0.58) (Figure 3). In addition, the prevalence of participants with a level of vitamin D < 20 ng/ml was 41% (95% CI: 0.30–0.52) and the level of 20–30 ng/ml was 50% (95% CI: 0.32–0.69) (Figure 4).

Publication bias and sensitivity analysis

Evaluation of publication bias by visual inspection of the funnel plot and Egger's test demonstrated the evidence for publication bias in the meta-analysis of the prevalence of vitamin D deficiency or insufficiency in patients with IBD ($p = 0.05$) (Supplementary Figure 4). However, the results of the meta-trim-and-fill analysis found no studies. Egger's linear regression test for case-control studies revealed no publication bias ($p = 0.31$) (Supplementary Figure 5). Sensitivity analysis revealed that removing some of the studies would not have a significant impact on the overall results (Supplementary Figures 6, 7).

Discussion

This systematic review and meta-analysis were conducted using 35 articles. Our study results showed that vitamin D is deficient in children with IBD when compared to the healthy control group. But the results were not statistically significant. These results were similar to another study. A meta-analysis was conducted, including 14 studies of 1,891 patients, and reported that patients with IBD had more vitamin D deficiency when compared to controls, and the results were statistically significant (18). Vitamin D regulates

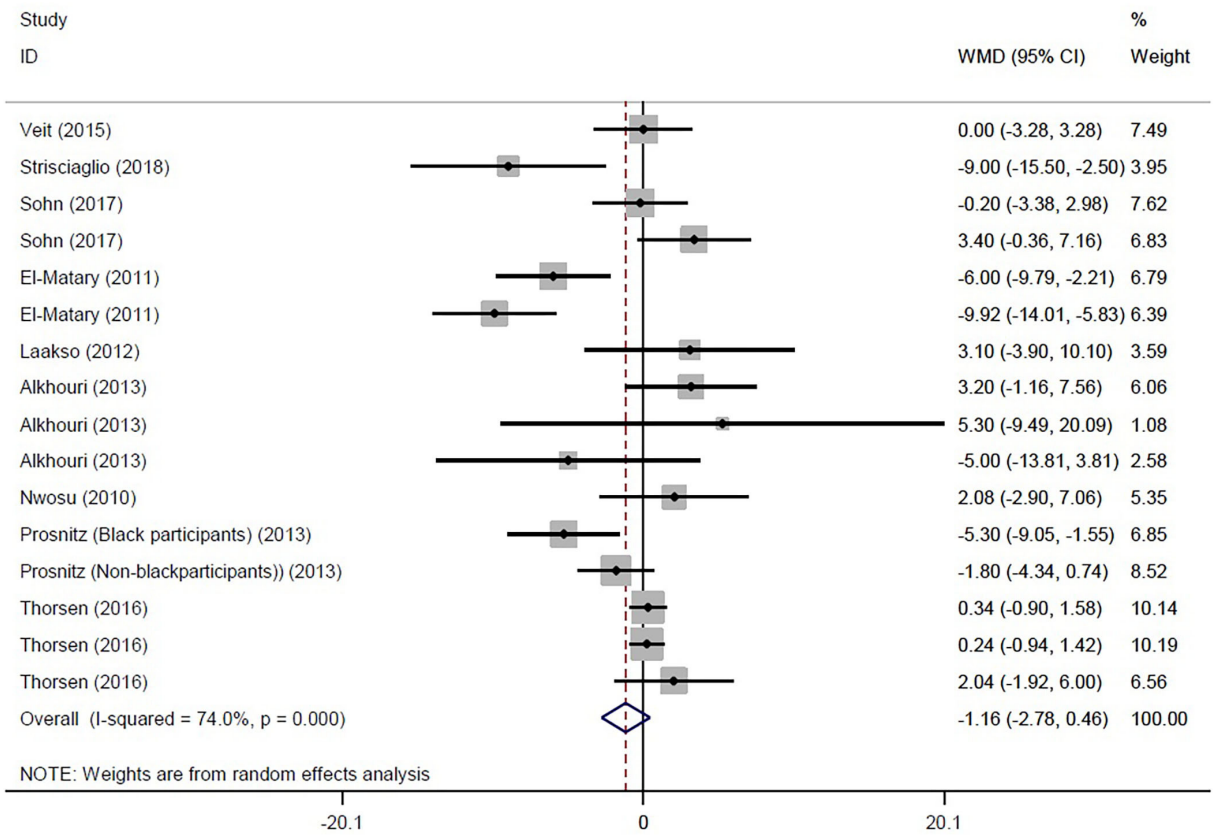


FIGURE 2
Forest plot show the WMD in serum vitamin D concentrations between participants with IBD and healthy control.

cytokine secretion, which is involved in the inflammatory response in immune systems (45). Many studies that were conducted previously have reported that the levels of vitamin D are lower in children with IBD (23, 46). Even there are contradictory results regarding the association of vitamin D with IBD (15). Sufficient vitamin D has been associated with clinical benefits in children with IBD. There was a reduction in disease activity in children with sufficient vitamin D (41). Guzman-Prado et al. also showed that vitamin D supplementation in patients with IBD and vitamin D deficiency is effective at correcting vitamin D levels and is associated with improvements in clinical and biochemical disease activity scores (47).

The subgroup analysis of our study did not reveal a statistically significant deficiency in vitamin D concentrations. The same results were obtained in a study conducted by Kim (41). El-Matary et al. (23) reported that the levels of vitamin D were lower in children with UC, but it was not statistically significant. Another study reported that the levels of vitamin D were higher in children with CD, and it was statistically significant (48). Sensitivity analysis revealed that removing some of the studies would not have a significant impact on the overall results. The quality of all the studies was assessed using the Newcastle-Ottawa scale.

The scientific evidence on the role of vitamin D in children with IBD was reviewed by studies conducted previously (6, 49–51). Although, the effective vitamin D supplementation in children with IBD remains controversial. Our meta-analysis evaluating vitamin D status in 2602 participants, a large cohort population, would provide consistent and useful results.

A low level of vitamin D is clinically significant. Both UC and CD are related to environmental factors (52). Hypovitaminosis D in IBD is related to malabsorption, which may be due to inflammation in the bowel or surgical resection (12); less exposure to sunlight (50); and higher intake of vitamin D by the inflammatory cells (53).

In our study, the prevalence of vitamin D deficiency or insufficiency in patients with IBD was 44%. The pooled prevalence of vitamin D deficiency or insufficiency in patients with IBD was 42%, UC was 51%, and CD was 42%. In addition, the prevalence of participants with a level of vitamin D of $<$ 20 ng/ml was 41%

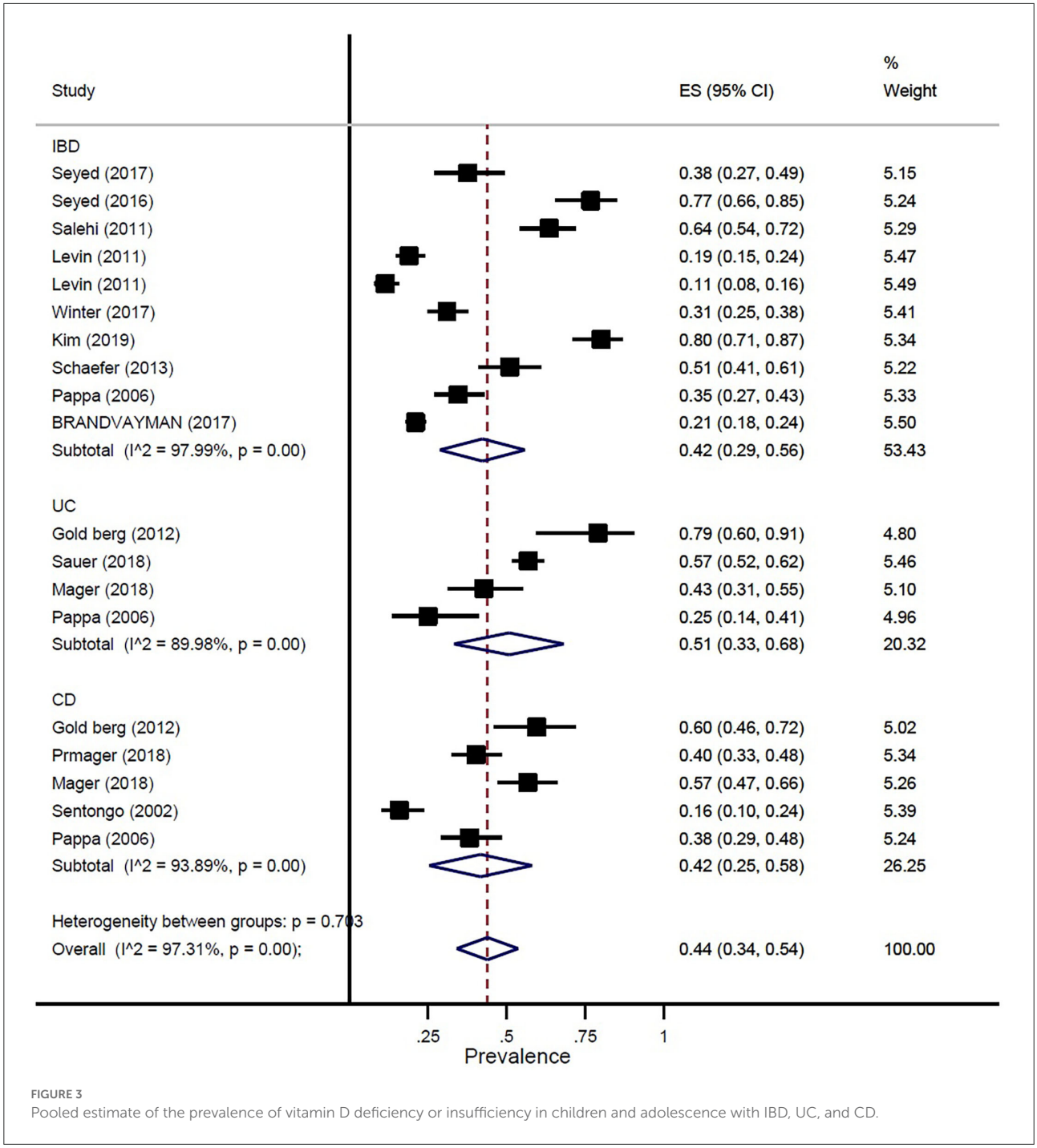


FIGURE 3
Pooled estimate of the prevalence of vitamin D deficiency or insufficiency in children and adolescence with IBD, UC, and CD.

and the level of vitamin D of 20–30 ng/ml was 50%. Our study results confirmed prior study findings that reported a high prevalence of vitamin D deficiency in IBD (13, 29).

There are a few major strengths of the present systematic review and meta-analysis. First, a large number of studies were included, which provided a large sample size. In addition, we successfully pooled the results of subgroup analyses, sensitivity analyses, and publication bias. Similarly, there are some limitations in our study that should be considered. First, there existed a substantial degree of heterogeneity in the designs of studies, with a wide range of methods of vitamin D assessment. Nonetheless, we performed subgroup analysis to find possible sources of heterogeneity. Another important limitation of this study is that we could not identify the relationship between the season of vitamin D level

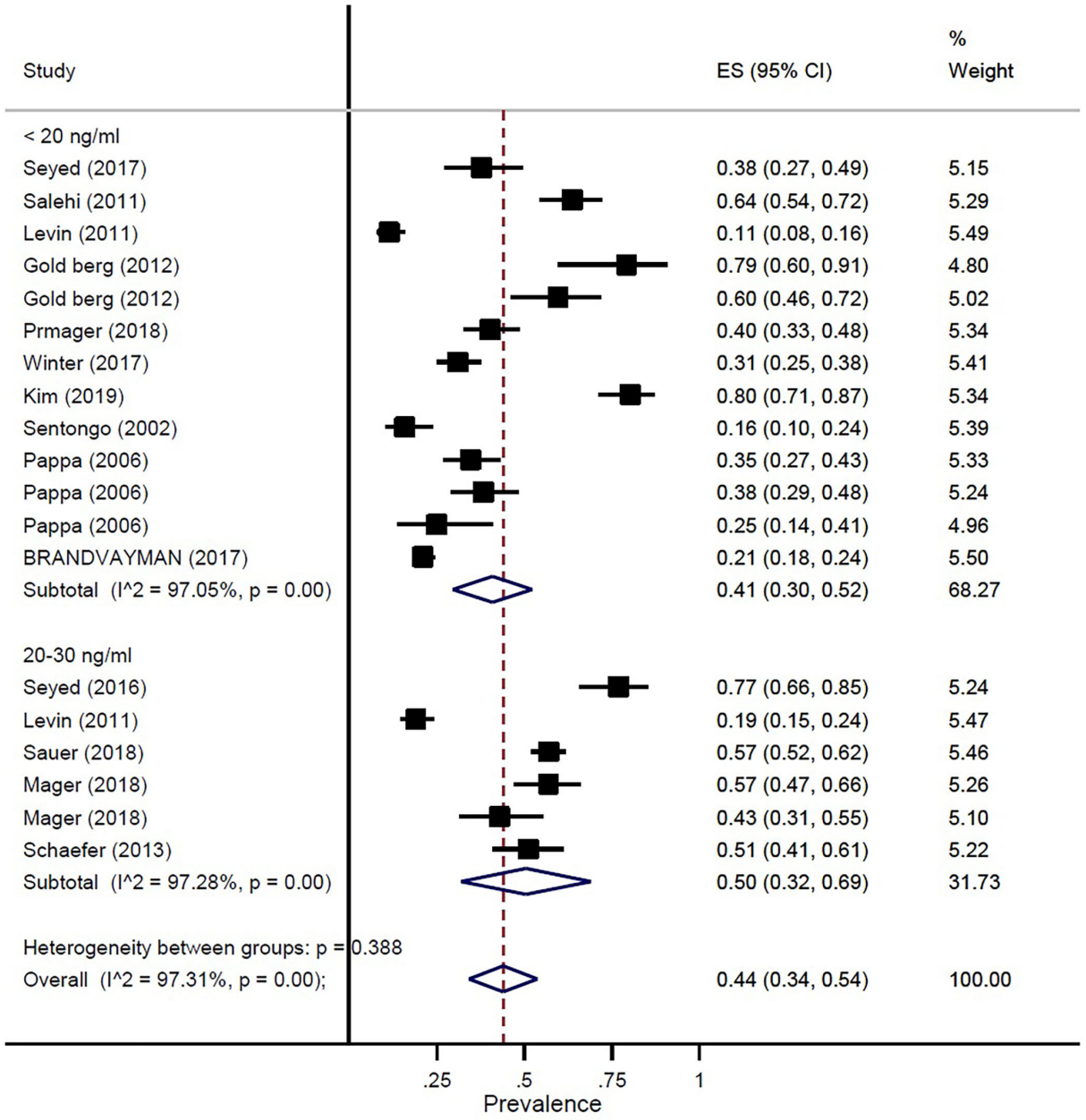


FIGURE 4
Pooled estimate of the prevalence of vitamin D status in children and adolescence with IBD.

measurement and IBD because many studies did not report this variable well.

Conclusion

This systematic review and meta-analysis study showed that patients with IBD were associated with vitamin D deficiency. Longitudinal studies should be conducted in the future to confirm our findings. Large randomized controlled trials assessing the doses of vitamin D supplementation would provide a better understanding of the association between vitamin D and IBD.

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 authors.

Author contributions

SF, NAly, NAlb, FM, and MS contributed to the conception, design, and statistical analysis. SF, KP, NAly, NAlb, FM, PR, NK, AH, and AS contributed to data collection and the manuscript draft. AS, AH, and AA-Z supervised the study. All authors contributed the manuscript draft, critical revision, and approved the final version of the manuscript.

Funding

This article is taken from the disease registry, titled Registration of inflammatory bowel diseases in children and with code number IR.SBMU.RETECH.REC.1397.1199 from the ethics committee, which was supported by the deputy of research and technology at Shahid Beheshti University of Medical Sciences (http://dregistry.sbmu.ac.ir).

Acknowledgments

We express our gratitude to the participants of this study.

Conflict of interest

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

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1007725/full#supplementary-material

References

1. Burisch J, Munkholm P. Inflammatory bowel disease epidemiology. *Curr Opin Gastroenterol.* (2013) 29:357–62. doi: 10.1097/MOG.0b013e32836229fb

2. Ye Y, Manne S, Treem WR, Bennett D. Prevalence of inflammatory bowel disease in pediatric and adult populations: recent estimates from large national databases in the United States, 2007–2016. *Inflamm Bowel Dis.* (2020) 26:619–25. doi: 10.1093/ibd/izz182

3. Ruel J, Ruane D, Mehandru S, Gower-Rousseau C, Colombel J-F, IBD. across the age spectrum—is it the same disease? *Nat Rev Gastroenterol Hepatol.* (2014) 11:88–98. doi: 10.1038/nrgastro.2013.240

4. Gower-Rousseau C, Vasseur F, Fumery M, Savoye G, Salleron J, Dauchet L, et al. Epidemiology of inflammatory bowel diseases: new insights from a French population-based registry (EPIMAD). *Digest Liver Dis.* (2013) 45:89–94. doi: 10.1016/j.dld.2012.09.005

5. Ananthakrishnan AN. Environmental risk factors for inflammatory bowel diseases: a review. *Dig Dis Sci.* (2015) 60:290–8. doi: 10.1007/s10620-014-3350-9

6. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1, 25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr.* (2000) 130:2648–52. doi: 10.1093/jn/130.11.2648

7. Di Rosa M, Malaguarnera G, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immuno-modulatory effects of vitamin D3 in human monocyte and macrophages. *Cell Immunol.* (2012) 280:36–43. doi: 10.1016/j.cellimm.2012.10.009

8. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyang Y, et al. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. *Endocrinology.* (2010) 151:2423–32. doi: 10.1210/en.2010-0089

9. Hewison M. Vitamin D and immune function: an overview. *Proc Nutr Soc.* (2012) 71:50–61. doi: 10.1017/S0029665111001650

10. Zhao H, Zhang H, Wu H, Li H, Liu L, Guo J, et al. Protective role of 1, 25 (OH) 2vitamin D3 in the mucosal injury and epithelial barrier disruption in DSS-induced acute colitis in mice. *BMC Gastroenterol.* (2012) 12:1–14. doi: 10.1186/1471-230X-12-57

11. Abraham BP, Prasad P, Malaty HM. Vitamin D deficiency and corticosteroid use are risk factors for low bone mineral density in inflammatory bowel disease patients. *Dig Dis Sci.* (2014) 59:1878–84. doi: 10.1007/s10620-014-3102-x

12. Farraye F, Nimitphong H, Stucchi A, Dendrinos K, Boulanger A, Vijjeswarapu A, et al. Use of a novel vitamin D bioavailability test demonstrates that vitamin D absorption is decreased in patients with quiescent Crohn's disease. *Inflamm Bowel Dis.* (2011) 17:2116–21. doi: 10.1002/ibd.21595

13. Bruyn JR, Ponsioen CY, Brink GRvd, Löwenberg M, Bredenoord AJ, et al. Vitamin D deficiency in Crohn's disease and healthy controls: a prospective case–control study in the Netherlands. *J Crohn's Colitis.* (2014) 8:1267–73. doi: 10.1016/j.crohns.2014.03.004

14. Jørgensen SP, Hvas CL, Agnholt J, Christensen LA, Heickendorff L, Dahlerup JF. Active Crohn's disease is associated with low vitamin D levels. *J Crohn's Colitis.* (2013) 7:e407–e13. doi: 10.1016/j.crohns.2013.01.012

15. Alkhouri RH, Hashmi H, Baker RD, Gelfond D, Baker SS. Vitamin and mineral status in patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* (2013) 56:89–92. doi: 10.1097/MPG.0b013e31826a105d

16. Veit LE, Maranda L, Fong J, Nwosu BU. The vitamin D status in inflammatory bowel disease. *PLoS ONE.* (2014) 9:e101583. doi: 10.1371/journal.pone.0101583

17. Harries A, Brown R, Heatley R, Williams L, Woodhead S, Rhodes J. Vitamin D status in Crohn's disease: association with nutrition and disease activity. *Gut.* (1985) 26:1197–203. doi: 10.1136/gut.26.11.1197

18. Del Pinto R, Pietropaoli D, Chandar AK, Ferri C, Cominelli F. Association between inflammatory bowel disease and vitamin D deficiency: a systematic review and meta-analysis. *Inflamm Bowel Dis.* (2015) 21:2708–17. doi: 10.1097/MIB.0000000000000546

19. Selçuk AA, A. guide for systematic reviews: PRISMA. *Turkish Arch Otorhinolaryngol.* (2019) 57:57. doi: 10.5152/tao.2019.4058

20. Brooke BS, Schwartz TA, Pawlik TM, MOOSE. reporting guidelines for meta-analyses of observational studies. *JAMA Surg.* (2021) 156:787–8. doi: 10.1001/jamasurg.2021.0522

21. Margulis AV, Pladevall M, Riera-Guardia N, Varas-Lorenzo C, Hazell L, Berkman ND, et al. Quality assessment of observational studies in a drug-safety

systematic review, comparison of two tools: The Newcastle-Ottawa scale and the RTI item bank. *Clin Epidemiol.* (2014) 6:359. doi: 10.2147/CLEP.S66677

22. Nwosu BU, Maranda L. Vitamin D status and adiposity in pediatric malabsorption syndromes. *Digestion.* (2010) 92:1–7. doi: 10.1159/000381895

23. El-Matary W, Sikora S, Spady D. Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease. *Dig Dis Sci.* (2011) 56:825–9. doi: 10.1007/s10620-010-1380-5

24. Laakso S, Valta H, Verkasalo M, Toiviainen-Salo S, Viljakainen H, Mäkitie O. Impaired bone health in inflammatory bowel disease: a case–control study in 80 pediatric patients. *Calcif Tissue Int.* (2012) 91:121–30. doi: 10.1007/s00223-012-9617-2

25. Prosnitz AR, Leonard MB, Shults J, Zemel BS, Hollis BW, Denson LA, et al. Changes in vitamin D and parathyroid hormone metabolism in incident pediatric Crohn's disease. Inflamm Bowel Dis. (2013) 19:45–53. doi: 10.1002/ibd.22969

26. Thorsen SU, Jakobsen C, Cohen A, Lundqvist M, Thygesen LC, Pipper C, et al. Perinatal vitamin D levels are not associated with later risk of developing pediatric-onset inflammatory bowel disease: a Danish case-cohort study. *Scand J Gastroenterol.* (2016) 51:927–33. doi: 10.3109/00365521.2016.1144218

27. Sohn J, Chang EJ, Yang HR. Vitamin D status and bone mineral density in children with inflammatory bowel disease compared to those with functional abdominal pain. *J Korean Med Sci.* (2017) 32:961–7. doi: 10.3346/jkms.2017.32.6.961

28. Strisciuglio C, Cenni S, Giugliano FP, Miele E, Cirillo G, Martinelli M, et al. The role of Inflammation on Vitamin D levels in a cohort of pediatric patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* (2018) 67:501–6. doi: 10.1097/MPG.0000000000002049

29. Sentongo TA, Semaeo EJ, Stettler N, Piccoli DA, Stallings VA, Zemel BS. Vitamin D status in children, adolescents, and young adults with Crohn disease. *Am J Clin Nutr.* (2002) 76:1077–81. doi: 10.1093/ajcn/76.5.1077

30. Pappa HM, Grand RJ, Gordon CM. Report on the vitamin D status of adult and pediatric patients with inflammatory bowel disease and its significance for bone health and disease. *Inflamm Bowel Dis.* (2006) 12:1162–74. doi: 10.1097/01.mib.0000236929.74040.b0

31. Salehi V, Small L, Yau M, Sockolow R. Vitamin D deficiency and abnormal bone density in pediatric patients with Inflammatory Bowel Disease. *Inflam Bowel Dis.* (2011) 17:S61–S. doi: 10.1097/00054725-201112002-00194

32. Levin AD, Wadhera V, Leach ST, Woodhead HJ, Lemberg DA, Czarina Mendoza-Cruz A, et al. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci.* (2011) 56:830–6. doi: 10.1007/s10620-010-1544-3

33. Goldberg L, Yau M, Salehi V, Sockolow RE, Ward MJ, Antal Z. Small bowel disease in children and adolescents with inflammatory bowel disease is associated with deteriorating vitamin D status. *Gastroenterology.* (2012) 142:S370–S1. doi: 10.1016/S0016-5085(12)61399-7

34. Schaefer M, Falaiye T. Monitoring Vitamin D levels in pediatric inflammatory bowel disease patients. *Inflamm Bowel Dis.* (2016) 22:S73–S4. doi: 10.1097/01.MIB.0000480336.60576.4b

35. Syed S, Smith EM, Tangpricha V, Chesdachai S, Kumar A, Prince J, et al. Assessing the relationship of vitamin D with iron status in children with inflammatory bowel disease. *Faseb J.* (2016) 30:128–7.

36. Syed S, Michalski ES, Tangpricha V, Chesdachai S, Kumar A, Prince J, et al. Vitamin D status is associated with hepcidin and hemoglobin concentrations in children with inflammatory bowel disease. *Inflamm Bowel Dis.* (2017) 23:1650–8. doi: 10.1097/MIB.0000000000001178

37. Brandvayman Y, Rinawi F, Shamir R, Assa A. Associations of seasonal patterns and vitamin D levels with onset and flares of pediatric inflammatory bowel disease. *Minerva Pediatr.* (2017) 73:42–49. doi: 10.23736/S2724-5276.17.04847-2

38. Winter RW, Lucci MB, Collins E, Cao B, Carrellas M, Crowell AM, et al. Higher 25-Hydroxyvitamin D levels are associated with a greater likelihood of remission with anti-tumor necrosis factor-alpha medications among patients with inflammatory bowel diseases. Gastroenterology. (2015) 148:S249–S. doi: 10.1016/S0016-5085(15)30819-2

39. Sauer CG, Loop MS, Venkateswaran S, Tangpricha V, Ziegler TR, Dhawan A, et al. Free and bioavailable 25-hydroxyvitamin D concentrations are associated with disease activity in pediatric patients with newly diagnosed treatment naive ulcerative colitis. *Inflamm Bowel Dis.* (2018) 24:641–50. doi: 10.1093/ibd/izx052

40. Mager DR, Carroll MW, Wine E, Siminoski K, MacDonald K, Kluthe CL, et al. Vitamin D status and risk for sarcopenia in youth with inflammatory bowel diseases. *Eur J Clin Nutr.* (2018) 72:623–6. doi: 10.1038/s41430-018-0105-2

41. Kim S, Kang Y, Park S, Koh H, Kim S. Association of Vitamin D with inflammatory bowel disease activity in pediatric patients. *J Korean Med Sci.* (2019) 34:e204. doi: 10.3346/jkms.2019.34.e204

42. Veit LE, Maranda L, Nwosu BU. The nondietary determinants of vitamin D status in pediatric inflammatory bowel disease. *Nutrition.* (2015) 31:994–9. doi: 10.1016/j.nut.2015.03.010

43. Santucci NR, Alkhouri RH, Baker RD, Baker SS. Vitamin and zinc status pretreatment and posttreatment in patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* (2014) 59:455–7. doi: 10.1097/MPG.0000000000000477

44. Strisciuglio C, Cenni S, Giugliano FP, Erasmo M, Cirillo G, Martinelli M, et al. The role of inflammation on vitamin D axis in a cohort of pediatric patients with inflammatory bowel disease. *Digestive Liver Dis.* (2017) 49:E260–E. doi: 10.1016/j.dld.2017.09.050

45. Ulitsky A, Ananthakrishnan AN, Naik A, Skaros S, Zadvornova Y, Binion DG, et al. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *J Parenteral Enteral Nutr.* (2011) 35:308–16. doi: 10.1177/0148607110381267

46. Bernstein CN, Blanchard JF, Leslie W, Wajda A, Yu BN. The incidence of fracture among patients with inflammatory bowel disease: a population-based cohort study. *Ann Intern Med.* (2000) 133:795–9. doi: 10.7326/0003-4819-133-10-200011210-00012

47. Guzman-Prado Y, Samson O, Segal JP, Limdi JK, Hayee BH. Vitamin D therapy in adults with inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis.* (2020) 26:1819–30. doi: 10.1093/ibd/izaa087

48. Raffner Basson A, Swart R, Jordaan E, Mazinu M, Watermeyer G. Vitamin D deficiency increases the risk for moderate to severe disease activity in Crohn's disease patients in South Africa, measured by the Harvey Bradshaw index. *J Am Coll Nutr.* (2016) 35:163–74. doi: 10.1080/07315724.2015.1039665

49. Mouli VP, Ananthakrishnan AN. vitamin D and inflammatory bowel diseases. *Aliment Pharmacol Ther.* (2014) 39:125–36. doi: 10.1111/apt.12553

50. Palmer MT, Weaver CT. Linking vitamin d deficiency to inflammatory bowel disease. *Inflamm Bowel Dis.* (2013) 19:2245–56. doi: 10.1097/MIB.0b013e31828a3b6f

51. Cantorna MT. Vitamin D, multiple sclerosis and inflammatory bowel disease. *Arch Biochem Biophys.* (2012) 523:103–6. doi: 10.1016/j.abb.2011.11.001

52. Molodecky N, Panaccione R, Ghosh S, Barkema H, Kaplan G. Alberta Inflammatory Bowel Disease Consortium Challenges associated with identifying the environmental determinants of the inflammatory bowel diseases. *Inflamm Bowel Dis.* (2011) 17:1792–9. doi: 10.1002/ibd.21511

53. Abreu M, Kantorovich V, Vasiliauskas E, Gruntmanis U, Matuk R, Daigle K, et al. Measurement of vitamin D levels in inflammatory bowel disease patients reveals a subset of Crohn's disease patients with elevated 1, 25-dihydroxyvitamin D and low bone mineral density. *Gut.* (2004) 53:1129–36. doi: 10.1136/gut.2003.036657

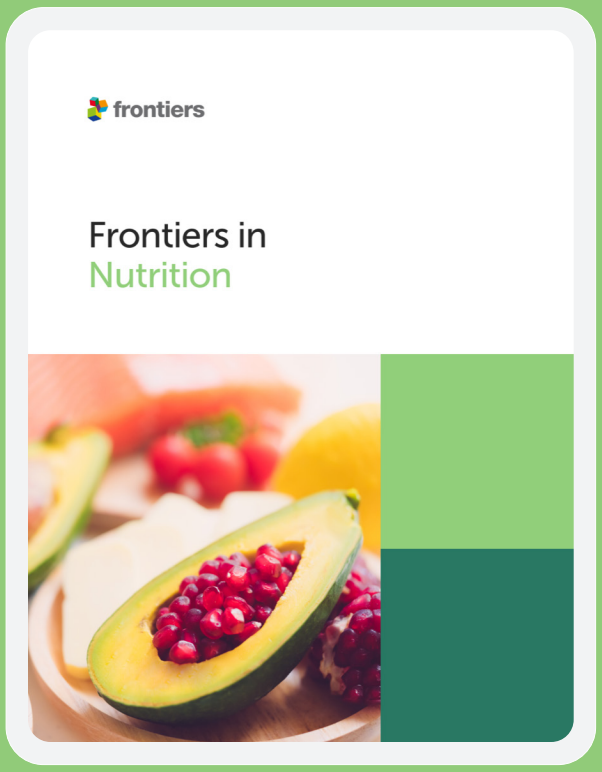
Frontiers in Nutrition

Explores what and how we eat in the context of health, sustainability and 21st century food science

A multidisciplinary journal that integrates research on dietary behavior, agronomy and 21st century food science with a focus on human health.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact