MUSCULOSKELETAL ADAPTATIONS TO TRAINING AND SPORTS PERFORMANCE: CONNECTING THEORY AND PRACTICE

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MUSCULOSKELETAL ADAPTATIONS TO TRAINING AND SPORTS PERFORMANCE: CONNECTING THEORY AND PRACTICE

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EDITORIAL
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Editorial: Musculoskeletal Adaptations to Training and Sports Performance: Connecting Theory and Practice

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Editorial on the Research Topic

Musculoskeletal Adaptations to Training and Sports Performance: Connecting Theory and Practice

We are pleased to present the Research Topic on Musculoskeletal Adaptations to Training and Sports Performance: Connecting Theory and Practice. With this Research Topic, we intend to increase knowledge on musculoskeletal adaptations to training with a strong focus on the connection between training practices and sports performance. To improve sports performance, coaches and researchers must better understand the training process, the dose-response relationship, and adaptations to training load. The scope of this research ranges from the general discussion of trainability and athlete responses to new assessment tools commonly used in other areas of investigation (e.g., medicine).

The adaptive process to training is discussed by Radak and Taylor and is focused on the specificity of trainability. The authors highlight some issues on the systemic nature of exercise-induced adaptation, alerting that the common use of the term "non-responders" could hide the view of a real specific response. The fact that a specific physiological function or system does not reveal positive changes to a training program does not mean that all other functions/systems will not respond positively. Thus, we should be aware of the specificity of the analysis that is performed and highlight one of the most valuable principles of training, which is specificity. The specificity of training responses can be influenced by preparatory activities (i.e., warm-up) and recovery mode (i.e., cooldown). Since early on, the sports community has been aware of the key role of warm-up and the literature has related the benefits of warm-up with changes in the physiological status of the athletes. Coaches and athletes have used different tasks during warm-up including dynamic and static stretching exercises, believing that these can improve performance later on. Moreover, it is suggested that some types of stretching may reduce muscle stiffness and increase the range of motion, thus decreasing the incidence of activity-related injuries. However, some discussion emerged in the literature when it was found that there is a loss of strength and power performance following stretching tasks. The opinion article by Afonso, Olivares-Jabalera, et al. debates the use of stretching during warm-up. Furthermore, the authors go further on the discussion about stretching, raising relevant issues such as *"Can I" Vs. "Do I Have" To Stretch in the Cool-Down Phase?; "Can I" Vs. "Do I Have To" Stretch To Chronically Improve Range of Motion? "Can I" Vs. "Do I Have To" Stretch To Reduce Injury Risk?* These questions create a link to other contributions to this Research Topic, such as the review presented by Afonso, Clemente, et al. In this review, the authors analyzed and discussed the effects of

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post-exercise stretching on muscle recovery markers (e.g., the range of motion and onset muscular soreness). Curiously, more than 70% of the analyzed studies comparing passive recovery (i.e., rest) and stretching found no differences for recovery markers. Stretching is usually recommended to enhance post-exercise recovery but the data is too scarce and heterogeneous to support guidelines.

Research on injury risk goes beyond questions about stretching exercises. This is clearly described by Afonso, Rocha-Rodrigues, et al. providing deeper insight into the individual risk factors for hamstring injuries. The authors presented an interesting perspective of the risk of injury based on modifiable factors (e.g., warm-up, load, fatigue, lumbopelvic hip stability, motor patterns, cardiovascular fitness, mobility, lower back pain, recovery strategies, strength, asymmetry, nutrition, and psychosocial factors), and non-modifiable factors (e.g., age, previous injury, specific anatomic variations). Physical training programs should be developed to mitigate the risk of injury with a focus on modifiable factors but keeping in mind that there is a high level of anatomic variability among different subjects, thus highlighting the need for better-individualized exercise interventions. In addition to being an influencing factor for training design, the anatomical characteristics of the athlete have been increasingly recognized as influencing performance in different sports. For instance, the architecture of gastrocnemius medialis and gastrocnemius lateralis is different between female cyclists and basketballers (May et al.). Some previous literature supports that these differences could be explained by the different stimulus that the gastrocnemius muscle is subjected to in the typical gesture performed in each sport. However, in this study, it appears that the distinct mechanical stimuli caused by cycling and basketball training did not influence gastrocnemius muscle architecture in male athletes. This emphasizes that there is still much to be understood about the anatomical and physiological adaptations to training and their relationship with performance. For example, contrary to expectations, Ando et al. found that 8 weeks of drop jump training resulted in decreased passive stiffness of the medial gastrocnemius. Nevertheless, drop jump performance was improved. Once again, this underlines the specificity of adaptations to training in each sport.

Research in sports science has been increasingly aware that different sports require different stimulations and, consequently, adaptations should also be different. The impact of the sport, different training methods, and evaluation tools have not been deeply explored in this Research Topic. However, some examples are provided. Specific strength training programs were developed for dancers (see Ávelia-Carvalho et al.) and young soccer players (see Falces-Prieto et al.) resulting in better jumping performance, aerobic endurance, and body composition. New training methods were explored, such as the combination of sprint interval exercises in hypoxia or with blood flow restriction (see Solsona et al.), and the use of new evaluation tools, such as arterial doppler ultrasound to analyze the arterial and venous diameters of the lower limbs in indoor soccer athletes (see Mateus et al.).

We strongly believe that increasing understanding of the adaptations to the exercise, from physiological, to biochemical–molecular, to structural-anatomical, should be a core aspect of sports science research to provide background knowledge to support coaches and athletes in their activities and, thus, enhance performance. Here emerges one of the most recent challenges for sport-related professionals, which is the translation of theoretical content and scientific findings into a practical setting.

AUTHOR CONTRIBUTIONS

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

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SYSTEMATIC REVIEW
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The Effectiveness of Post-exercise Stretching in Short-Term and Delayed Recovery of Strength, Range of Motion and Delayed Onset Muscle Soreness: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background: Post-exercise (i.e., cool-down) stretching is commonly prescribed for improving recovery of strength and range of motion (ROM) and diminishing delayed onset muscular soreness (DOMS) after physical exertion. However, the question remains if post-exercise stretching is better for recovery than other post-exercise modalities.

Objective: To provide a systematic review and meta-analysis of supervised randomized-controlled trials (RCTs) on the effects of post-exercise stretching on short-term (≤1 h after exercise) and delayed (e.g., ≥24 h) recovery makers (i.e., DOMS, strength, ROM) in comparison with passive recovery or alternative recovery methods (e.g., low-intensity cycling).

Methods: This systematic review followed PRISMA guidelines (PROSPERO CRD42020222091). RCTs published in any language or date were eligible, according to P.I.C.O.S. criteria. Searches were performed in eight databases. Risk of bias was assessed using Cochrane RoB 2. Meta-analyses used the inverse variance random-effects model. GRADE was used to assess the methodological quality of the studies.

Results: From 17,050 records retrieved, 11 RCTs were included for qualitative analyses and 10 for meta-analysis (n = 229 participants; 17–38 years, mostly males).

The exercise protocols varied between studies (e.g., cycling, strength training). Post-exercise stretching included static stretching, passive stretching, and proprioceptive neuromuscular facilitation. Passive recovery (i.e., rest) was used as comparator in eight studies, with additional recovery protocols including low intensity cycling or running, massage, and cold-water immersion. Risk of bias was high in ~70% of the studies. Between-group comparisons showed no effect of post-exercise stretching on strength recovery (ES = −0.08; 95% CI = −0.54–0.39; $p = 0.750$; $I^2 = 0.0\%$; Egger's test $p = 0.531$) when compared to passive recovery. In addition, no effect of post-exercise stretching on 24, 48, or 72-h post-exercise DOMS was noted when compared to passive recovery (ES = −0.09 to −0.24; 95% CI = −0.70–0.28; $p = 0.187$–629; $I^2 = 0.0\%$; Egger's test $p = 0.165$–0.880).

Conclusion: There wasn't sufficient statistical evidence to reject the null hypothesis that stretching and passive recovery have equivalent influence on recovery. Data is scarce, heterogeneous, and confidence in cumulative evidence is very low. Future research should address the limitations highlighted in our review, to allow for more informed recommendations. For now, evidence-based recommendations on whether post-exercise stretching should be applied for the purposes of recovery should be avoided, as the (insufficient) data that is available does not support related claims.

Systematic Review Registration: PROSPERO, identifier: CRD42020222091.

Keywords: flexibility, post exercise recovery, myalgia, cool-down, delayed onset muscular soreness, stretching, muscle stretching exercises, articular range of motion

INTRODUCTION

Exercise sessions typically begin with a warm-up period, followed by the main workout, and end with a cool-down phase, including a progressive reduction of effort and intensity (ACSM, 2018). Stretching is prescribed as an essential component of the cool-down phase by the guidelines of ACSM (2018) and the American Heart Association (2020). The main goals of stretching exercises applied during the cool-down phase (i.e., post-exercise stretching) are to enhance range of motion (ROM) and to reduce stiffness and delayed onset muscle soreness (DOMS) (Sands et al., 2013). There are different post-exercise stretching methods, such as passive static, active static, dynamic, proprioceptive neuromuscular facilitation (PNF), among others (Lima et al., 2019). Despite its wide adoption in exercise protocols, its effectiveness is not well-understood (Van Hooren and Peake, 2018).

Past research has a mixed and often contradicting set of results, with numerous studies indicating post-exercise stretching is not effective for improving recovery. Indeed, in one study with 10 healthy men (Mika et al., 2007), the participants performed three sets of leg extension and flexion at 50% of maximum voluntary contraction (MVC). Post-exercise recovery protocols were used, including light-intensity cycle ergometer and PNF stretching for 5 min. Light-intensity cycle ergometer exercise (10 W at 60 rpm) induced greater short-term recovery (i.e., immediately after the post-exercise protocol) than stretching as measured by MVC, total effort time, motor unit activation and EMG frequency ($p < 0.05$). In another study (Robey et al., 2009), club (8 men, 6 women; age: 20.2 ± 2.2 years) and elite level rowers (4 men, 2 women, age: 18.6 ± 0.8 years) performed a strenuous stair-climb running protocol. Post-exercise recovery protocols were applied at 15-min, 24 and 48 h, including stretching, hot/cold water immersion and passive recovery (i.e., rest). Compared to passive recovery, stretching and hot/cold water immersion induced no recovery effect on leg extension concentric peak torque, 2 km rowing ergometer times, creatine kinase levels, or DOMS, at any time-point. Further, nine physically active men (age, 23 ± 1 years) performed a fatiguing exercise protocol (i.e., 8-min of cycle ergometer at 90% maximum oxygen uptake), followed by a post-exercise stretching protocol (i.e., 10 min) (Cè et al., 2013). After 1 h of performing the stretching protocol, mechanical and physiological assessments (e.g., MVC, EMG amplitude, and lactate kinetics) were similar between the stretching group and the passive recovery group.

Moreover, stretching may be ineffective in relieving perceived muscle pain or in reducing DOMS (Wessel and Wan, 1994; Cheung et al., 2003; Xie et al., 2018). Also, recovery may not simply mean a return to basal values. In other words, to be effective, post-exercise stretching should recover and improve participants function over basal condition (Sands et al., 2013; Van Hooren and Peake, 2018).

Furthermore, potential short-term positive effects of post-exercise stretching on recovery should be balanced with long-term adaptations. For example, Fuchs et al. (2020) recently demonstrated that post-exercise cooling (i.e., cold-water immersion) accelerated acute recovery after training sessions; however, it impaired myofibrillar protein synthesis rates after 2-weeks of training compared to not performing cold-water immersion. In this sense, to comprehensively assess the effectiveness of post-exercise stretching, both short-term and delayed recovery should probably be considered.

In order to bring clarity to conflicting results, systematic reviews and meta-analysis (SRMA) are usually performed as a cornerstone for evidence-based practices (Higgins et al., 2019). Indeed, studies in the field tend to use small samples with reduced statistical power (Abt et al., 2020). In contrast, SRMA provide greater statistical power. In fact, some attempts were performed to synthesize current literature related to post-exercise stretching and recovery. A SRMA of randomized and quasi-randomized studies showed that stretching before or after exercise did not protect from DOMS (Herbert and Gabriel, 2002), and two independent updates reinforced the same conclusions (Henschke and Lin, 2011; Herbert et al., 2011). However, relevant databases such as PubMed and Web of Science were not included in the searches of the aforementioned SRMAs, and potentially relevant search terms such as "mobility" and "post-exercise" or "post-training" were not applied. Likewise, external experts were not consulted after automated searches, as suggested in high-standard protocols (Moher et al., 2009, 2015; Shea et al., 2017). Moreover, nearly a decade has passed since the publication of the aforementioned SRMAs, and a cursory search of articles in Google Scholar from 2011 to present date suggests that several new studies have been done on the topic. An updated SRMA focused solely on post-exercise stretching and limited to randomized controlled trials (RCTs) may provide a more homogeneous and high-quality data set (Hariton and Locascio, 2018), while an expanded set of relevant databases and search terms may provide a more representative sample of existing studies.

Therefore, our goal was to review supervised RCTs on the effects of post-exercise stretching on recovery makers (i.e., DOMS, strength, ROM), in comparison with passive recovery or alternative recovery methods (e.g., low-intensity cycling). Short-term ($\leq$1 h after exercise) and delayed recovery (24, 48, and 72 h) markers were considered.

METHODS

Protocol and Registration

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009, 2015), the Cochrane Collaboration guidelines for evaluation of risk of bias (RoB) in randomized studies (Sterne et al., 2019), and the AMSTAR 2 recommendations (Shea et al., 2017). Quality of studies was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Guyatt et al., 2011). The review methods were established before initiating the research, and protocol registration preceded the search. Protocol was published in PROSPERO with the reference CRD42020222091.

Eligibility Criteria

Studies were eligible if consisting of original research or replication studies published in peer-reviewed journals, with full-text not limited to any particular language or publication date. Beyond English language, the authors also have a deep understanding of Portuguese and Spanish, as well as a good understanding of French and Italian. If studies were written in different languages, professional translators were hired. Based on scope, P.I.C.O.S. and timeframe for follow-up, **Table 1** presents the inclusion and exclusion criteria. The limitation to RCTs was decided because randomization reduces the RoB and balances participants distribution between groups (Hariton and Locascio, 2018). Indeed, RCTs are the gold standard for evidence-based practices (Spieth et al., 2016). Supervision was considered if explicit information was available stating that at least one qualified professional oriented the post-exercise protocol. No studies were excluded on the basis of RoB as assessed through RoB 2 (Sterne et al., 2019).

Information Sources

Search was programmed to start on January 1st, 2021, but since protocol approval occurred earlier (December 2nd, 2020), we conducted the automated searches on December 23 and 24, 2020, with search results being exported to EndNote X9 for Mac (v.9.3.3., Clarivate Analytics). The following electronic databases were searched: Cochrane Library (including CENTRAL), EBSCO (all available databases), PEDro, PubMed, Scielo, Scopus, SPORTDiscus (all databases), and Web of Science (all databases/collections). Search protocol used Boolean operators and required the title, abstract, or keywords had to include *("stretch*" OR "flex*" OR "mobility" OR "range of motion")* AND *("post-exerci*" OR "post-workout" OR "post-exertion" OR "post-train*" OR "after exerci*" OR "after workout" OR "after exertion" OR "after training" OR "recover*" OR "warm-down" OR "cool-down")* AND *"random.*"* Similar terms or synonyms were used to guarantee a more inclusive initial search and avoid an excessively narrow scope of analyzed studies. Searches were updated on February 16, 2021, for inclusion of records with date of entry from December 25, 2020, onwards. Where date of entry was not a feature (e.g., EBSCO, Scielo, Scopus, SPORTDiscus, Web of Science), publication date was limited to 2021, since the year 2000 would be practically all covered until the search was completed.

A manual search was conducted within the reference list of the records included in the sample after full text analysis, to retrieve potentially relevant studies that had not emerged in the initial search. After completion of this stage, the list of studies, as well as inclusion and exclusion criteria were sent to eight independent experts in the field, to check if they were aware of additional papers. The experts were university professors with a Ph.D. and with peer-reviewed publications within the scope of our SRMA. Search strategy was not provided, to avoid biasing the experts' search. After the final list of studies was completed, all the databases were again consulted to retrieve errata, corrigenda/corrections, or retractions of the included studies, as some may have been found to be fraudulent or retracted (Higgins et al., 2019).

TABLE 1 | Inclusion and exclusion criteria based on scope, PICOS and timeframe for follow-up.

Rule	Inclusion criteria	Exclusion criteria
Study type	Original research or replication studies published in peer-reviewed journals. No limitations imposed regarding language or publication date.	Conference abstracts, books and book chapters, editorials, letters to the editor, feasibility and pilot studies, trial registrations, reviews, essays, or original research in non-peer-reviewed journals.
Participants	Participants of any age, sex, health, and training status.	Non-human animals (e.g., rats).
Interventions	Stretching (e.g., static passive, static active, dynamic, PNF, other) immediately after any type of exercise session (e.g., strength training, endurance, multimodal, sports). No co-interventions.	Stretching as the training intervention *per se*. Pre-exercise stretching (e.g., warm-up). Post-exercise multimodal interventions (e.g., stretching combined with low-intensity cycling). Post-exercise stretching with co-interventions (e.g., massage).
Comparators	Passive recovery (i.e., rest) or alternative recovery protocols (e.g., low-intensity aerobic activities, massage).	Absence of comparators. Multimodal comparators that include stretching.
Outcomes	*Primary outcomes* Effects on short-term post-exercise recovery (≤1 h post-exercise): strength, DOMS, ROM. Effects on delayed post-exercise recovery (24, 48, 72 h)*: strength, DOMS, ROM. *Secondary outcomes* Biochemical markers of muscle damage; muscle and tendon stiffness; adverse effects from the post-exercise interventions.	No outcomes related to strength and/or ROM for short-term recovery. AND No outcomes related to DOMS, strength and/or ROM for delayed recovery.
Study design	Supervised RCTs (parallel or cross-over) (Elbourne et al., 2002; Spieth et al., 2016).	Non-randomized studies. Non-supervised intervention and/or comparators. Case reports, case series, observational studies (e.g., case-control and cohort studies).
Timeframe for follow-up	Maximum 72 h post-intervention, based on the existing literature (Van Hooren and Peake, 2018).	No study will be excluded if presenting values >72 h, but these will not be considered for analysis.

DOMS, delayed onset muscular soreness; PNF, proprioceptive neuromuscular facilitation; RCT, randomized controlled trial; ROM, range of motion.
**If an additional exercise bout or an active recovery protocol is included between the initial session and the delayed markers (e.g., application of a second exercise bout at 48 h while providing data regarding recovery from the initial bout at 72 h), then only values until that second bout (i.e., 48 h) will be considered.*

Study Selection

The screening process started on January 4, 2021 for the first wave of searches. The screening process for the updated searches started on February 17, 2021. JA and FMC conducted the initial search, screening of titles and abstracts and analysis of full texts independently. HS and PM later reviewed the entire process. Thirdly, a step-by-step comparison of the whole process was conducted, and any disagreements motivated a new analysis of the records in question. Discussion regarding manuscripts suitability was performed with all the involved authors in the study selection process, until consensus was achieved. The same process was then used to analyze the reference lists of the included studies to verify if additional relevant studies were available. External experts were contacted to provide additional suggestions of relevant studies based on inclusion criteria and on our preliminary list. JA and FMC independently verified the list to decide on inclusion of the suggested studies. HS and PM then reviewed this process. The same process was applied to search for errata of the included studies.

Data Extraction

All extracted data were defined *a priori*, to avoid biased analyses (Spieth et al., 2016). Study characteristics: (i) sample size and features (e.g., age, sex, health, training status, country, continent; single or multicenter study); (ii) length and characteristics of the interventions and comparators (e.g., weekly frequency, type/modality of stretching and comparators, volume, intensity, duration, supervision ratio, qualification of supervisors, description of co-interventions); (iii) adherence rates to training (i.e., attendance percentage); (iv) funding sources and potential conflicts of interest. Data specific to cross-over studies (Elbourne et al., 2002; Spieth et al., 2016): (i) length of wash-in and wash-out periods; (ii) carryover effects, if there were any.

Primary outcomes for short-term recovery (≤1-h post-intervention): strength levels (e.g., maximum voluntary contraction) and joint ROM immediately or until 1 h after exertion. *Primary outcomes for delayed recovery*: DOMS, strength levels, and joint ROM at 24, 48, and 72 h, which are considered theoretically relevant (Van Hooren and Peake, 2018) and are commonly assessed periods on studies investigating this subject matter (Bonfim et al., 2010; Torres et al., 2013).

Secondary outcomes: Biochemical markers (e.g., plasma creatine kinase; blood lactate concentration); muscle and tendon stiffness; adverse effects during the post-exercise interventions (type, intensity or severity, time points). The timings described in the previous paragraph were considered for secondary outcomes as well.

Outcomes were only considered for analysis in case there was no additional exercise bout between the initial session and the delayed recovery timeframe. For all primary and secondary outcomes, description of measurement tools and metrics was included (Higgins et al., 2019) and both significant and non-significant results were considered (Spieth et al., 2016). Furthermore, parallel and cross-over trials were combined as long as the latter did not have significant carryover effects (Elbourne et al., 2002). JA and FMC completed initial data extraction independently. HS and PM later reviewed the entire process and consensus had to be achieved. The data required for meta-analysis was fulfilled by JA and FMC and then reviewed by HS and PM. RRC provided a final verification of the quality of data inserted into the table.

Risk of Bias in Individual Studies

Bias refers to systematic errors that can threaten the internal validity of an RCT (Spieth et al., 2016). RoB was assessed using the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) (Sterne et al., 2019), which consists of five dimensions, i.e., bias arising due to: (i) the randomization process; (ii) deviations from intended interventions; (iii) missing outcome data; (iv) measurement of the outcome; and (v) selection of the reported result. JA and FMC independently assessed RoB for all studies. After the first assessment, tables were compared and disagreements were discussed, with a subsequent re-analysis of the situation. Finally, HS and PM reviewed the assessments to ensure the quality of the evaluations. For assessing RoB in parallel trials, the Excel tool ROB2_IRPG_beta_v7 (Cochrane) was used. For crossover trials, the Excel tool ROB2.0_IRCX_beta (MRC | Hubs for Trials Methodology Research) was planned to be used. However, this tool is outdated. Following the most up-to-date Cochrane guidelines for applying RoB 2 to individual cross-over trials (Higgins et al., 2020), the five domains can be assessed following the structure of parallel trials. However, an extra dimension (Domain S) is added. Therefore, we used the ROB2_IRPG_beta_v7, with manual addition of Domain S.

Summary Measures

It is possible to use two studies in a meta-analysis (Valentine et al., 2010), but we chose to establish a minimum of three studies (Moran et al., 2018; García-Hermoso et al., 2019; Skrede et al., 2019) to avoid small sample sizes (Abt et al., 2020; Lohse et al., 2020). Pre- and post-intervention means and standard deviations (SDs) were converted to Hedge's *g* effect size (ES) (García-Hermoso et al., 2019; Skrede et al., 2019). In case the study instead provides 95% confidence intervals (CIs) or standard errors of mean (SEM), means and standard deviations were obtained from 95% CI or SEM, using Cochrane's RevMan Calculator for Microsoft Excel (Drahota and Beller, 2020). In case data for primary outcomes was presented only in graphical form, a validated software ($r = 0.99$, $p < 0.001$), WebPlotDigitizer, version 4.4 (Rohatgi, 2020) was used to extract data, with all values rounded to two decimal places. In these cases, the main author extracted data from the graphs, and an outside researcher, not involved in this work (see section Acknowledgments), performed an independent data extraction. Reliability was calculated through Cronbach's Alpha, using SPSS Statistics version 27 for Mac (IBM).

The inverse variance random-effects model for meta-analyses was used because it allocates a proportionate weight to trials based on the size of their individual standard errors (Deeks et al., 2008) and enables analysis while accounting for heterogeneity across studies (Kontopantelis et al., 2013). The ESs were presented alongside 95% CIs and interpreted using the following thresholds (Hopkins et al., 2009): <0.2, trivial; 0.2–0.6, small; >0.6–1.2, moderate; >1.2–2.0, large; >2.0–4.0, very large; >4.0, extremely large. Heterogeneity was assessed using the I^2 statistic, with values of <25, 25–75, and >75% considered to represent low, moderate, and high levels of heterogeneity, respectively (Higgins and Thompson, 2002). Publication bias was explored using the extended Egger's test (Egger et al., 1997). To adjust for publication bias, a sensitivity analysis was conducted using the trim and fill (Duval and Tweedie, 2000), with L0 as the default estimator for the number of missing studies (Shi and Lin, 2019). Analyses were performed in the Comprehensive Meta-Analysis program (version 2; Biostat, Englewood, NJ, USA). Statistical significance was set at $p \leq 0.05$.

Moderator Analyses

These analyses were planned but could not be performed. Details on planned moderator analysis can be found in the **Supplementary Materials**.

Confidence in Cumulative Evidence

For RCTs, GRADE starts assuming high quality, which can be downgraded according to five dimensions (Zhang et al., 2019). In addition to RoB, inconsistency (heterogeneity) and publication bias, which have already been addressed, indirectness and imprecision (using 95% CIs) were assessed independently by JA and FMC and verified by HS. These authors also estimated the overall quality and confidence in cumulative evidence.

RESULTS

Study Selection

Initial search retrieved 16,851 results [Cochrane Library: 13 reviews and 621 trials; EBSCO: 1,704; PEDro: 21; PubMed: 2,421; Scielo: 12; Scopus: 5,253; SPORTDiscus: 734; Web of Science (all collections): 6,072]. Automated removal (EndNote function) of 6,635 duplicates resulted in 10,216 records. Manual removal of additional 2,333 duplicates resulted in 7,882 records to be screened. The first stage of screening titles and abstracts was based on study type (first inclusion criteria) and resulted in the exclusion of 2,101 records. The second stage of screening started with 5,781 records and 5,481 studies that were clearly out of scope (e.g., exercise-related studies not addressing the theme of our work, non-exercise related studies) were removed. Finally, starting with 300 records, the third stage of screening applied the PICOS criteria, and further excluded 278 studies. In these three stage-screening processes, exclusion criteria were defined hierarchically, i.e., if a paper had several reasons for exclusion, its exclusion would be based on the first criteria it failed to fit. Finally, two records had untraceable full texts, with discontinued links, disappearance from databases from where they were retrieved, and even not emerging in searches within the journals where they were supposedly published.

The updated searches retrieved 199 new records [Cochrane Library: 1 review and 8 trials; EBSCO: 49; PEDro: 0; PubMed: 53; Scielo: 3; Scopus: 25; SPORTDiscus: 7; Web of Science (all collections): 53]. Removal of duplicates results in 121 records, of which 14 were excluded due not fitting study type, 60 being non-related to exercise, 40 being related to exercise but out of scope, and six did not comply with PICOS criteria. More in-depth information concerning the screening can be found in **Supplementary Table 1**. Therefore, 21 records were considered eligible for analysis of the full text (20 in the initial searches and one in the updated searches). While most were written in English, one was in Portuguese (Bonfim et al., 2010), one in Greek (Kokkinidis et al., 1998), and three in Korean (금동민 et al., 2010; Oh, 2013; Kang and Park, 2018). A translator was hired for the Korean studies, and another for the Greek study.

At this stage, 12 records were excluded, with reasons. The study by Apostolopoulos et al. (2018) was excluded because the interventions were not supervised. However, they have interesting results that we will explore briefly here. Since they applied stretching for three consecutive days after the eccentric exercise protocol, only results at 24 h were considered. The authors used a 90% CIs (and not the more common 95% CIs) to compare low-intensity and high-intensity stretching to a control group using passive rest. Despite the authors' claims, all 90% CIs passed through zero, and no differences were observed at 24 h between the stretching groups and the controls for DOMS, eccentric and isometric peak torques of knee extensors, creatine kinase (U/L), and high-sensitivity C-reactive protein. The study of Boobphachart et al. (2017) was excluded because the stretching intervention was performed three times per day and, furthermore, was unsupervised.

The study of Cha and Kim (2015) was excluded because both groups included some form of stretching, therefore inhibiting the comparison of stretching with alternative protocols and failing our PICOS criteria. The study of Duffield et al. (2014) was excluded because both the training interventions and the protocols were applied twice a day. Furthermore, one of the protocols included not only immediate measures (15-min cold-water immersion), but also ongoing measures such as 3 h of wearing full-body compression garments, plus abiding by sleep-hygiene recommendations in that night. The study of Gulick et al. (1996) was excluded because randomization was compromised. The authors created seven groups with 10 participants each. When a participant would quit, they would simply recruit a new participant to the group, therefore compromising both randomization and baseline values for each group. In addition, no details were provided concerning how these new subjects changed the values for each variable.

The study of Kang and Park (2018) was excluded because the exercise intervention lasted 20 min, while the post-exercise stretching protocol consisted of 5 min of so-called preparation exercises, followed by 30 min of stretching, followed by 5 min of so-called clean-up exercises. Therefore, not only did post-exercise recovery last 200% more than the exercise intervention (thereby, being akin to a stretching intervention *per se* and failing our inclusion criteria), but also the recovery intervention was not exclusively reliant on stretching (again, falling our inclusion criteria). The study of McGlynn et al. (1979) was excluded because stretching was applied immediately post-exercise, but also repeated at 6, 25, 30, 49, and 54 h post-exercise. Therefore, even the 24 h assessments could not be attributed to stretching performed immediately following an exercise bout. Incidentally, the authors reported that both the stretching and biofeedback groups observed a reduction in EMG muscle activity on the biceps brachii in comparison with a passive control group, but they had no effect on perceived pain.

The study of Oh (2013) was excluded because the cool-down protocols were not stretching-based. The study of Pooley et al. (2020) was a cross-over study that was excluded because randomization was compromised: while after "home" fixtures, the participants were randomized to cold-water immersion or cycle ergometer, in "away" fixtures stretching was always prescribed. The study of Robey et al. (2009) was excluded because the authors detail, in the manuscript, that the crossover was only semi randomized, and therefore does not meet our inclusion criteria. In any case, the main characteristics and results from this study have been addressed in the introduction, which was written prior to our searches. The study of Xanthos et al. (2013) was excluded because the so-called traditional recovery group was multimodal. The study of 금동민 et al. (2010) was excluded because the cool-down protocol was multimodal.

Therefore, nine studies fulfilled all inclusion criteria (Kokkinidis et al., 1998; Mika et al., 2007; Bonfim et al., 2010; Cè et al., 2013; Torres et al., 2013; McGrath et al., 2014; Muanjai and Namsawang, 2015; Cooke et al., 2018; César et al., 2021). As per protocol, in studies where the recovery methods were applied in multiple sessions (e.g., stretching after exercise and repeated at 24 and 48 h), only data before the second application was considered. To illustrate, in the studies of Cooke et al. (2018) and Kokkinidis et al. (1998), only the results at 24 h post-exercise were considered. Since a new recovery session was applied at 24 h, the results at 48 h and longer were not considered in the meta-analysis since results might not be attributable to the immediate post-exercise stretching protocol. In addition, and following protocol, multimodal recovery groups also including stretching were excluded from analysis (e.g., the group combining stretching followed by cold water immersion in the study of Muanjai and Namsawang, 2015). In the study of Torres et al. (2013), two groups were considered: the group performing eccentric exercise, and the group performing eccentric exercise followed by a single bout of stretching. The group that only performed stretching and the group that performed eccentric exercise followed by repeated bouts of stretching in the following days were excluded as they did not conform to our inclusion criteria.

A manual search within the reference lists of included studies revealed 26 potentially fitting titles (including updated searches). Of these, two had already been included in our final sample, and five had been excluded during the process. Nineteen studies had not appeared in our searches; screening of their abstracts resulted in the exclusion of five based on study type (e.g., abstract, review), and 10 based on failure to fulfill PICOS criteria. Of the four studies that required full text analysis, two fulfilled all PICOS criteria and were therefore added to our sample (Torres et al., 2005; West et al., 2014). In relation to Torres et al. (2005), and following the rules applied to Torres et al. (2013), only the two groups meeting the criteria were considered for analysis. Subsequently, eight experts were invited to contribute with additional relevant studies. Two experts declined the invitation due to lack of time, while five experts did not respond. One expert responded that our list was thorough and did not make any additional recommendation. Finally, errata, corrigenda, corrections, and retractions were searched for the included studies, but none was found. Therefore, 11 studies were included for qualitative analysis ($n = 289$), of which 10 could integrate quantitative analysis ($n = 280$, $n = 229$ after exclusion of groups that did not fulfill PICOS criteria). The process is summarized in the PRISMA flow diagram (**Figure 1**).

Study Characteristics

Study characteristics are provided in **Table 2**. Three studies used a cross-over design (Mika et al., 2007; Cè et al., 2013; West et al., 2014), while the remaining used a parallel design. Sample size ranged from 9 (Cè et al., 2013) to 57 (McGrath et al., 2014), with ages ranging from 17 to 38 years-old, i.e., all studies were performed with adults or near-adulthood (i.e., the usual legal age of 18 years old). The studies of Bonfim et al. (2010) and McGrath et al. (2014) had a mixed sample of men and women. The remaining studies only used male participants. All participants were healthy, but varied considerably in terms of training status: described as sedentary or untrained in four studies (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Bonfim et al., 2010), "physically active," "recreationally active," or "not involved in intense physical conditioning" in five studies (Mika et al., 2007; Cè et al., 2013; McGrath et al., 2014; Muanjai and Namsawang, 2015; Cooke et al., 2018), one study assessed the effects in aerobically trained, recreational cyclists (West et al., 2014), and only one study assessed athletes (César et al., 2021). Geographically, five studies were performed in Europe (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Mika et al., 2007; Cè et al., 2013), three in North America (McGrath et al., 2014; West et al., 2014; Cooke et al., 2018), two in South America (Bonfim et al., 2010; César et al., 2021) and one in Asia (Muanjai and Namsawang, 2015).

The studies purposefully applied soreness-inducing exercise protocols for the upper limbs (César et al., 2021) or lower limbs (all other articles), using diverse means such as cycling (Cè et al., 2013; West et al., 2014), running-based activities (Cooke et al., 2018), plyometrics (Muanjai and Namsawang, 2015), simulated jiu-jitsu fights (César et al., 2021), and more commonly, some form of strength training, usually with an emphasis on the eccentric component (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Mika et al., 2007; Bonfim et al., 2010; McGrath et al., 2014). Familiarization with the soreness-inducing protocols was described in three studies (Mika et al., 2007; Cè et al., 2013; Cooke et al., 2018; César et al., 2021), stated but not described in one (Muanjai and Namsawang, 2015), and not performed or unreported in six (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Bonfim et al., 2010; McGrath et al., 2014; West et al., 2014). In most studies, the duration of the soreness-inducing protocol was unclear (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Mika et al., 2007; Bonfim et al., 2010; McGrath et al., 2014; West et al., 2014; Muanjai and Namsawang, 2015), but unlikely to have surpassed 30 min, considering the descriptions provided. In the remaining studies, soreness-inducing protocols lasted between 10 min (César et al., 2021) and 55 min (Cooke et al., 2018), including warm-up when applied. The only study to report a co-intervention stated that a nutritional bar was provided pre-fatiguing exercise (West et al., 2014).

All studies had at least one group performing post-exercise stretching as an attempt to mitigate the negative effects of the soreness-inducing protocols. Active static stretching was used in four studies (Kokkinidis et al., 1998; Bonfim et al., 2010; West et al., 2014; Cooke et al., 2018), passive stretching in six (Torres et al., 2005, 2013; Cè et al., 2013; McGrath et al., 2014; Muanjai and Namsawang, 2015; César et al., 2021), and PNF in two (Mika et al., 2007; McGrath et al., 2014). McGrath et al. (2014) used both passive static stretching and PNF. No study used dynamic stretching. Almost all the post-exercise stretching protocols targeted the lower limbs, with one study targeting the upper limbs (César et al., 2021), and lasted between ∼1 min (McGrath et al., 2014) and 30 min (West et al., 2014; Cooke et al., 2018). Intensity of stretching was measured using only subjective feelings during the exercise, ranging from "subjects perceiving a slight feeling of stretching (...), without generating discomfort" (Bonfim et al., 2010) to "until subjects felt a *maximal* stretch of the hamstrings" (McGrath et al., 2014) or "until the greatest discomfort was reported by the participants" (César et al., 2021).

The comparator post-exercise interventions were also varied across studies, with some studies having more than one comparator group. Passive recovery (i.e., rest) was used as comparator in eight studies (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Mika et al., 2007; Bonfim et al., 2010; Cè et al., 2013; McGrath et al., 2014; César et al., 2021). Additional recovery protocols included low-intensity cycling (Mika et al., 2007; Cè et al., 2013; West et al., 2014) or running/jogging (West et al., 2014; Cooke et al., 2018), superficial and deep massage (Cè et al., 2013), cryotherapy and/or cold-water immersion (Kokkinidis et al., 1998; Muanjai and Namsawang, 2015; César et al., 2021).

One study explicitly stated that there were no adverse effects to report (Muanjai and Namsawang, 2015), while the other studies made no mention to it. We further highlight that two studies had potentially relevant conflicts of interest, as the company manufacturing the anti-gravity treadmill provided financing for the research (West et al., 2014; Cooke et al., 2018).

Risk of Bias Within Studies

Cochrane's RoB 2 tool evaluates RoB in five different dimensions (Sterne et al., 2019), the second of which subdivided into two parts. Here, an intention-to-treat analysis was considered. In terms of outcomes, RoB was only assessed for the primary outcomes (i.e., strength, ROM, and DOMS). None of the included studies had a pre-registered protocol. However, one had a specific reference to a grant (Torres et al., 2013), and another to an approval number by an Ethics Committee (Bonfim et al., 2010). In both cases, a pre-study protocol had to exist,

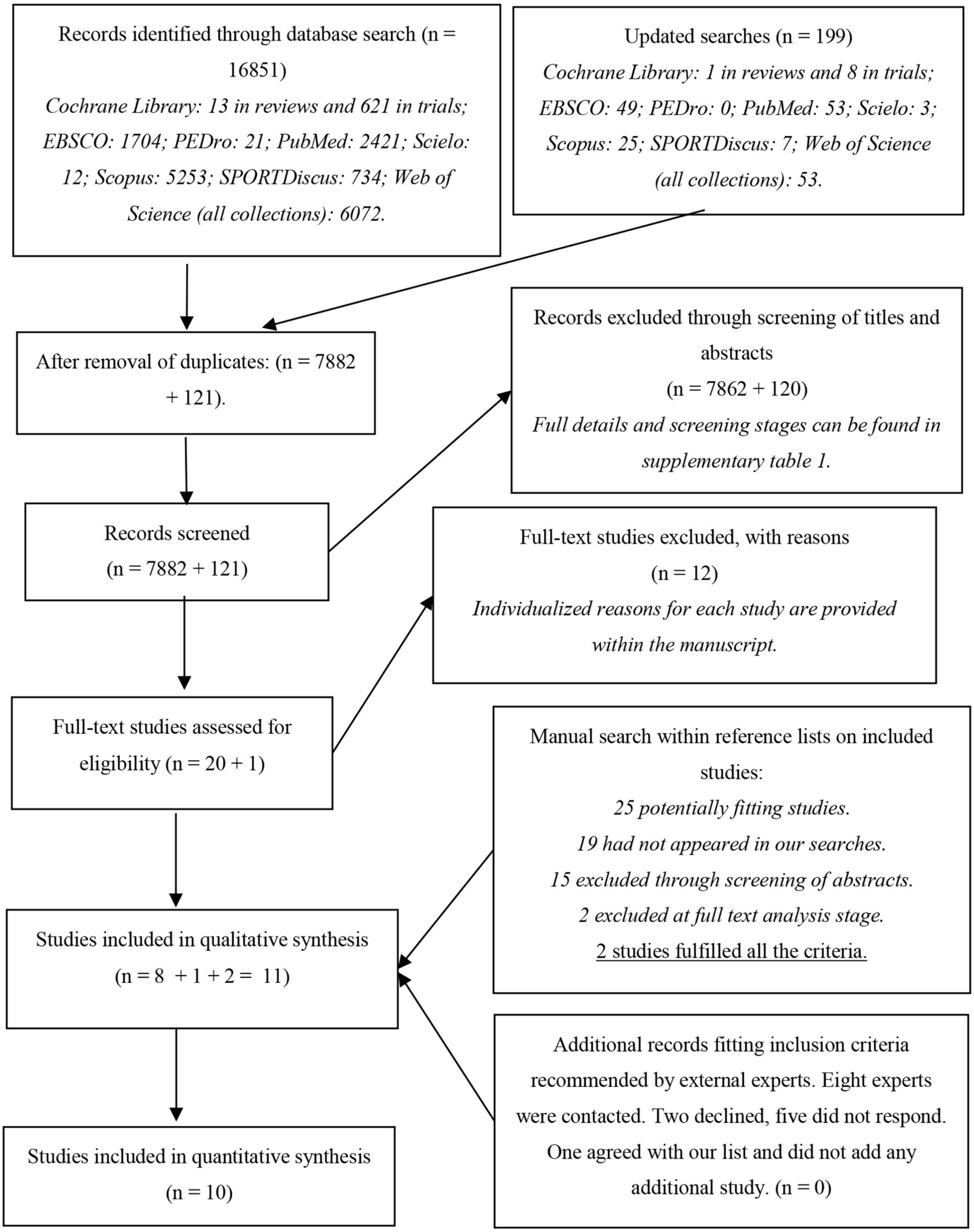

FIGURE 1 | Flowchart describing the study selection process.

and so we have contacted the authors. The corresponding author of Bonfim et al. (2010) provided the trial protocol, which also contained a statistical analysis plan. The main author of Torres et al. (2013), which was the recipient of the grant, was contacted, but unfortunately did not have the original project, which is comprehensible given the timeline. Since some studies had more than one outcome, assessments for domains 4 and 5 could have multiple assessments for each study. The complete assessments (i.e., one assessment per outcome per study) can be found in **Supplementary Table 2**. **Table 3** presents the worst-case scenario

TABLE 2 | Study characteristics.

Authors (year)/country	Trial design	Sample	Intervention	Length	Intervention (stretching group)	Comparators	Funding and potential conflicts of interest
Bonfim et al. (2010) Brazil	P	20 M/F healthy sedentary [17–30 years]	After 5 × 20 rep (with 30-s rest) of plantar/dorsiflexion, while standing and hands providing anchoring, EG (n = 10) performed active static stretching for ± 3 min.	Single application	n = 10. Active static stretching, 3 × 30-s per limb, per set. Until subjects perceived a slight feeling of stretching of the triceps surae, without generating discomfort.	n = 10. Passive recovery (rest).	No mention to funding. Explicit statement reporting no potential conflicts of interest.
Cè et al. (2013) Italy	CO	9 male healthy active (23 ± 1.0 years)	10-min warm-up followed by 8-min of cycling in ergometer at 90% VO_2max. Then, 10-min of passive static stretching.	Unclear, but estimated 2–3 sessions per week, during ~3 weeks	n = 9. Passive static stretching, 5 × 30-s per set, with 30-s rest between sets. Stretching to the point of discomfort.	n = 9. In a CO manner, for 10 min: superficial massage, deep massage, passive recovery (rest), low-intensity cycling (50% VO_2max).	No mention to funding. Explicit statement reporting no potential conflicts of interest.
César et al. (2021) Brazil	P	21 male healthy jiu-jitsu fighters (27.0 ± 5.9 years)	After 10-min of fight, EG (n = 7) performed passive static stretching for 10-min.	1 week	n = 7. Passive static stretching, 9 × 30-s per limb, per set. Until the greatest discomfort was reported by the participants.	n = 7 + 7. CWI (3 × 3 min at 10°C) and passive recovery (rest).	No mention to funding. No mention to potential conflicts of interest.
Cooke et al. (2018) USA	P	25 male healthy active (22.2 ± 3.5 years)	After 10-min warm-up and 45-min downhill running exercise, EG (n = 9) performed active static stretching for 30-min.	4 visits to the lab	n = 9. Active static stretching, 3 × 30-s per set. Stretching to the point of mild discomfort, but not pain.	n = 8 + 8. 30-min jogging at 60% VO_2peak and 0° incline. Treadmill + anti-gravity treadmill (at 75% bodyweight).	Partially funded by authors' lab, and by the company manufacturing the anti-gravity treadmill. Conflicts of interest not mentioned by the authors, but with financial support by the treadmill company.
Kokkinidis et al. (1998) Greece	P	12 male healthy sedentary [18–27 years]	After 6 × 10 rep (with 3-min rest) of leg curl focusing on the eccentric part of the movement, EG (n = 4) performed active static stretching for ±12-min.	3 days	n = 4. Active static stretching, 10 × 30-s per set, with 10-s rest between sets. Until subjects felt a painless sensation of stretching.	n = 4 + 4. Cryotherapy (cold compresses for 20 min) + passive recovery (rest).	No mention to funding. No mention to potential conflicts of interest.
McGrath et al. (2014) USA	P	29 M and 28 F healthy active ["Substantial majority" between 18–25 years]	After 5–10 min warm-up, 3 × 8–12 rep leg curl with an 8–12 RM load, focusing on the eccentric phase, EG (n = 20) performed passive static stretching for ±1-min.	Single application	n = 20 (14 male, 6 female). Passive static stretching, 2 × 10-s per limb, per set, with 4-s rest between sets. Until subjects felt a maximal stretch of the hamstrings.	n = 19 (9 male, 10 female) + 18 (5 male, 13 female). PNF (5-s each phase) + passive recovery (rest).	No mention to funding. No mention to potential conflicts of interest.
(Mika et al., 2007) Poland	CO	10 male healthy active [24–38 years]	3 sets (30-s rest) of dynamic leg extension and flexion (20–110°) at 50% MVC. Subjects had to perform as many repetitions as possible, stopping only when full ROM was no longer achieved. Following, 5-min of PNF stretching was applied.	1 session per week, for 5 weeks	n = 10. PNF stretching of unclear duration: 5-s performing isometrics, but unknown time during each passive stretching phase. Passive stretch to the point of onset of resistance, followed by isometric contraction, followed by relaxation and new passive stretch.	n = 10 + 10. Cycle ergometer (10 W, 60 rpm, 5-min) + passive recovery.	No mention to funding. No mention to potential conflicts of interest.

(Continued)

TABLE 2 | Continued

Authors (year)/country	Trial design	Sample	Intervention	Length	Intervention (stretching group)	Comparators	Funding and potential conflicts of interest
Muanjai and Namsawang (2015) Thailand	P	27[a] male healthy active (20.9 ± 1.1 years)	After plyometric training (3 sets of single leg bound, 6 sets of 30 m double leg bounds, 6 sets of 10 m tuck jumps and 5 sets of 10 drop jumps on a 60 cm box; maximum 10-s rest between each jump and 2-min rest between each set), EG (*n* = 13) performed passive static stretching for 20-min.	Single application	*n* = 13. Passive static stretching, 2× (5 × 30-s, with 5-s rest) per limb, with 1-min rest between sets. Until a sensation of stretch or resistance against the movement was felt; if this was not achieved, hip extension was added.	*n* = 14. 20-min CWI at 15 ± 1°C. [Stretching + CWI group excluded following PICOS criteria.]	Grant attributed by the National Research Council of Thailand. No mention to potential conflicts of interest.
Torres et al. (2005) Portugal	P	17[b] male healthy sedentary (21.2 ± 2.2 years)	After eccentric contractions for knee extensors, performed in an isokinetic dynamometer (2 sets of eccentric contractions until fatigue, 30-s rest in-between, at 80% maximum peak torque and 60°/s; range of motion fixed between 20° and 90° of knee flexion), EG (*n* = 9) performed passive static stretching for 6.5-min.	Single application	*n* = 9. Passive static stretching, 10 × 30-s per set, with 10-s rest between sets. Until resistance and/or discomfort were felt; if it was not felt, hip extension was added to knee flexion.	*n* = 8. Passive recovery (rest).	No mention to funding. No mention to potential conflicts of interest.
Torres et al. (2013) Portugal	P	28[c] male healthy untrained (21.4 ± 1.9 years)	After eccentric contractions for knee extensors, performed in an isokinetic dynamometer (2 sets of eccentric contractions until fatigue, 30-s rest in-between, at 80% maximum peak torque and 60°/s; range of motion fixed between 20° and 90° of knee flexion), EG (*n* = 14) performed passive static stretching for 6.5-min.	Single application	*n* = 14. Passive static stretching, 10 × 30-s per set, with 10-s rest between sets. Until resistance and/or discomfort were felt; if it was not felt, hip extension was added to knee flexion.	*n* = 14. Passive recovery (rest).	Grant attributed by the Foundation for Science and Technology, Portugal. No mention to potential conflicts of interest.
West et al. (2014) USA	CO	12 male healthy trained (21.3 ± 2.3 years)	29-km stationary cycling time trial, followed by 30-min of active static stretching.	1 session every 2 weeks, during ∼45 days	*n* = 12. Active static stretching, 3 × 30-s per set. Stretches held to the point of mild discomfort, but not pain.	*n* = 12 + 12. 30-min running in antigravity treadmill (40% VO_2peak, 75% bodyweight) + cycle ergometer (40% VO_2peak).	Partially funded by authors' lab, and by the company manufacturing the anti-gravity treadmill. Conflicts of interest not mentioned by the authors, but with financial support by the treadmill company.

P, Parallel; CO, Crossover; M/F, Male and Female; CWI, Cold-water immersion; EG, Experimental Group; HG, Handgrip; MVC, Maximum voluntary contraction; PNF, Proprioceptive neuromuscular facilitation; ROM, range of motion.
[a]After excluding the subjects of the multimodal recovery group, because it also included stretching and therefore had to be excluded due to PICOS; [b]After exclusion of Group 1, since stretching was the intervention per se, and not a post-exercise application; [c]After exclusion of Group 1, since stretching was the intervention per se, and not a post-exercise application, and after exclusion of group 4, which had multiple application/bouts of the recovery intervention.

TABLE 3 | Risk of bias in individual studies (worst-case scenario).

References	D1	D2	D3	D4	D5	DS	Overall
Bonfim et al. (2010)	!	!	+	-	+	N/A	-
Cè et al. (2013)	!	+	+	-	!	-	-
César et al. (2021)	!	+	+	+	!	N/A	!
Cooke et al. (2018)	!	+	+	-	!	N/A	-
Kokkinidis et al. (1998)	-	+	+	-	!	N/A	-
McGrath et al. (2014)	-	+	+	-	!	N/A	-
Mika et al. (2007)	!	+	+	+	!	+	!
Muanjai and Namsawang (2015)	-	+	-	-	!	N/A	-
Torres et al. (2005)	!	+	+	-	!	N/A	-
Torres et al. (2013)	!	+	+	-	!	N/A	-
West et al. (2014)	!	+	+	+	!	+	!

D1, Randomization process; D2, Deviations from intended intervention, effect of assignment to intervention; D3, Missing outcome data; D4, Measurement of the outcome; D5, Selection of the reported result; DS, Domain S, Bias arising from period and crossover effects; specific to crossover designs and not applicable to parallel trials; VAS, Visual analog scale; N/A, Not applicable; Colors: green means low risk of bias; yellow means some concerns; red means high risk of bias.

for each study, i.e., considering the outcome for which the risk of bias was higher.

These results can be visualized in **Figure 2,** which exhibits the percentage distribution of RoB for domains 1–5 and overall bias considering the worst assessment for each study. Overall RoB was high in 72.7% of the studies and presented some concerns in 27.3%. All studies presented problems with the randomization process: no description of how randomization was achieved and whether allocation sequence was properly concealed and, in 27.3% of the studies, baseline values suggested problems with the randomization process. Moreover, 72.7% of studies had high RoB in measurement of the outcome, mostly because testers were usually not blinded, and some outcomes were particularly prone to being influenced by knowledge of the intervention received.

There was low RoB arising from deviations from intended interventions and from missing outcome data in 90.9% of the papers. Finally, although 90.9% of papers presented some concerns for RoB arising from selection of the reported result, this resulted mostly from lack of pre-registered protocols, and our opinion upon reading the studies is that the authors provided an honest and complete reporting. Of the crossover studies, one had high RoB for carry-over effects (Cè et al., 2013) and, following protocol, was excluded from meta-analysis. However, it still integrated the qualitative review.

Results of Individual Studies

Primary outcomes were registered on the form of means ± SDs, except for Cè et al. (2013), that used means ± SEM. This study, in particular, had a graph from which we felt we could not extract reliable data. Allied to the fact that this study could not enter the meta-analytical calculations, we chose not to extract the data from the graph, and only present the qualitative results provided by the authors. For values extracted from graphs (Mika et al., 2007; Bonfim et al., 2010; Muanjai and Namsawang, 2015; Cooke et al., 2018; César et al., 2021), Cronbach's Alpha values were 0.991 (means) and 0.981 (SDs). The results of individual studies are compiled in **Table 4**.

Primary outcomes were any assessments related to strength, ROM and/or soreness, both short-term (i.e., until ≤1-h post-recovery) and delayed (24, 48, and 72 h post-recovery). These outcomes were useful only if there were pre-exercise and post-recovery assessments. Short-term effects were reported for strength-related measures in six studies (Torres et al., 2005, 2013; Mika et al., 2007; Cè et al., 2013; Muanjai and Namsawang, 2015; César et al., 2021), ROM in one study (McGrath et al., 2014; Muanjai and Namsawang, 2015), and DOMS in three studies (Torres et al., 2005, 2013; Muanjai and Namsawang, 2015). Three studies had no short-term assessments (Kokkinidis et al., 1998; Bonfim et al., 2010; West et al., 2014). One study mentioned having data at 15- and 30-min after recovery, but that data only applied to secondary outcomes (Cooke et al., 2018). With the exception of César et al. (2021), all strength-related assessments were performed for the lower limbs, and this was valid also for delayed assessments.

Delayed assessments were performed for strength-related variables in five studies (Torres et al., 2005, 2013; West et al.,

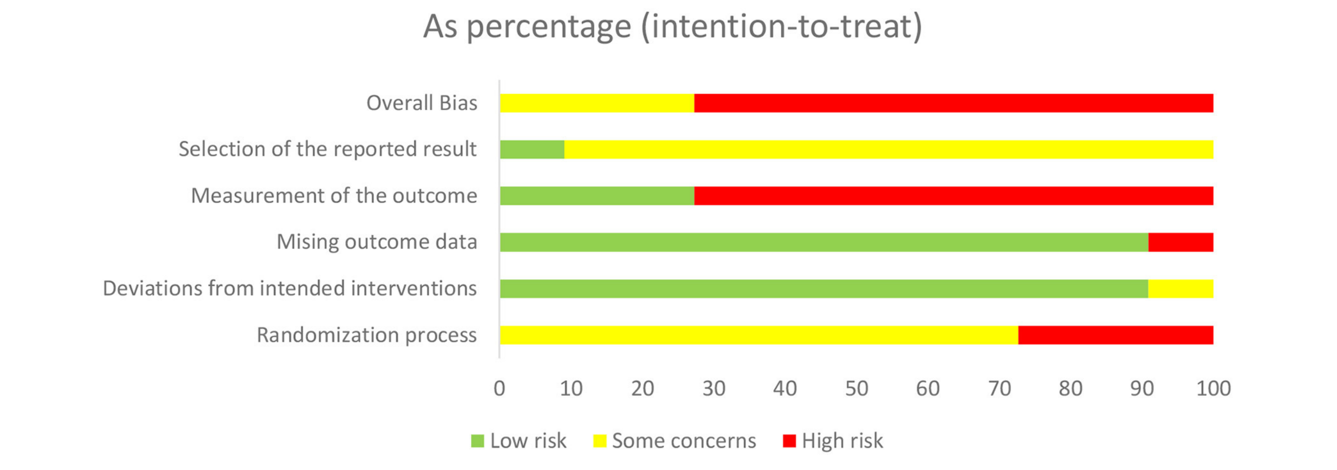

FIGURE 2 | Percentage distribution of risk of bias in individual studies (RoB 2).

2014; Muanjai and Namsawang, 2015; Cooke et al., 2018), ROM in one (Muanjai and Namsawang, 2015), and DOMS in seven (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Bonfim et al., 2010; McGrath et al., 2014; Muanjai and Namsawang, 2015; Cooke et al., 2018). Three studies did not have delayed outcomes (Mika et al., 2007; Cè et al., 2013; César et al., 2021). Although Kokkinidis et al. (1998) assessed delayed effects on strength and ROM, they presented only means, without any measure of variation that could help to better interpret the results. As previously explained, if the delayed assessments were conducted after a new bout of the recovery protocol, they would be discarded, as the effects of the first bout could no longer be assessed. Of the studies including delayed assessments, four had data for the three timepoints defined in our protocol (i.e., 24, 48, and 72 h) (Torres et al., 2005, 2013; Bonfim et al., 2010; Muanjai and Namsawang, 2015), one study had data for 24 and 48 h post-recovery protocol (McGrath et al., 2014), and three had data for 24 h post-recovery only (Kokkinidis et al., 1998; West et al., 2014; Cooke et al., 2018).

Based on their data, some studies concluded that post-exercise stretching was not an effective recovery strategy, and was not superior to comparator interventions (West et al., 2014; Cooke et al., 2018), including passive recovery, i.e., rest (Bonfim et al., 2010; Cè et al., 2013; César et al., 2021). In the study of Kokkinidis et al. (1998), the authors stated that stretching and cryotherapy were superior to passive rest, but these effects were not observed at 24 h, only at 48 h; moreover, after 24 h, the experimental groups had an additional recovery bout applied, but without the soreness-inducing exercise. In study of McGrath et al. (2014), PNF was not superior to passive recovery, and the static stretching group was the only one not showing significant decreases in DOMS at 24 or 48 h.

In the study of Mika et al. (2007), short-term strength levels recovered faster in the low-intensity cycling group than in the stretching or passive rest groups. In two studies (Torres et al., 2005, 2013), the authors stated that post-exercise stretching did not impair recovery in terms of strength and DOMS when compared to a passive rest group, but it did not improve recovery either. Finally, Muanjai and Namsawang (2015) concluded that both stretching and cold-water immersion could be used to improve post-exercise recovery. However, this conclusion is not sustained on their data, as DOMS only returned to baseline at 96 h post-recovery protocol, strength levels and ROM after 48 h, and vertical jump was still not back to baseline even after 96 h. Moreover, without a passive recovery group to compare to, no statement can be provided regarding acceleration of recovery.

Synthesis of Results

As stipulated in the protocol, cross-over trials would only be combined with parallel trials if there were no significant carryover effects (Elbourne et al., 2002). This was not guaranteed in the study of Cè et al. (2013), which was therefore excluded from meta-analysis. Across the remaining nine studies, as previously presented, there was considerable variation concerning the soreness-inducing protocols, the comparators to stretching, the outcome domains, the measurements within those outcome domains, and the timepoints of assessing the outcomes. Our protocol had stipulated three primary outcomes (strength, ROM, and DOMS) across four different timepoints (short-term, i.e., maximum 1 h after the recovery intervention; and 24, 48, and 72 h after the recovery intervention). After analyzing the outcomes and timepoints in each study, and also considering the comparator protocols, we found that only a few meta-analytical comparisons were feasible.

Short-Term Effects on Strength, Stretching vs. Passive Recovery (Rest)

Three studies had comparable data (i.e., strength measures of the knee extensors) to afford this meta-analysis (Torres et al., 2005, 2013; Mika et al., 2007). One study used PNF stretching (Mika et al., 2007) and the others used passive static stretching and compared this intervention to passive rest. Although the study of César et al. (2021) had strength assessments, they were for the upper limbs, more specifically grip strength, and so we decided not to compare it with the remaining studies. In RoB assessments considering this outcome, these studies had an

TABLE 4 | Results of individual studies.

References	Primary outcomes and timepoints post-recovery[a]	Intervention (pre-post)	Comparators (pre-post)	Main findings
Bonfim et al. (2010)	DOMS assessed through perceived pain at 24, 48, and 72 h. Two methods: VAS (0–10 scale) + pain dorimeter applied to medial gastrocnemius.	VAS: Pre = 0; 24 h = 2.87 ± 2.14; 48 h[c] = 3.47 ± 2.61; 72 h = 2.18 ± 2.04. Pain dorimeter: Pre = 8.48 ± 2.84; 24 h[c] = 6.55 ± 2.79; 48 h = 6.89 ± 5.02; 72 h = 7.4 ± 2.39.	*Passive recovery (rest)* VAS: Pre = 0; 24 h = 2.85 ± 1.70; 48 h = 3.49 ± 1.90; 72 h = 1.47 ± 1.38. Pain dorimeter: Pre = 9.26 ± 3.55; 24 h = 5.74 ± 1.87; 48 h = 6.89 ± 2.13; 72 h = 8.4 ± 1.98.	No between-group differences. Stretching was not effective in alleviating DOMS. No reporting of adverse effects.
Cè et al. (2013)	MVC of knee extensor muscles until 60-min post-exercise, stand-and-reach test [excluded due to insufficient information].	MVC remained depressed after the exercise bout until 60' after recovery in all trials, regardless of recovery protocol. Passive stretching cannot be considered an alternative to active recovery in accelerating lactate kinetics after fatiguing exercise. *Concrete data was not extracted for this study due to reasons explained in the manuscript. Also, potential problems with carry-over effects motivated the exclusion of meta-analysis.*		MVC remained depressed after the exercise bout until 60' after recovery in all trials, regardless of recovery protocol. No difference between conditions at any time point. No reporting of adverse effects.
César et al. (2021)	HG strength and HG muscle endurance, immediately post-recovery.	Maximal HG strength: Pre = 33.56 ± 7.19; Post = 28.25 ± 8.39. HG endurance: Pre = 54.87 ± 12.56; Post = 48.43 ± 18.7.	*CWI* Maximal HG strength: Pre = 33.22 ± 4.79; Post = 35.79 ± 4.45. HG endurance: Pre = 45.36 ± 13.18; Post = 44.75 ± 13.49. *Passive recovery* Maximal HG strength: Pre = 35.62 ± 6.67; Post = 34.42 ± 4.79. HG endurance: Pre = 47.82 ± 10.42; Post = 35.25 ± 14.41.	CWI promoted regeneration of HG strength and endurance, while stretching and passive recovery did not. No reporting of adverse effects.
Cooke et al. (2018)	MVC of knee extensor and flexor muscles, perceived DOMS (0–13 scale). 24 h post-recovery.	MVC ext. 60°/s: pre-exercise = 0.33 ± 0.09; 24 h = 0.31 ± 0.08. MVC ext. 180°/s: pre-exercise = 0.31 ± 0.05; 24 h = 0.29 ± 0.09. MVC fle. 60°/s: pre-exercise = 0.21 ± 0.05; 24 h = 0.20 ± 0.05. MVC fle. 180°/s: pre-exercise = 0.16 ± 0.06; 24 h = 0.16 ± 0.06. DOMS: pre-exercise = 1.2 ± 1.67; 24 h = 7.34 ± 1.60.	*Treadmill* MVC ext. 60°/s: pre-exercise = 0.35 ± 0.08; 24 h = 0.29 ± 0.08. MVC ext. 180°/s: pre-exercise = 0.39 ± 0.24; 24 h = 0.27 ± 0.12. MVC fle. 60°/s: pre-exercise = 0.21 ± 0.05; 24 h = 0.20 ± 0.07. MVC fle. 180°/s: pre-exercise = 0.16 ± 0.03; 24 h = 0.16 ± 0.05. DOMS: pre-exercise = 0.52 ± 1.36; 24 h = 7.75 ± 1.77. *Anti-gravity treadmill* MVC ext. 60°/s: pre-exercise = 0.32 ± 0.10; 24 h = 0.27 ± 0.12. MVC ext. 180°/s: pre-exercise = 0.24 ± 0.10; 24 h = 0.22 ± 0.09. MVC fle. 60°/s: pre-exercise = 0.20 ± 0.07; 24 h = 0.17 ± 0.06. MVC fle. 180°/s: pre-exercise = 0.14 ± 0.04; 24 h = 0.14 ± 0.04. DOMS: pre-exercise = 1.44 ± 1.43; 24 h = 6.52 ± 1.84.	MVC decreased in all groups until 24 h post-exercise, while DOMS increased. No between-group differences for any outcome at any time point. No reporting of adverse effects.

(Continued)

TABLE 4 | Continued

References	Primary outcomes and timepoints post-recovery[a]	Intervention (pre-post)	Comparators (pre-post)	Main findings
Kokkinidis et al. (1998)	Muscle pain/DOMS (VAS 1–10). *1 RM knee flexion and sit-and-reach were excluded because only the mean was presented, without variation measures. 24 h post-recovery*.	DOMS: pre-exercise = 1 ± 0; 24 h = 4.8 ± 1.1.	*Passive recovery (rest)* DOMS: pre-exercise = 1 ± 0; 24 h = 5.0 ± 1.2. *Cryotherapy* DOMS: pre-exercise = 1 ± 0; 24 h = 4.3 ± 0.4.	All groups had increased DOMS at 24 h post-exercise. Stretching and cryotherapy were not effective in diminishing DOMS in comparison with passive recovery. No reporting of adverse effects.
McGrath et al. (2014)	Sit-and-reach, muscle Soreness Scale (1–6) at 24 and 48 h. Sit-and-reach also immediately post-recovery.	Sit-and-reach: pre-exercise = 6.0 ± 9.7; immediately post-exercise = 8.5 ± 9.7. DOMS: pre-exercise = 0; 24 h = 2.3 ± 1.1; 48 h = 2.0 ± 1.1	*PNF* Sit-and-reach: pre-exercise = 5.2 ± 10.2; immediately post-exercise = 6.2 ± 10.3. DOMS: pre-exercise = 0; 24 h = 2.2 ± 0.8; 48 h = 1.6 ± 1.0. *Passive recovery (rest)* Sit-and-reach: pre-exercise = 7.9 ± 9.7; immediately post-exercise = 9.0 ± 9.4. DOMS: pre-exercise = 0; 24 h = 1.9 ± 0.9; 48 h = 1.7 ± 0.9.	Stretching group had no significant decrease in DOMS at 24 and 48 h, while the comparator groups had at 48 h. Static stretching impaired recovery in comparison with PNF and passive recovery. No reporting of adverse effects.
Mika et al. (2007)	Isometric knee extension at 50% of MVC to the point of fatigue, static knee extension at 78°, while sitting (MedX leg-extension dynamometer), immediately post-recovery.	MVC: pre-exercise = 224.21 ± 70.43; immediately post-exercise = 205.84 ± 78.85.	*Cycling* MVC: pre-exercise = 224.21 ± 70.43; immediately post-exercise = 214.26 ± 97.99. *Passive recovery (rest)* MVC: pre-exercise = 224.21 ± 70.43; immediately post-exercise = 207.37 ± 55.12.	MVC was significantly higher after cycling than after stretching or passive recovery. No reporting of adverse effects.
Muanjai and Namsawang (2015)	Soreness sensation (0–100 VAS) during knee extensor MVC and stretching, active ROM (knee flexion), knee extensors isometric MVC at 90°, vertical jump. Immediately post-recovery, as well as at 24, 48, and 72 h[b].	MVC: pre-exercise = 0.39 ± 0.1; post-recovery = 0.27 ± 0.04; 24 h = 0.21 ± 0.04; 48 h = 0.31 ± 0.02; 72 h = 0.35 ± 0.04. Knee flexion ROM: pre-exercise = 131.7 ± 5.5; post-recovery: 128.2 ± 3.5; 24 h = 123.9 ± 3.0; 48 h = 126.6 ± 3.0; 72 h = 129.4 ± 1.9. Vertical jump high: pre-exercise = 56.5 ± 8.9; post-recovery = 51.4 ± 5.7; 24 h = 48.4 ± 5.7; 48 h = 49.9 ± 3.3; 72 h = 51.8 ± 5.1. Soreness on MVC: pre-exercise = 0 ± 0; post-recovery = 5.33 ± 7.61; 24 h = 20.05 ± 15.48; 48 h = 14.21 ± 19.55; 72 h = 7.11 ± 9.64. Soreness on passive stretching: pre-exercise = 0 ± 0; post-recovery = 2.94 ± 3.64; 24 h = 16.62 ± 16.8; 48 h = 3.98 ± 5.2; 72 h = 3.64 ± 6.23.	*CWI* MVC: pre-exercise = 0.38 ± 0.1; post-recovery = 0.30 ± 0.04; 24 h = 0.25 ± 0.04; 48 h = 0.30 ± 0.04; 72 h = 0.35 ± 0.03. Knee flexion ROM: pre-exercise = 133.8 ± 4.4; post-recovery = 129.2 ± 3.4; 24 h = 126.3 ± 6.6; 48 h = 127.2 ± 4.7; 72 h = 130.1 ± 2.9. Vertical jump height: pre-exercise = 54.6 ± 6.3; post-recovery = 43.4 ± 5.5; 24 h = 45.0 ± 5.1; 48 h = 46.9 ± 4.9; 72 h = 50.0 ± 4.2. Soreness on MVC: pre-exercise = 0 ± 0; post-recovery = 8.63 ± 11.93; 24 h = 25.89 ± 25.89; 48 h = 17.13 ± 17.39; 72 h = 10.15 ± 13.20. Soreness on passive stretching: pre-exercise = 0 ± 0; post-recovery = 12.99 ± 21.99; 24 h = 15.93 ± 20.43; 48 h = 15.76 ± 22.85; 72 h = 8.83 ± 15.59.	For both groups, soreness increased after exercise, peaked at 24 h, and gradually returned to baseline levels at 96 h. MVC of knee extensors had the lowest peak value at 24 h, having returned to baseline at 48 h. Vertical jump started recovering immediately post-exercise, but was not back to baseline even at 96 h. No differences between groups. Explicit statement reporting there were no adverse effects.
Torres et al. (2005)	DOMS through perceived pain (VAS), maximal eccentric peak torque (knee extensors). 1, 24, 48, and 72 h[b].	Maximal eccentric peak torque: pre-exercise = 303.1 ± 59.96; 1 h = 231.1 ± 49.1; 24 h = 266.5 ± 57.5; 48 h = 275.3 ± 54.2; 72 h = 285.7 ± 63.7. DOMS: pre-exercise = 0.0 ± 0.0; 1 h = 0.6 ± 0.7; 24 h = 4.1 ± 1.2; 48 h = 5.7 ± 1.8; 72 h = 3.6 ± 2.0.	*Passive recovery (rest)* Maximal eccentric peak torque: pre-exercise = 352.6 ± 76.49; 1 h = 276.1 ± 66.3; 24 h = 294.9 ± 65.7; 48 h = 308.2 ± 65.5; 72 h = 318.1 ± 75.1. DOMS: pre-exercise = 0.0 ± 0.0; 1 h = 0.6 ± 0.5; 24 h = 3.2 ± 0.6; 48 h = 5.3 ± 1.5; 72 h = 2.8 ± 1.6.	DOMS began in the first hour post-exercise, achieved a peak at 48 h, and pain to palpation was still present at 96 h. No differences between the groups at any time point. No reporting of adverse effects.

(Continued)

TABLE 4 | Continued

References	Primary outcomes and timepoints post-recovery[a]	Intervention (pre-post)	Comparators (pre-post)	Main findings
Torres et al. (2013)	Muscle soreness (VAS), maximal concentric peak torque (knee extensors). 1, 24, 48, and 72 h[b].	Muscle soreness: pre-exercise = 0.0 ± 0.0; 1 h = 1.2 ± 0.7; 24 h = 2.3 ± 1.1; 48 h = 3.5 ± 1.4; 72 h = 1.8 ± 1.3. Maximal concentric peak torque (60°/s): pre-exercise = 216.9 ± 33.5; 1 h = 173.4 ± 33.7; 24 h = 183.2 ± 42.5; 48 h = 189.7 ± 44.5; 72 h = 204.7 ± 42.6.	*Passive recovery (rest)* Muscle soreness: pre-exercise = 0.0 ± 0.0; 1 h = 0.1 ± 0.4; 24 h = 2.3 ± 0.8; 48 h = 3.8 ± 1.8; 72 h = 1.8 ± 1.2. Maximal concentric peak torque (60°/s): pre-exercise = 221.3 ± 16.7; 1 h = 181.7 ± 30.7; 24 h = 186.8 ± 27.0; 48 h = 188.5 ± 40.3; 72 h = 203.8 ± 29.3.	Significant reduction in maximal concentric peak torque and significant increases in muscle soreness. No differences between groups at any time points. No reporting of adverse effects.
West et al. (2014)	Peak power output, mean power output, time to peak power and rate to fatigue (supramaximal 30-s cycle ergometer test) at 24 h post-exercise.	Peak power: pre-exercise = 1,323 ± 323; 24 h = 1,431 ± 429. Mean power: pre-exercise = 731 ± 114.2; 24 h = 718.5 ± 125.7. Time to peak: pre-exercise = 4.68 ± 0.83; 24 h = 4.43 ± 0.49. Rate to fatigue: pre-exercise = 36.17 ± 12.5; 24 h = 40.71 ± 15.5.	*Anti-gravity treadmill* Peak power: pre-exercise = 1,323 ± 323; 24 h = 1,372 ± 364. Mean power: pre-exercise = 731 ± 114.2; 24 h = 707.7 ± 115.8. Time to peak: pre-exercise = 4.68 ± 0.83; 24 h = 4.59 ± 0.70. Rate to fatigue: pre-exercise = 36.17 ± 12.5; 24 h = 38.72 ± 12.8 *Cycle ergometer* Peak power: pre-exercise = 1,323 ± 323; 24 h = 1,411 ± 355. Mean power: pre-exercise = 731 ± 114.2; 24 h = 705.3 ± 127.6. Time to peak: pre-exercise = 4.68 ± 0.83; 24 h = 4.38 ± 0.53. Rate to fatigue: pre-exercise = 36.17 ± 12.5; 24 h = 40.15 ± 12.3.	In all groups, no differences in relation to baseline. No differences between groups. No reporting of adverse effects.

CWI, Cold-water immersion; DOMS, Delayed onset muscular soreness (more is worse); HG, Handgrip; MVC, Maximum voluntary contraction; ROM, Range of motion; VAS, Visual Analog Scale (greater values mean worse outcomes).
[a]As defined in our protocol. [b]96 h not considered, as per protocol.

overall classification of "some concerns," meaning none of the domains presented high RoB. In domain 4 (measurement of the outcome), they had low RoB.

For within-group effects, three studies provided data for short-term strength recovery, involving three stretching groups (pooled $n = 33$). Results showed that post-exercise stretching protocols did not allow participants to recover their basal strength level (ES = −0.85; 95% CI = −1.53 to −0.17; $p = 0.015$; $I^2 = 80.4\%$; Egger's test $p = 0.396$; **Figure 3**).

In addition, three studies provided data for short-term strength recovery, involving three passive recovery groups (pooled $n = 32$). Results showed that post-exercise passive recovery protocols did not allow participants to recover their basal strength level (ES = −0.81; 95% CI = −1.46 to −0.15; $p = 0.016$; $I^2 = 78.7\%$; Egger's test $p = 0.435$; **Figure 4**).

Between-group comparisons (pooled $n = 65$) showed no effect of post-exercise stretching protocols on strength recovery (ES = −0.08; 95% CI = −0.54 to 0.39; $p = 0.750$; $I^2 = 0.0\%$; Egger's test $p = 0.531$; **Figure 5**) when compared to control condition (i.e., passive recovery).

Delayed Effects (24 h) on Delayed Onset Muscle Soreness, Stretching vs. Passive Recovery (Rest)

Five studies had comparable data to assess DOMS at 24 h (Kokkinidis et al., 1998; Torres et al., 2005, 2013; Bonfim et al., 2010; McGrath et al., 2014). Two used active static stretching (Kokkinidis et al., 1998; Bonfim et al., 2010) and three passive static stretching (Torres et al., 2005, 2013; McGrath et al., 2014). All had at least one comparator that passively recovered (i.e., rest). The study of Bonfim et al. (2010) had two assessments of DOMS; here, we used the assessment through the visual analog scale, as the other studies also used similar scales. The four studies had high RoB in measurement of this outcome, so all results should be considered with caution.

For within-group comparisons, five studies provided data for 24-h post-exercise DOMS, involving five experimental groups (pooled $n = 57$). Results showed that post-exercise DOMS remained significantly above basal levels after post-exercise stretching protocols (ES = 1.55; 95% CI = 1.12–1.97; $p < 0.001$; $I^2 = 48.3\%$; Egger's test $p = 0.231$; **Figure 6**).

In addition, five studies provided data for 24-h post-exercise DOMS, involving five control groups (pooled $n = 54$). Results showed that passive recovery protocols did not allow participants to recover their basal DOMS level (ES = 1.87; 95% CI = 1.28–2.46; $p < 0.001$; $I^2 = 64.6\%$; Egger's test $p = 0.119$; **Figure 7**).

Between-group comparisons involved five experimental and five control groups (pooled $n = 111$). Results showed no effect of post-exercise stretching protocols on 24-h post-exercise DOMS (ES = −0.24; 95% CI = −0.60–0.12; $p = 0.187$; $I^2 = 0.0\%$; Egger's test $p = 0.880$; **Figure 8**) when compared to control conditions (i.e., passive recovery).

Delayed Effects (48 h) on Delayed Onset Muscle Soreness, Stretching vs. Passive Recovery (Rest)

Four studies had comparable data (Torres et al., 2005, 2013; Bonfim et al., 2010; McGrath et al., 2014). One used active static stretching (Bonfim et al., 2010) and three passive static stretching (Torres et al., 2005, 2013; McGrath et al., 2014). All had at least one comparator that passively recovered (i.e., rest). With regard to RoB, four studies had high RoB in measurement of this outcome.

Four studies provided data for within-group comparisons on 48-h post-exercise DOMS, involving four experimental groups (pooled $n = 53$). Results showed that post-exercise DOMS remained significantly above basal levels after post-exercise stretching protocols (ES = 1.50; 95% CI = 1.02–1.98; $p < 0.001$; $I^2 = 59.8\%$; Egger's test $p = 0.257$; **Figure 9**).

Four studies provided data for 48-h post-exercise DOMS, involving four control groups (i.e., passive recovery) (pooled $n = 50$). Results showed that post-exercise passive recovery protocols did not allow participants to recover their basal DOMS level (ES = 1.52; 95% CI = 1.17–1.87; $p < 0.001$; $I^2 = 18.3\%$; Egger's test $p = 0.120$; **Figure 10**).

For between-group comparisons, four studies provided data for 48-h post-exercise DOMS, involving four experimental and four control groups (pooled $n = 103$). Results showed no effect of post-exercise stretching protocols on 48-h post-exercise DOMS (ES = −0.09; 95% CI = −0.47–0.28; $p = 0.629$; $I^2 = 0.0\%$; Egger's test $p = 0.777$; **Figure 11**) when compared to control conditions (i.e., passive recovery).

Delayed Effects (72 h) on Delayed Onset Muscle Soreness, Stretching vs. Passive Recovery (Rest)

Three studies had comparable data for DOMS at 72 h (Torres et al., 2005, 2013; Bonfim et al., 2010). One used active static stretching (Bonfim et al., 2010) and two passive static stretching (Torres et al., 2005, 2013). With regard to RoB, the three studies had high RoB in measurement of this outcome.

For within-group analysis, three studies provided data for 72-h post-exercise DOMS, involving three experimental groups (pooled $n = 33$). Results showed that post-exercise DOMS remained significantly above basal levels after post-exercise stretching protocols (ES = 0.98; 95% CI = 0.67–1.28; $p < 0.001$; $I^2 = 0.0\%$; Egger's test $p = 0.525$; **Figure 12**).

Three studies provided data for 72-h post-exercise DOMS, involving three passive recovery groups (pooled $n = 32$). Results showed that post-exercise passive recovery protocols did not allow participants to recover their basal DOMS level (ES = 0.99; 95% CI = 0.68–1.30; $p < 0.001$; $I^2 = 0.0\%$; Egger's test $p = 0.641$; **Figure 13**).

For between-group comparisons, three studies provided data for 72-h post-exercise DOMS, involving three experimental and three control groups (pooled $n = 65$). Results showed no effect of post-exercise stretching protocols on 72-h post-exercise DOMS (ES = −0.23; 95% CI = −0.70–0.24; $p = 0.337$; $I^2 = 0.0\%$; Egger's test $p = 0.165$; **Figure 14**) when compared to control conditions (i.e., passive recovery).

Additional Analysis

Due to the small number of studies included in each meta-analysis, additional analysis, and sensitivity analyses were not performed. In each analysis, RoB was similar in all studies, and so

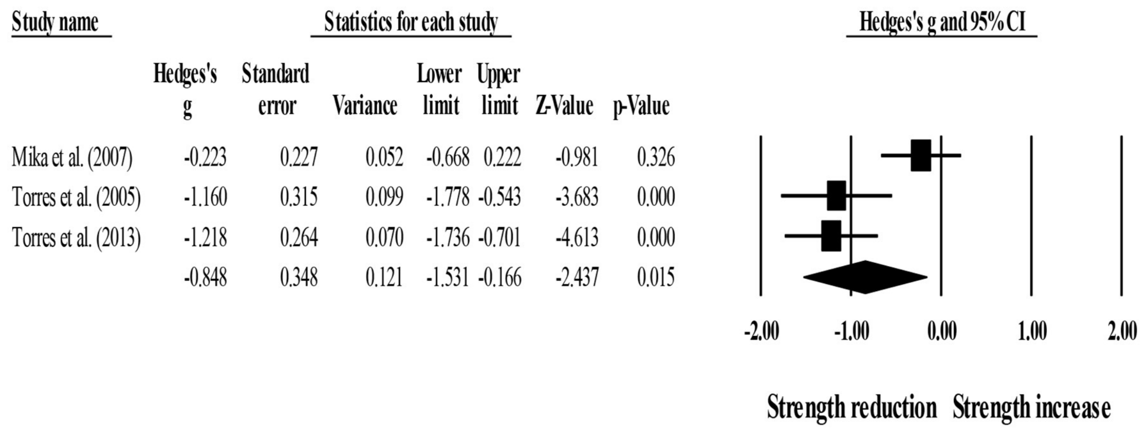

FIGURE 3 | Forest plot denoting short-term strength recovery level in participants that completed post-exercise stretching protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: negative values denote that post-exercise stretching protocols did not allow participants to recover their basal strength level (i.e., 0.00 in the figure).

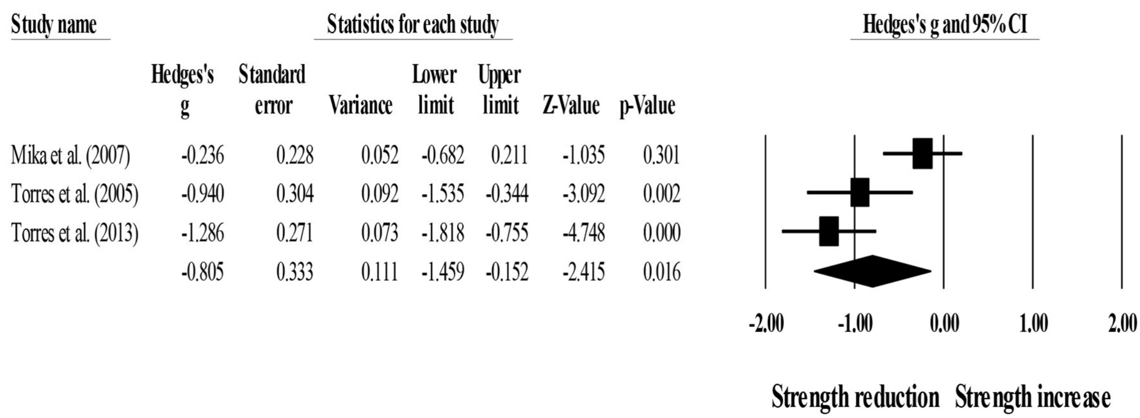

FIGURE 4 | Forest plot denoting short-term strength recovery level in participants that completed post-exercise passive recovery protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: negative values denote that post-exercise passive recovery protocols did not allow participants to recover their basal strength level (i.e., 0.00 in the figure).

we decided not to assess the effects of RoB on the results. Meta-regression was not performed due to having <10 studies with sufficient commonalities.

Confidence in Cumulative Evidence

Confidence in cumulative is equivalent to quality of the evidence (Higgins et al., 2019). GRADE assessments are presented in **Table 5**. Overall, we have very little confidence in the effect estimate, and the true effect is likely to be substantially different from the estimate of effect.

DISCUSSION

Summary of Evidence

Stretching has been traditionally prescribed for the cool-down phase of training sessions, under the premise that it enhances recovery (ACSM, 2018; American Heart Association, 2020). But this premise has been questioned by previous assessments of the literature (Herbert and Gabriel, 2002; Henschke and Lin, 2011; Herbert et al., 2011). Therefore, we have conducted a systematic review with meta-analysis of supervised RCTs on the effects of post-exercise stretching on short-term (i.e., ≤1 h) and delayed (24, 48, and 72 h) recovery of strength levels, ROM, and DOMS. Searches were conducted in eight electronic databases post-protocol approval, on December 23 and 24 of 2020, and updated on February 16, 2021. Of the 17,050 records emerging from the searches and 25 additional records emerging from manual searches within reference lists, 11 RCTs were eligible for qualitative analysis ($n = 289$), and 10 for quantitative analyses ($n = 280$, with $n = 229$ after excluding groups not fulfilling PICOS criteria). Due to the overall small sample size, generalization to a broader population is not advised.

Active static stretching, passive stretching and PNF were used for post-exercise recovery, but no protocol adopted dynamic

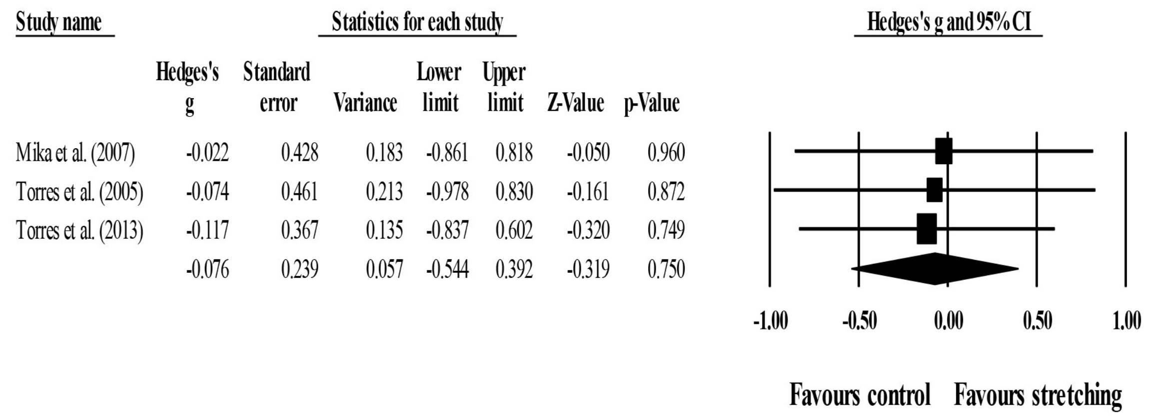

FIGURE 5 | Forest plot of changes in short-term strength recovery after participating in post-exercise stretching protocols compared to control conditions (i.e., passive recovery). Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result.

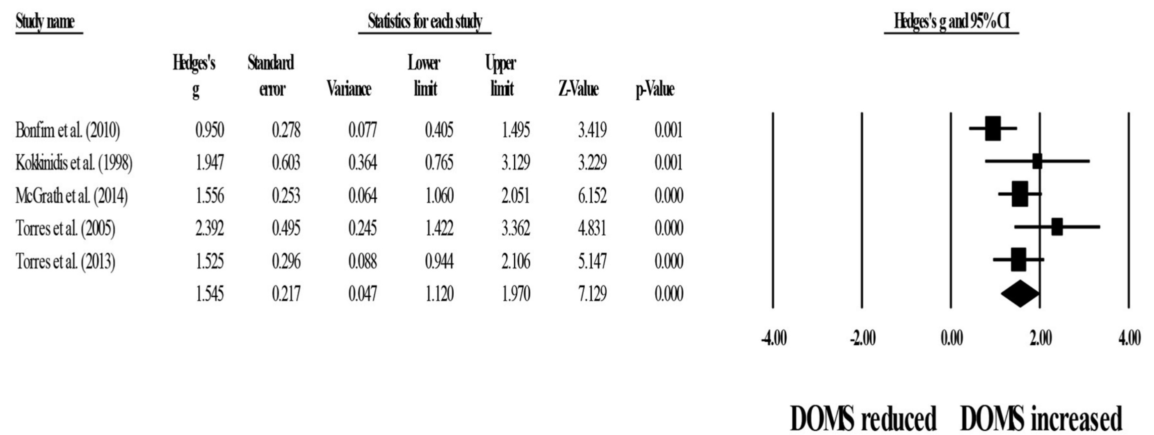

FIGURE 6 | Forest plot denoting 24-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed post-exercise stretching protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that post-exercise stretching protocols did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

stretching. Overall, analysis of individual studies showed that there was no evidence that stretching enhanced recovery in comparison to passive recovery (i.e., rest) or to alternative recovery modalities, such as cycling and cold-water immersion. There was no evidence to the contrary, i.e., that stretching impaired recovery. Even for secondary outcomes, such as blood lactate and serum creatine kinase, for example, no strong case can be made for stretching accelerating or improving recovery. Furthermore, overall RoB was high, meaning that this field of research is lacking in terms of methodological design. Especially problematic was the wide use of unblinded testers, even for outcomes with greater degree of subjectivity.

Due to the diversity of outcomes and timepoints of assessments, only four meta-analytical comparisons were possible, all between stretching and passive recovery (i.e., rest): strength levels at ≤1 h, and DOMS at 24, 48, and 72 h. Overall, stretching was no more effective than passive recovery in returning strength levels and DOMS to baseline values. Heterogeneity of the meta-analysis (I^2) was high for within-group (pre-post) comparisons and low for between-group comparisons for strength outcomes at ≤1 h of recovery, moderate (within) and low (between) for DOMS at 24 h, low to moderate (within) and low (between) for DOMS at 48 h, and low (within and between) for DOMS at 72 h. Information in terms of recovery of ROM after different recovery protocols was insufficient to run a meta-analysis. There was no evidence of publication bias.

Poor External Validity

Overall, the studies included in our analysis may be considered to have poor external validity. In terms of population, they only apply to adults under 40-years-old, with no studies being performed in children, teenagers or adults older ≥40-years-old. And only two of the 11 studies included women in their sample:

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	1.188	0.304	0.092	0.592	1.784	3.906	0.000
Kokkinidis et al. (1998)	1.879	0.587	0.344	0.729	3.029	3.203	0.001
McGrath et al. (2014)	1.563	0.267	0.071	1.040	2.086	5.861	0.000
Torres et al. (2005)	3.676	0.752	0.566	2.202	5.151	4.886	0.000
Torres et al. (2013)	2.098	0.364	0.132	1.385	2.811	5.768	0.000
	1.867	0.302	0.091	1.275	2.459	6.177	0.000

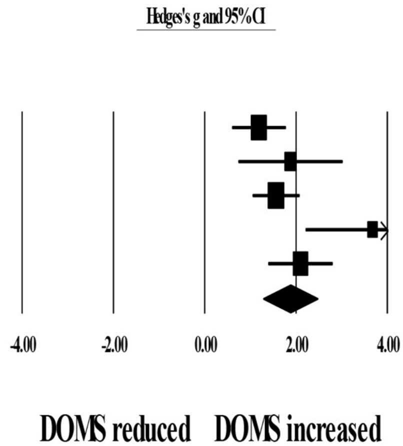

FIGURE 7 | Forest plot denoting 24-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed passive recovery (control conditions) protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that passive recovery protocols did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	-0.010	0.428	0.183	-0.849	0.830	-0.023	0.982
Kokkinidis et al. (1998)	0.151	0.616	0.379	-1.056	1.358	0.245	0.806
McGrath et al. (2014)	-0.388	0.321	0.103	-1.017	0.242	-1.207	0.228
Torres et al. (2005)	-0.883	0.485	0.236	-1.834	0.068	-1.819	0.069
Torres et al. (2013)	0.000	0.367	0.135	-0.719	0.719	0.000	1.000
	-0.243	0.184	0.034	-0.604	0.118	-1.319	0.187

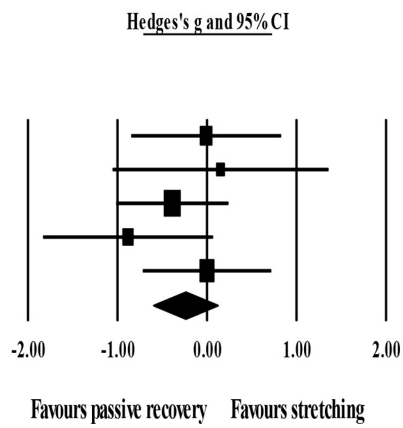

FIGURE 8 | Forest plot of changes in 24-h post-exercise delayed onset of muscle soreness (DOMS) after participating in post-exercise stretching protocols compared to control conditions (i.e., passive recovery). Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result.

50% of the sample in one study (McGrath et al., 2014) and unclear in another (Bonfim et al., 2010). As such, current results derive mainly from studies with men. As all subjects were healthy, it is unclear how subjects with injuries and/or pathologies would respond. Furthermore, only two studies included recreationally trained subjects (West et al., 2014) or athletes (César et al., 2021).

The nature of the exercise protocols (pre-recovery) presents a number of problems that limit their external validity as well. While most studies used protocols that were likely to induce DOMS, in real-life settings coaches are unlikely to regularly try to elicit DOMS in their athletes or patients. And since most studies did not assess athletes, it is possible that results from the fatigue-inducing protocols have been somewhat artificial, as most were conducted with populations not engaged in regular, structured physical activity, and thereby less well-adapted to the acute effects of fatiguing exercise. Lack of familiarity with the protocols may have exacerbated this effect. Moreover, the protocols were single-component or even single exercise, while real-life exercise sessions will more likely involve multiple components and/or multiple exercises. Also, most of the knowledge derives from studies focusing on the lower limbs, with only one study having assessed the effects of the upper limbs (César et al., 2021).

With one exception (Cooke et al., 2018), the fatigue-inducing protocols had very short durations, usually well below 30 min. Hardly will a real-life exercise session last ≤30 min, especially with athletic populations. Conversely the duration of recovery protocols was excessive in many cases, even reaching 30 min in duration (West et al., 2014; Cooke et al., 2018). The combination of very short exercise sessions with long recovery sessions does not seem practical. Also, six studies (∼55%) used individualized passive stretching. This means that one supervisor is required

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	0.942	0.277	0.077	0.399	1.485	3.399	0.001
McGrath et al. (2014)	1.353	0.235	0.055	0.893	1.813	5.763	0.000
Torres et al. (2005)	2.216	0.467	0.218	1.301	3.132	4.746	0.000
Torres et al. (2013)	1.823	0.330	0.109	1.176	2.471	5.518	0.000
	1.499	0.247	0.061	1.015	1.982	6.074	0.000

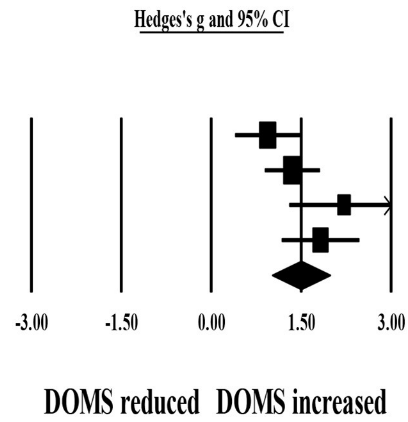

FIGURE 9 | Forest plot denoting 48-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed post-exercise stretching protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that post-exercise stretching protocols did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	1.301	0.318	0.101	0.679	1.924	4.096	0.000
McGrath et al. (2014)	1.399	0.251	0.063	0.907	1.891	5.572	0.000
Torres et al. (2005)	2.434	0.530	0.281	1.394	3.474	4.588	0.000
Torres et al. (2013)	1.540	0.298	0.089	0.956	2.124	5.168	0.000
	1.520	0.177	0.031	1.173	1.868	8.572	0.000

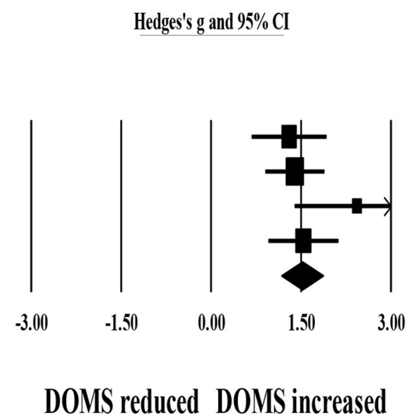

FIGURE 10 | Forest plot denoting 48-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed post-exercise passive recovery. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that post-exercise passive recovery did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

for every practitioner, something that will hardly be possible to implement in physical education classes, sports training, and even for the general gym-going population (exceptions would be those with access to a personal trainer).

Data Is Scarce, Heterogeneous, and Does Not Support Existing Guidelines

Considering that stretching is so often prescribed as a valid protocol for enhancing post-exercise recovery (ACSM, 2018), the reduced number of studies ($n = 11$) and small overall sample ($n = 289$) emerging from our searches, allied with a considerable diversity of exercise and post-exercise recovery protocols, demonstrate that data is too scarce and heterogeneous to support existing guidelines. Although absence of evidence is not evidence of absence, world-leading organizations should encourage further research in this field before promoting more definitive recommendations. Recommendations should not be provided in the absence of empirical support. At a minimum, guidelines should acknowledge that prescribing post-exercise stretching as a means of improving recovery is based on belief and not on data. In fact, enhancing recovery implies that recovery is accelerated and/or improved if post-exercise stretching is applied than if passive recovery (i.e., rest) is used. Our data does not sustain this belief. Indeed, >70% of the analyzed studies had one group performing passive recovery (i.e., rest), and stretching did not prove to improve recovery when compared to those controls. Perhaps the eventual benefits of post-exercise stretching are balanced by the extra fatigue that they add, although further research is required to better explore the mechanistic phenomena underlying these effects.

We strongly suggest that science should abide by the burden of proof. Until more (and better) data is collected, no case should be built for (or against) post-exercise stretching with the goal

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	0.008	0.428	0.183	-0.831	0.848	0.020	0.984
McGrath et al. (2014)	-0.291	0.320	0.102	-0.917	0.336	-0.909	0.363
Torres et al. (2005)	-0.228	0.463	0.214	-1.135	0.679	-0.492	0.623
Torres et al. (2013)	0.181	0.368	0.135	-0.540	0.901	0.491	0.623
	-0.092	0.191	0.037	-0.468	0.283	-0.483	0.629

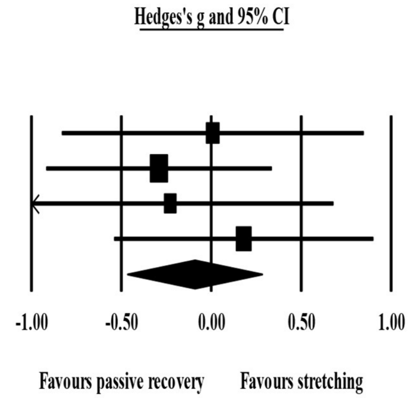

FIGURE 11 | Forest plot of changes in 48-h post-exercise delayed onset of muscle soreness (DOMS) after participating in post-exercise stretching protocols compared to control conditions (i.e., passive recovery). Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result.

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	0.757	0.260	0.067	0.248	1.266	2.917	0.004
Torres et al. (2005)	1.260	0.328	0.107	0.618	1.902	3.846	0.000
Torres et al. (2013)	1.010	0.245	0.060	0.531	1.489	4.129	0.000
	0.975	0.156	0.024	0.669	1.282	6.235	0.000

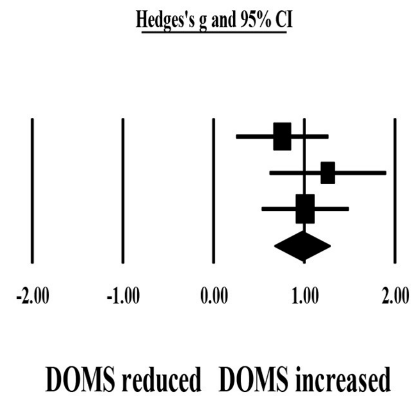

FIGURE 12 | Forest plot denoting 72-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed post-exercise stretching protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that post-exercise stretching protocols did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

of improving recovery. Admittedly, post-exercise stretching may have other goals than improving recovery, but these were not addressed in our analysis.

What's Different in Relation to Previous Systematic Reviews on the Topic?

As mentioned in the introduction, previous SRMA addressed the topic of post-exercise stretching (Herbert and Gabriel, 2002; Henschke and Lin, 2011; Herbert et al., 2011). However, important differences in design exist in comparison with our review, beyond the natural update: (i) these reviews assessed the effects of both post- and pre-exercise stretching, while we focused solely on post-exercise stretching; (ii) they assessed the effects of stretching on DOMS and risk of injury, while we focused on DOMS, strength levels, and ROM; (iii) finally, they accepted non-randomized studies, while our review was limited to randomized studies; (iv) furthermore, we consulted more databases than those reviews. Therefore, it is not surprising that the list of included articles is largely different. Still, our review reinforces previous conclusions that post-exercise stretching does not confer protection from DOMS, while also showing that it does not accelerate (nor impairs) recovery and strength levels or ROM.

LIMITATIONS

The limited number of studies; the high RoB and high heterogeneity, allied to the diversity of designs and poor external validity advise against more definitive conclusions. Moreover, the included studies solicited extremely varied stretching intensities, but all were based in vague sentences to suggest the subjects the degree of stretching intended. And if stretching intensity is not properly described, any comparisons can be limited (Sands et al., 2013). Instead, we believe that stretching intensity could be more rigorously assessed with instruments such as the Stretching Intensity Scale (Freitas et al., 2015).

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	0.755	0.259	0.067	0.247	1.263	2.911	0.004
Torres et al. (2005)	1.205	0.337	0.114	0.544	1.866	3.574	0.000
Torres et al. (2013)	1.094	0.252	0.064	0.600	1.589	4.338	0.000
	0.991	0.159	0.025	0.679	1.303	6.218	0.000

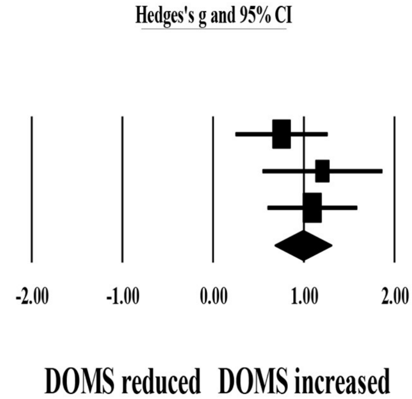

FIGURE 13 | Forest plot denoting 72-h post-exercise delayed onset of muscle soreness (DOMS) in participants that completed post-exercise passive recovery protocols. Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result. Note: positive values denote that post-exercise passive recovery protocols did not allow participants to recover their basal DOMS level (i.e., 0.00 in the figure).

Study name	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bonfim et al. (2010)	-0.390	0.433	0.187	-1.239	0.458	-0.902	0.367
Torres et al. (2005)	-0.416	0.467	0.218	-1.331	0.498	-0.892	0.372
Torres et al. (2013)	0.000	0.367	0.135	-0.719	0.719	0.000	1.000
	-0.230	0.240	0.058	-0.701	0.240	-0.959	0.337

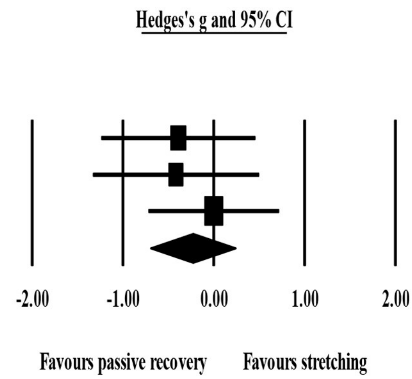

FIGURE 14 | Forest plot of changes in 72-h post-exercise delayed onset of muscle soreness (DOMS) after participating in post-exercise stretching protocols compared to control conditions (i.e., passive recovery). Values shown are effect sizes (Hedges's g) with 95% confidence intervals (CI). The size of the plotted squares reflects the statistical weight of each study. The black diamond reflects the overall result.

TABLE 5 | GRADE assessment for the certainty of evidence.

Outcomes[a]	Study design	Risk of bias in individual studies	Publication bias	Inconsistency	Indirectness	Imprecision	Confidence in evidence	Recommendation
Strength, ROM and DOMS	11 RCTs and 289 participants.	High[b]	No publication bias detected[c]	High[d]	High[e]	High[f]	⊕ Very low.	No recommendation can be provided on the basis of existing data.

[a] *Outcomes were grouped as their assessments were not different.*
[b] *Detailed assessments in* ***Table 3****.*
[c] *Assessed through extended Egger's test.*
[d] *Assessed through I^2, but also considering qualitative analysis from studies not included in meta-analysis. Because the outcomes are continuous variables, high heterogeneity was expected. Heterogeneity also likely emerged from very distinct study designs in terms of soreness-inducing protocols, as well as modality and dosage of post-exercise recovery protocols. Adverse effects were mostly unreported.*
[e] *Studies were mainly limited to sedentary or recreationally active subjects, while athletes and populations with pathologies are not included. Second, all measures provide only indirect assessments of the more complex phenomena of recovery.*
[f] *While some imprecision is expected due to referring to continuous variables, two additional factors weighted on this decision: small sample size and wide confidence intervals, generating uncertainty about magnitude of effect.*
ROM, Range of motion; DOMS, Delayed onset muscular soreness; RCTs, randomized controlled trials.

CONCLUSIONS

Overall, our data does not support nor contradicts the utilization of post-exercise stretching. Notwithstanding, if post-exercise stretching does not seem to enhance recovery in relation to passive recovery (i.e., rest), the implementation of the former among participants or athletes is, at least, questionable. Still, data is scarce, heterogenous, and overall confidence in cumulative evidence is very low. For now, recommendations on whether post-exercise stretching should be applied for the purposes of recovery are misleading, as the (insufficient) data that is available does not support those claims.

We suggest that future research on post-exercise recovery always pre-registers the protocol and adopts a randomized design, with proper description of how randomization was performed and whether allocation sequence was concealed. A passive recovery (i.e., rest) control group should always be included. Multi-component exercise sessions lasting ≥60 min, with recovery protocols lasting ≤15 min, would provide greater external validity to the findings. Studies with women and athletes should be reinforced, as studies with children, teenagers, adults ≥40 years and populations with pathologies and/or injuries are lacking and should be prioritized.

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

All authors had substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data for the work, drafting the work or revising it critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.677581/full#supplementary-material

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Evaluation of Lower Limb Arteriovenous Diameters in Indoor Soccer Athletes: Arterial Doppler Ultrasound Study

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The purpose of this study was to analyze the arterial and venous diameters of lower limbs in indoor soccer athletes and non-athletes using Doppler ultrasound to identify the differences in the variation of arterial and venous diameters between groups. Additionally, we intended to verify the differences of arterial and venous diameters between the skilled member (right member) and the not skilled member in each group. 74 male volunteers, aged between 19 and 30 years old, were divided in a group of athletes (n = 37, 24 ± 2.7 years, soccer players from national championship), and a group of non-athletes (n = 37, 26 ± 2.83 years). Vascular lower limb was assessed using Doppler ultrasound (Philips HD7 echograph with linear transducer 7–12 MHz). The athletes showed higher diameters of right common femoral artery ($p = 0.009$; moderate), left common femoral artery ($p = 0.005$; moderate), right deep femoral artery ($p = 0.013$; moderate), right popliteal artery ($p = 0.003$; moderate), and left popliteal artery ($p = 0.017$; small) than non-athletes. Veins' diameters were also higher in athletes, specifically the right deep femoral vein ($p \leq 0.001$; large), left deep femoral vein ($p \leq 0.001$; large), right popliteal vein ($p \leq 0.001$; large), and left popliteal vein ($p \leq 0.001$; large). Differences were found between the skilled and non-skilled leg in athletes in the popliteal vein (7.68 ± 1.44 mm vs. 7.22 ± 1.09 mm, respectively, $p < 0.003$). It seems that futsal athletes have superior mean diameters of lower limbs arteries and veins of the deep venous system to non-athletes. Moreover, the veins presented greater dilation, namely of the leg of the skilled lower limb.

Keywords: arteries, athletes, lower limb, ultrasound, veins

INTRODUCTION

Arterial and venous systems are different systems, namely in their constitution, giving them very specific characteristics, such as plasticity. It is agreed that the peripheral arterial system has arterial remodeling (Thijssen et al., 2011). This remodeling occurs in different ways regarding the structure and arterial function of athletes, initially dilating the vessel and maintaining it later, depending on physical training (Thijssen et al., 2010). According to Brown (2003) the impact of training endurance exercises on the expansion of vessels, namely the capillaries, is well established, with an increase in the size of the arteries that supply the muscles in effort in individuals who practice endurance exercises (Brown, 2003). These increases can be found in the arteries that nourish the active muscles, suggesting that the increase in arterial diameter is associated with the repeated increase in blood pressure caused by the peak blood flow responses to exertion (Sugawara et al., 2007). This process results in the remodeling of the diameter of the vessel that is mediated by the endothelium (Sugawara et al., 2007). Contrarily, while at rest, the diameter may not be increased, due to compensatory increases in vasoconstrictor tone to maintain blood pressure (Sugawara et al., 2007). These changes in the diameter of the vessels cause significant changes in their ability to conduct blood during effort and rest, namely when there is a reduction in turbulent flow passing to a laminar pattern, consequently increasing the conductance of the vessel in proportion to the fourth power of its diameter (Green et al., 2010; Naylor et al., 2011). The vasodilation capacity of vessels during and after exercise allows blood pressure to be maintained, reducing total peripheral resistance and its plasticity in response to physical exercise, and suggests that arterial adaptation is an essential condition for performance in endurance exercise (Calbet et al., 2004; Saltin and Calbet, 2006; Taylor et al., 2008). According to the literature, it is also possible to observe changes in the venous system. Veins are vessels that, compared to arteries, have a thinner middle layer, with fewer muscle fibers, and therefore less plasticity (Cunha da Silva et al., 2010).

It was previously described that some anaerobic exercises with muscle overload might aggravate venous disorders. However, no similar studies focusing on aerobic exercise were found (Cunha da Silva et al., 2010). According to the literature, exercise increases blood return and the veins of the superficial venous system and communicating veins dilate and become more visible. At the level of the deep venous system, the effects are not clear. It is known that this system houses about 80–90% of the circulating blood and the superficial the rest, so the increase in return will also be greater in the deep system, as well as the possible dilation (Rowland, 2001). Adequate physical exercise is considered an effective measure for the prevention and treatment of chronic venous diseases that occur in the superficial venous system. It is also important to understand the repercussions in the deep venous system, where the most frequent pathology is venous thrombosis, which in turn can result in pulmonary embolism and be fatal (Beebe-Dimmer et al., 2005). These adaptations should be further understood in some specific popular exercises, such as team sports. Among these, indoor soccer emerges as an intermittent sport that requires high-intensity short-duration activities of approximately 3 s, such as sprints, changes of direction, dribbles, jumps, shots, tackling, and short periods of recovery (20–30 s) during the game (Marques et al., 2019). It is one of the most physically demanding team sports, taking into account the concentric and eccentric muscular movements of the lower limbs that were a consequence of the specific actions of the game (Nunes et al., 2018; Torres-Torrelo et al., 2018).

In order to qualitatively and quantitatively assess vessel morphology and flow dynamics in the main arteries and veins of the lower limbs, there are complementary diagnostic tests such as peripheral arterial and venous Doppler ultrasound. The latter is a non-invasive, painless examination, which does not involve the use of radiation and has high applicability and reproducibility (Hwang, 2017). The main objective of the present study was to analyze the arterial and venous diameters of the lower limbs in indoor soccer athletes and non-athletes, using arterial and venous lower limbs Doppler ultrasound was used to identify the differences in the variation of arterial and venous diameters between groups. Additionally, we intended to verify the differences of arterial and venous diameters between the skilled member (right member) and the not skilled member in each group (athletes and non-athletes). We believe that we can verify vessels of greater caliber in athletes, as they are subject to greater blood pressure during exercise and observe larger diameters at the venous level because they are vessels with less elastic properties and less capacity for retraction.

MATERIALS AND METHODS

Participants

An analytical cross-sectional study was carried out on a sample consisting of 74 male individuals aged between 19 and 30 years old who were divided into two groups: a group of athletes ($n = 37$) (mean age 24 ± 2.7 years) and a group of non-athletes ($n = 37$) (mean age 26 ± 2.83 years). Only the participants in which the right lower limb was the skilled one were considered for the study. Athletes were considered individuals who practiced indoor soccer three or more times a week playing in the second national league in central Portugal with the same level of practice (16 ± 2.6 years of practice). The sample was collected in two indoor soccer clubs in the central region. In the group of athletes, goalkeepers were excluded. Inclusion criteria were male athletes or non-athletes without musculoskeletal or neurological injuries, conditions, or syndromes diagnosed in the last 6 months who agreed to participate in the study and underwent arterial and venous lower limb Doppler ultrasound. The group of non-athletes answered the Physical Habitual Activity Questionnaire (Rowland, 2001; Beebe-Dimmer et al., 2005), where it was observed that some individuals had practiced different sports for at least 3 years and at a recreational level, including tennis ($n = 8$), football ($n = 14$), basketball ($n = 4$), handball ($n = 6$), and volleyball ($n = 5$). Those who practice less than three times a week and were not federated in any sport were considered non-athletes, consistent with the criteria adopted in previous studies (Hirsch et al., 2006; Gerhard-herman et al., 2017). All individuals were informed about the purpose of the study and signed the informed consent form. This study was conducted in accordance with the Helsinki Declaration (World Medical Association [WMA], 2008), and all procedures were approved by the local Ethics Committee (14/CE-ESALD/2016).

Instruments

In order to carry out this study, everyone underwent arterial and venous lower limb Doppler ultrasound and, for this, a Philips ultrasound device and a linear probe 7–12 megahertz (MHz) were necessary. Three techniques were applied, namely ultrasound, pulsed Doppler, and color-coded Doppler, which together allowed for the evaluation of the anatomy and morphology of the vessels and to evaluate the flow of blood vessels (arteries or veins) (Hirsch et al., 2006; Hwang, 2017).

An ultrasound device is a device that uses the reflection of an ultrasound beam through an anatomical structure or erythrocytes to make medical images. The probe through piezoelectric crystals emits and receives ultrasounds with different frequencies, depending on several characteristics of the structure under study (Hirsch et al., 2006; Hwang, 2017).

Procedures

Some questions were asked by the researchers, to all individuals, and information about physical activity and the number of times they exercised were recorded. Information on the results of the examinations was also recorded. To perform the exams, it was necessary to have an adequate, quiet physical space, with a low light environment, a tilt table of 30°, an experienced technician, and an ultrasound with a linear probe of 7–12 MHz. The examinations were performed in the supine position and three techniques were applied: ultrasound, pulsed Doppler, and color-coded Doppler. The ultrasound technique allows the visualization of the arterial and venous lumen of the lower limb, allowing an anatomical and morphological analysis, where the diameters of each artery were measured in the longitudinal axis, three times in centimeters (cm), about 2 cm after their origin, with the later calculation using the average of the three measurements (**Figures 1A,B**).

Similarly, for the diameters of the veins, but in transverse axis, we also obtained three measurements, with subsequent calculation of the average, in centimeters (cm): the deep femoral vein 2 cm before the femoral bifurcation and the popliteal vein 2 cm before the saphenous-popliteal cross (**Figure 2**).

In total, eight arteries and four veins of the deep venous system were studied in all individuals: common femoral arteries (CFA), superficial femoral arteries (SFA), deep femoral arteries (DFA), popliteal arteries (popA), deep femoral veins (DFV), and popliteal veins (popV). The selection of the studied vessels was made to obtain measurements with greater precision and also because they represent the main vessels that carry blood to the thigh muscles. Regarding the veins, the veins of the smaller venous deep system were chosen, but which still allow measurements to be performed with good accuracy representing the thigh and leg venous return, where blood flow during the knee extension exercise is the high point stress during exercise. All artery measurements were made with a longitudinal axis with correct *preset* corrections and all venous measurements were made with a transversal axis with correct veins *preset* corrections. Examinations and results were performed based on the protocols and normality criteria of a study published in 2017 (Hwang, 2017). To assist in conducting the exams, the DGS (2015) protocol on clinical indications and execution methodology was also used (George, 2015).

Statistical Analysis

Preliminary Analysis

An inspection of the data revealed no missing values, nor were univariate outliers found. *A priori* power analysis through G*Power (3.1.9.2) (Faul et al., 2007) was used to determine the required sample size considering the following input parameters: (effect size $d = 0.8$; $\alpha = 0.05$; statistical power = 0.90). The required sample size was 68 (34 for each group) which was respected in the present study.

Main Statistical Analysis

Descriptive statistics including mean and standard deviation were performed for all variables under analysis. The coefficient of variation (CoV) and the interclass correlation coefficient (ICC) was computed to evaluate the variability of the data. Then, a Shapiro–Wilk test ($n < 50$) to analyze data distribution was performed, considering $p > 0.05$ as a normal distribution (Hair et al., 2019). Initially, a *T*-Test for Independent Samples (variables with normal distribution) and Mann–Whitney (variables with non-normal distribution) was used to verify differences between groups (athletes and non-athletes). Secondly, the Paired Sample *T*-test (variables with normal distribution) and Wilcoxon (variables with non-normal distribution) were used to verify the differences of arterial and venues diameters between the skilled member (right member) and the non-skilled member in each group (athletes and non-athletes) (Hopkins et al., 2009). Finally, an effect size (Cohen d) analysis was used to determine the magnitude of effect and the following cut-off values were considered: 0–0.2, trivial; 0.21–0.6, small; 0.61–1.2, moderate; 1.21–2.0, big; and >2.0, very big (George, 2015). In the variables with non-normal distribution, the effect size was calculated based on the eta square value (η^2) (Lenhard and Lenhard, 2016). All statistical analysis was performed using SPSS software v. 25.0 (IBM, Chicago, IL, United States), and the significance level was set at $p \leq 0.05$ to reject the null hypothesis (Ho, 2014; Hair et al., 2019).

RESULTS

Table 1 shows the differences in the studied variables between the two groups (athletes and non-athletes) on the diameter of the right and left arteries and veins. Differences between groups ($p \leq 0.05$) in the diameter of right common femoral artery ($p = 0.009$; moderate), in the diameter of left common femoral artery ($p = 0.005$; moderate), in the diameter of right deep femoral artery ($p = 0.013$; moderate), in the diameter of right popliteal artery ($p = 0.003$; moderate), and in the diameter of

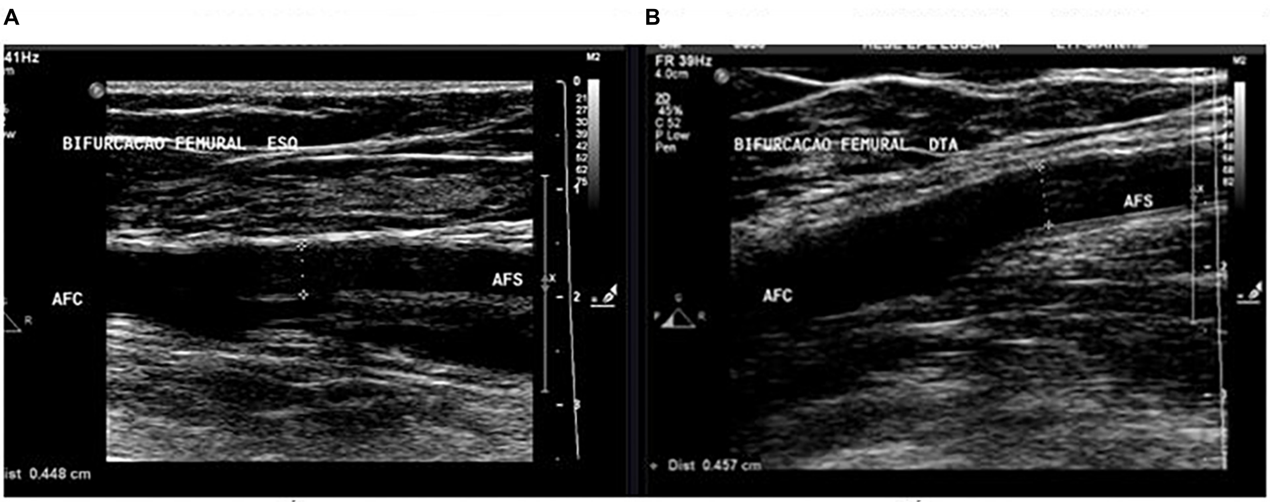

FIGURE 1 | Measurement of arterial diameter of the left **(A)** and right **(B)** superficial femoral artery by lower limb arterial Doppler ultrasound.

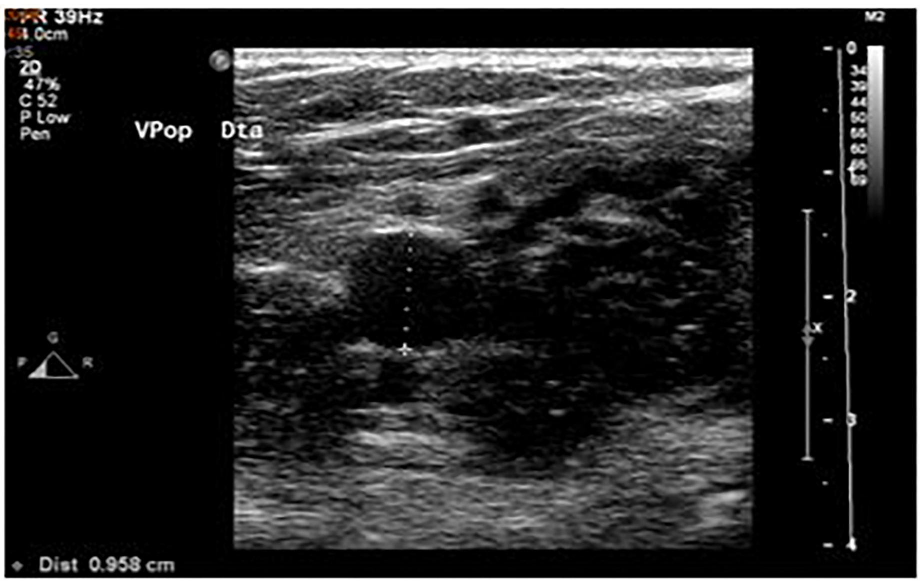

FIGURE 2 | Measurement of venous diameter of right popliteal vein by lower limb venous Doppler ultrasound.

left popliteal artery (p = 0.017; small) were found. We also found differences in the diameter of the right deep femoral vein ($p \leq 0.001$; big), in the diameter of the left deep femoral vein ($p \leq 0.001$; big), in the diameter of the right popliteal vein ($p \leq 0.001$; big), and in the diameter of the left popliteal vein ($p \leq 0.001$; big), showing that the group of athletes had the highest values.

Figure 3 shows the variance analyses related to the diameter of the right and left arteries and veins in the athletes. Statistically acceptable values ($p \leq 0.05$) were found in popliteal vein.

Figure 4 shows the variance analyses related to the diameter of the right and left arteries and veins in the non-athletes. Statistically acceptable values were not found.

DISCUSSION

The main objective of the present study was to analyze the arterial and venous diameters of the lower limbs in indoor soccer athletes, who play an intermittent high-intensity team sport, and non-athletes (Ramos-Campo et al., 2016), using arterial and venous lower limb Doppler ultrasound to identify the differences in the variation of the arterial and venous diameters between groups. Additionally, we intended to verify the differences in the arterial and venous diameters between the skilled member (right member) and the not skilled member in each group (athletes and non-athletes). Like other studies, Doppler ultrasound was the diagnostic test selected due to

TABLE 1 | Descriptive statistics, differences between groups, and effect size in the variation of diameter of arteries and veins of left and right limbs.

Variables	Groups*N*	M	M CI 95%	SD	*p*	Effect size	ICC (CI 95%)	CoV
Diameter – right common femoral artery	Non-athletes37	7.52	7.18–7.86	1.01	0.009[a]*	0.621	0.998 (0.996–0.999)	1.2%
	Athletes37	8.14	7.81–8.47	0.99			0.993 (0.988–0.996)	2.1%
Diameter – left common femoral artery	Non-athletes37	7.41	7.12–7.68	0.85	0.005[a]*	0.663	0.997 (0.994–0.998)	1.3%
	Athletes37	7.97	7.69–8.25	0.84			0.983 (0.971–0.991)	2.8%
Diameter – right deep femoral artery	Non-athletes37	5.19	4.99–5.40	0.61	0.013[a]*	0.605	0.966 (0.942–0.982)	3.1%
	Athletes37	5.68	5.35–6.00	0.97			0.994 (0.99–0.997)	3.2%
Diameter – left deep femoral artery	Non-athletes37	5.23	4.98–5.48	0.75	0.581[b]	0.451	0.99 (0.982–0.994)	2.5%
	Athletes37	5.62	5.34–5.89	0.82			0.96 (0.931–0.978)	3.8%
Diameter – right superficial femoral artery	Non-athletes37	6.24	6.02–6.45	0.65	0.197[a]	0.292	0.988 (0.979–0.993)	2.3%
	Athletes37	6.40	6.26–6.55	0.43			0.961 (0.933–0.979	3.1%
Diameter – left superficial femoral artery	Non-athletes37	6.40	6.13–6.67	0.80	0.228[a]	0.227	0.989 (0.981–0.994)	2.6%
	Athletes37	6.59	6.41–6.78	0.55			0.966 (0.942–0.981)	3.6%
Diameter – right popliteal artery	Non-athletes37	5.69	5.46–5.93	0.72	0.003[a]*	0.734	0.989 (0.981–0.994)	2.9%
	Athletes37	6.12	5.99–6.26	0.41			0.95 (0.913–0.972)	3.7%
Diameter – left popliteal artery	Non-athletes37	5.84	5.60–6.07	0.70	0.017[a]*	0.568	0.985 (0.975–0.992)	3.0%
	Athletes37	6.16	6.03–6.28	0.38			0.952 (0.918–0.974)	3.5%
Diameter – right deep femoral vein	Non-athletes37	6.75	6.11–7.39	1.91	<0.001[a]*	1.323	0.997 (0.996–0.999)	2.7%
	Athletes37	9.31	8.65–9.96	1.96			0.998 (0.996–0.999)	2.7%
Diameter – left deep femoral vein	Non-athletes37	6.39	5.69–7.08	2.09	<0.001[b]*	1.692	0.999 (0.998–0.999)	2.4%
	Athletes37	9.68	9.09–10.28	1.79			0.998 (0.997–0.999)	2.2%
Diameter – right popliteal vein	Non-athletes37	5.32	4.76–5.88	1.68	<0.001[a]*	1.508	0.998 (0.996–0.999)	3.0%
	Athletes37	7.68	7.20–8.15	1.44			0.997 (0.994–0.998)	2.5%
Diameter – left popliteal vein	Non-athletes37	5.32	4.88–5.77	1.33	<0.001[a]*	1.563	0.997 (0.995–0.998)	2.9%
	Athletes37	7.22	6.86–7.58	1.09			0.983 (0.971–0.991)	4.6%

**p ≤ 0.05 – a student's t-test and b Mann–Whitney U test significance level; p > 0.05 – Levene's test equal variances assumed. N, subjects' number; M, mean; M CI, Confidence interval for the mean values; SD, standard deviation; CI, confidence interval; diameter – mm; ICC, intra-class correlation coefficient; CoV, coefficient of variation.*

its high sensitivity in arterial and venous studies. It is the gold standard diagnostic exam in venous evaluation. The Doppler ultrasound is a technique that allows the anatomical and morphological evaluation of vessels and hemodynamic study of blood qualitatively and quantitatively, being non-invasive and painless for the patient (Hussain et al., 1996; Thrush and Hartshorne, 2005).

Previous studies have reported increases in the diameter of the arteries that supply the muscles during endurance exercises (Thijssen et al., 2010). Nevertheless, they have not reported a study of this variation in diameters between lower limb arteries in indoor soccer athletes. Regarding the venous system, only studies were found regarding anaerobic exercises with muscle overload (Cunha da Silva et al., 2010).

In the current study, the average diameter of the arteries and veins studied in athletes was higher than non-athletes in both limbs. These differences were statistically significant in almost all studied arteries and all studied veins. At the same time, the minimum values of arterial and venous diameters are also always higher in the group of athletes. We think that a possible explanation of these findings is the specific demands of indoor soccer that involves high-intensity short duration activities that require very high concentric and eccentric muscular movements in the lower limbs (Marques et al., 2019). These results corroborate with previous studies that show that athletes' arteries have larger lumen diameters than healthy non-athletes' arteries (Green et al., 2012). Also, two studies from Tinken et al. (2008, 2010) stated that athletes' arteries may be normal in function, but different in some structural characteristics. These characteristics can happen through changes in arterial structure and function, where dilation occurs initially, which, depending on the intensity of the training, will be maintained later. According to the last study, there is sufficient evidence to conclude that endurance athletes have enlarged arteries and may also have decreased wall thickness (Tinken et al., 2010). In this sample, the fact that both SFA do not present statistically significant differences may be related to the artery having a greater length and a greater number of branches of the thigh, with these anatomical characteristics allowing for a better distribution and dispersion of the increased blood gradient during exercise, creating less impact on the arterial vascular wall, and thereby decreasing dilation (Hwang, 2017). Regarding the studied veins, the results are in agreement with the fact that the middle layer of the venous wall has less muscle and elastic fibers, decreasing its plasticity, with the blood pressure being very low compared to the arterial system in the veins. According to literature, the athletes' venous system during training is subject to an increase in blood return and, consequently, pressure, which may dilate the veins of the superficial venous system too much, which, being

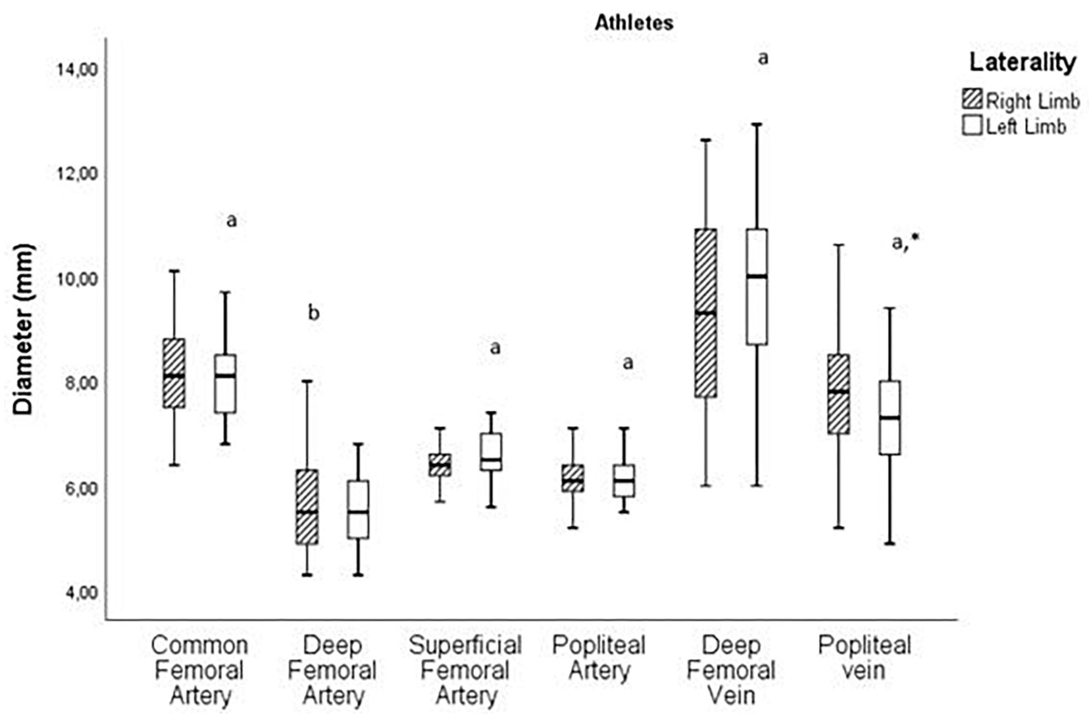

FIGURE 3 | Analysis of the diameter variance pairwise of right and left arteries and veins in athletes (*$p \leq 0.05$; [a] Paired *T*-Test; [b] Wilcoxon).

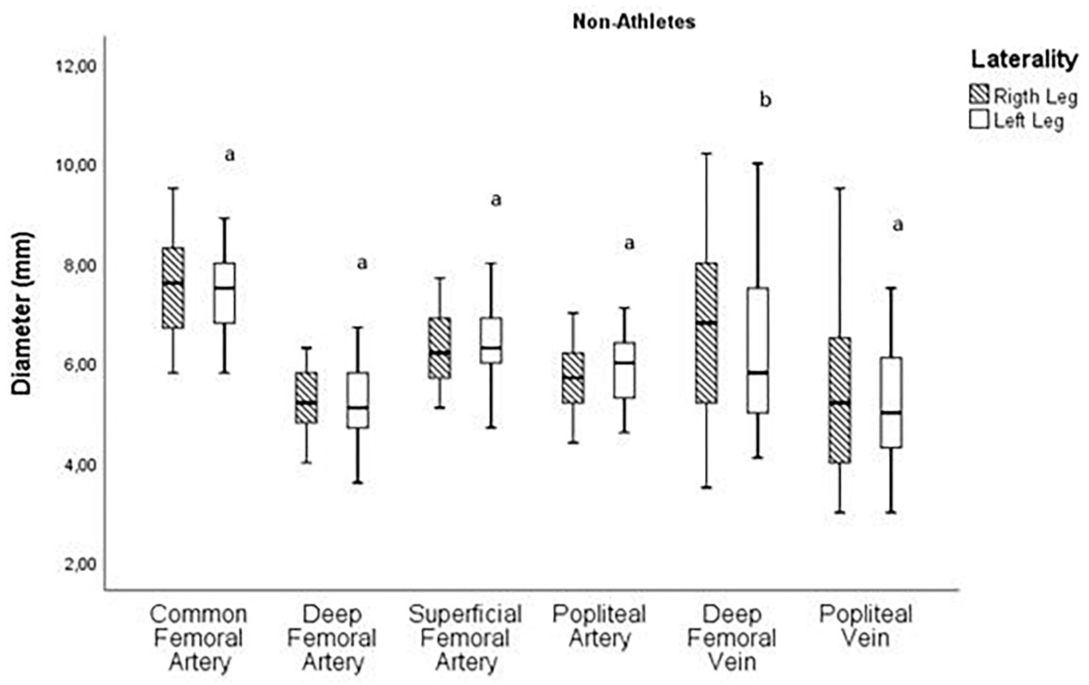

FIGURE 4 | Analysis of the diameter variance pairwise of right and left arteries and veins in non-athletes ([a] Paired *T*-Test; [b] Wilcoxon).

connected to the deep venous system by perforating veins, will suffer equal pressure increases and consequent expansion (Cunha da Silva et al., 2010).

In the study of the comparison of arteries between left and right lower limbs, it is observed that in both groups the diameters are higher in the left lower limb, considered the support leg, except for the mean diameter of the CFA which in both groups is higher in the right lower limb, which may be related to this being the skillful leg and anatomically being the artery of shorter length, with a lower number of branches, greater blood volume, and, consequently, an increase in diameter (Hwang, 2017). However, these results were not statistically significant.

As for the comparison in the veins between lower limbs, in the group of non-athletes, the average diameter of the studied veins tends to be higher in the right lower limb, the result being significant in PFVs, veins that reflect the venous return at the level of the thigh muscles of individuals who, despite not being athletes, practice some physical exercise, namely exercises with different characteristics in terms of muscular effort. In the athletes' group, the mean diameter of the studied veins is higher in the left lower limb at the level of PFV, with the opposite occurring at the level of the popV, which has mean diameters greater in the right, which is a significant result. This may again be related to the skillful leg and greater pressure spikes and to training being more directed at strengthening leg muscle groups (Cunha da Silva et al., 2010).

In the analysis of arteries by members, in the group of non-athletes, the variation between the different arteries is significant in the right and left lower limbs. However, in the athletes' group, the variation between the different arteries is not statistically significant between SFA and popA bilaterally, which can be justified because they are anatomically similar in caliber to the others studied (Hwang, 2017).

In the analysis of the venous system by members, in the non-athletes' group, there was statistical significance in the variation between the means of the venous diameters on the left and in the right. In the athletes' group, statistical significance was only found in the right. This means that there are differences in blood pressure between the members of futsal athletes. This can be justified by the right limb in this sample being the skilled member, with the left leg being the supporting leg. Remembering that the veins have less plasticity, the veins of the skilled member are subject to greater peak pressure and pressure variations, dilate more and stay dilated, not returning to the initial diameter. The veins of the support member suffer fewer pressure peaks and are likely to experience smaller increases in diameter (Cunha da Silva et al., 2010). The results of this study, given the fragility of the vein walls and the possible irreversibility of the dilation they suffer during training, point to the need for a supervised training program and the application of preventive measures. There is some evidence that the practice of exercise is related to the worsening of venous disorders (Cunha da Silva et al., 2010). According to a study published in 2006, venous diameters are strongly related to chronic insufficiency of the veins of the peripheral venous system (Eifell et al., 2006). The dilation of a blood vessel can cause venous reflux and, consequently, an increase in local venous pressure, especially in the lower limbs where, due to the action of the force of gravity, greater pumping is needed for its upward path to the right atrium (Padberg et al., 2004). However, physical exercise can promote benefits in the functionality of the venous system. It is not related to the type of exercise, but to the amount and intensity of training. Aerobic exercise is recommended by the literature for individuals with venous diseases, since it is able to improve the venous pressure drop mechanism and increase venous flow (Kahn et al., 2003; Padberg et al., 2004). During exercise, muscle contraction provides perfusion pressure, helping blood flow from the lower limbs to the heart, opposing forces against venous return such as gravity (Kahn et al., 2003; Padberg et al., 2004). A study where 100 adults over 50 years of age, of both sexes, were prospectively studied to verify the relationship between lower limbs chronic venous insufficiency and physical activity concluded that exercise did not influence the occurrence of venous insufficiency, having prevented the evolution to more advanced stages (Tinken et al., 2010). However, the type of exercise was not clarified, and it was a study applied to individuals older than the present study. The scarce literature on the effect of aerobic and anaerobic exercise on the arterial system, and especially on the venous system, is a restriction that remains.

Although the present study contributes to knowing how sports practice influences the caliber of arteries and veins, it has some limitations. One of the limitations was the comparison of one single sport modality (futsal) against multiple heterogeneous sports activities and the fact that the cofound variables were not analyzed (e.g., hydration, diet, and previous physical activities) (Duarte-Mendes et al., 2020). Another limitation is the fact that objective measures were not used to directly assess one or more dimensions of physical activity (e.g., frequency, intensity, time, or type) (Alberti et al., 2010), and all variables were assessed at one moment (cross-sectional design). Therefore, we cannot draw causality associations. Longitudinal and/or experimental studies are needed to further examine the effects of the analyzed variables. In order to increase knowledge on the variability of lower limb artery and vein diameters in athletes, we suggest future studies considering the wide range of sport modalities and physical activity. As for the practical implications, coaches and players should take into account the observed changes. To minimize them, vasoconstrictor techniques can be applied, such as the use of compression stockings or the application of ice in high-intensity training (Armstrong et al., 2015).

CONCLUSION

In this study, the group of futsal athletes had superior mean diameters of the arteries and veins of the deep venous system of the lower limbs to non-athletes. Nevertheless, it was concluded that in the veins that there was greater dilation, namely of the leg of the skilled lower limb. This variation in the diameter of veins in the venous system should be monitored to prevent the functional impact that may lead to chronic venous diseases, with medical monitoring and convenient prevention measures.

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 Comissão de Ética Escola Superior de Saúde Dr. Lopes Dias. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SM, RP, PC, FR, VM, HN, and PD-M participated in study design, data collection, and the writing of the first draft manuscript. SM, PC, FR, and PD-M participated in article collection and analysis. SM, RP, PC, FR, HN, and PD-M participated in the writing of the methodology and results. SM, RP, HN, and PD-M participated in final revisions of the manuscript. All authors have read and approved the final version of the manuscript and agreed with the order of presentation of the authors.

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frontiers in Physiology

REVIEW
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The Hamstrings: Anatomic and Physiologic Variations and Their Potential Relationships With Injury Risk

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The incidence and recurrence of hamstrings injuries are very high in sports, posing elevated performance and financial-related costs. Attempts to identify the risk factors involved in predicting vulnerability to hamstrings injury is important for designing exercise-based programs that aim to mitigate the rate and severity of hamstrings injuries and improve rehabilitation strategies. However, research has shown that non-modifiable risk factors may play a greater role than modifiable risk factors. Recognizing non-modifiable risk factors and understanding their implications will afford the prescription of better suited exercise programs, i.e., that are more respectful of the individual characteristics. In a nutshell, non-modifiable risk factors can still be acted upon, even if indirectly. In this context, an underexplored topic is how intra and inter- individual anatomic and physiologic variations in hamstrings (e.g., muscle bellies, fiber types, tendon length, aponeurosis width, attachment sites, sex- and age-related differences) concur to alter hamstrings injuries risk. Some anatomic and physiologic variations may be modifiable through exercise interventions (e.g., cross-sectional area), while others may not (e.g., supernumerary muscle bellies). This apparent dichotomy may hide a greater complexity, i.e., there may be risk factors that are partially modifiable. Therefore, we explored the available information on the anatomic variations of the hamstrings, providing a deeper insight into the individual risk factors for hamstrings injuries and contributing with better knowledge and potential applications toward a more individualized exercise prescription.

Keywords: hamstrings injuries, interindividual variation, kinesiology, muscle architecture, exercise prescription, hamstrings anatomy

INTRODUCTION

The hamstrings are a hot topic in Sports Sciences, with a PubMed search using the terms "hamstring*" and "sport*" from the year 2020 to the present showing >500 entries. This is because hamstrings injuries are common in the athletic community (Monajati et al., 2016; Sheean et al., 2021). While hamstrings strain injury (HSI) is a commonly used term, it does not encompass all hamstrings injuries; therefore, HSI will only be used if the cited authors specifically refer to that subset of hamstrings injuries. The number of non-contact training-related hamstrings injuries in different sports have increased gradually and systematically [e.g., an annual average 2.3% increase in the total hamstrings injuries rate over the 13-year period (2001–2014)] (Ekstrand et al., 2016; Ertelt and Gronwald, 2017). Anatomic and functional aspects of the hamstrings, including the fact that muscles cross two joints (except the short head of the biceps femoris) and that eccentric action during running or stretching carried out to extreme joint positions, make it prone to vulnerable to strain-related injuries (Edouard et al., 2016).

A systematic review including 13 studies with more than 3,800 athletes and two million sport exposure hours reported an incidence of acute hamstrings injuries ranging from 0.3 to 0.5 per 1,000 exposure hours in women, to 0.3–1.9 per 1,000 exposure hours in men, accounting for 5% to 15% of all soccer-related injuries, with recurrence rates varying from 4 to 68% (Diemer et al., 2021). The prevalence of hamstrings injuries were reported to be more than 60% (unilateral) and more than 30% (bilateral) in baseball players, with ∼30% recurrence rates (Zachazewski et al., 2019), while prevalence rates of 40% were reported for professional soccer players (Ribeiro-Alvares et al., 2020). This is challenging in terms of recovery time and financial costs (Pickering and Kiely, 2018; Shield and Bourne, 2018; Metcalf et al., 2019). In the Australian Football League, data from 10 competitive seasons determined that the financial cost of HSI per club increased by 71% from 2003 to 2012 (Hickey et al., 2014).

A wide body of research explored the implementation of specific exercise-based programs for mitigating hamstrings injury risk (Al Attar et al., 2017; Chebbi et al., 2020). Due to intra- and inter-muscular differences in this group of muscles, exercises designed for the hamstrings may not provide equal stimuli for semitendinosus (ST), semimembranosus (SM), and biceps femoris (BF) (Kellis, 2018). Relevant works suggested that non-modifiable factors (i.e., older age, previous injury) may have a greater impact on risk for hamstrings injuries compared to modifiable ones (Freckleton and Pizzari, 2013; Green et al., 2020). Non-modifiable factors could also extend to aspects of human anatomy which are not possible to modify, such as variations in insertions sites and the presence of supernumerary muscle bellies (Tubbs et al., 2016).

A better knowledge of interindividual variation in hamstrings anatomy and geometry could allow the design of better-individualized interventions, eventually reducing the likelihood of suffering (and aid in the recovery from) an injury (Ertelt and Gronwald, 2017). Interindividual variation in strength levels (specifically eccentric strength), hamstrings muscle fascicle length, muscle-tendon architecture, muscle fiber type and region-specific innervation have been hypothesized as relevant factors for risk of HSI (Timmins, 2017; Huygaerts et al., 2021), and genetic variations have been explored in response to loading and post-exercise recovery (Pickering and Kiely, 2018). However, anatomic and physiologic variations of the hamstrings are more diversified than highlighted here (Tubbs et al., 2016).

Our goal was to explore the available information on the anatomic variations of the hamstrings, provide a deeper insight into the individual risk factors for hamstrings injuries and contribute evidence-based applications for more individualized intervention regimens. Although acute injury prediction, given its multifactorial nature, will probably never be possible (Bahr, 2016), some risk factors can be controlled for. In the first part of this commentary, an overview of hamstrings injuries is provided, including injury mechanisms, both non- and modifiable risk factors, exercise-based strategies to mitigate risk factors, as well as topics of rehabilitation and return to play (RTP). While the literature often dichotomizes risk factors into modifiable and non-modifiable, some non-modifiable risk factors may be partially modifiable, as will be discussed. In the second part, "normal" anatomy of the hamstrings is described, and the inter- and intraindividual variations of hamstrings anatomy and how they might be related to injury risk are explored, considering modifiable and non-modifiable anatomic variations and how to recognize them to prescribe better adjusted exercise. Non-modifiable risk factors can still be acted upon, by designing exercise interventions that are more respectful of those factors.

A systematic review of the literature was not performed, given the scope and goals of this commentary. However, we selected literature published in peer-reviewed journals, most of which indexed in PubMed. The goal was to gather relevant information on a wide variety of topics related to our theme. Where controversy existed, we attempted to provide a balanced discussion, selecting studies with contradictory findings. Where appropriate, renowned textbooks were used (Standring, 2015; Tubbs et al., 2016).

PART 1: AN OVERVIEW OF HAMSTRINGS INJURIES

Hamstrings Functions and Injury Mechanisms

The hamstrings are involved in major movements of daily life, such as standing and walking. In the standing position, the line of gravity passes behind the hip, producing an extension moment that needs to be counterbalanced by the hip flexors (Houglum and Bertoti, 2012). On the contrary, the hamstrings need to act toward knee flexion, since the line of gravity falls in front of the knee inducing hyperextension (Smith, 1957). During walking, the hamstrings (and gluteus maximus) act on the hip during the final leg swing phase by generating an eccentric action to decelerate the rate of hip flexion, and concentrically in the following hip extension (Hogervorst and Vereecke, 2015). During the leg swing phase, the hamstrings act eccentrically to decelerate the rate of knee extension (Houglum and Bertoti, 2012).

The hamstrings are important for sport activities involving sprints, jumps, tackles, cutting maneuvers and kicking (Ekstrand et al., 2012). The motion of the kicking leg (e.g., soccer kick) includes a backward and forward motion (Lees and Nolan, 1998). During the backward motion, the hamstrings act using concentric action in hip extension (together with gluteus maximus) and knee flexion and lateral rotation (only the BF) (Fields et al., 2005). During the forward motion, the hamstrings act eccentrically to decelerate hip and knee during respective hip flexion and knee extension motions (Fields et al., 2005). The decelerating action of hamstrings occur at a position (hip flexion and knee extension) where these muscles are passively insufficient (i.e., the muscles reach their maximal length since they are biarticular muscles passing through hip and knee) (Coole and Gieck, 1987). For these reasons, the hamstrings are prone to injury during high-velocity running or sprinting, due to eccentric over-loading at the end of the swing phase (Jönhagen et al., 1994).

A systematic review with 26 studies explored the mechanisms behind the hamstrings injuries (Danielsson et al., 2020); the authors stratified the mechanisms according to the methods used to determine the injury: (i) stretch-related injuries; (ii) kinematic analysis; (iii) electromyography-based kinematic analysis; and (iv) strength-related injuries. In the first group (i.e., stretch-related injuries), all studies reported that hamstrings injuries occurred upon extensive hip flexion with hyperextension of the knee (i.e., the hamstrings were strongly stretched at both joints it crosses, which are the hip and knee). In the review (Danielsson et al., 2020), hamstrings injuries were associated with running-based actions and especially to the late swing phase of the running gait cycle, which may imply a powerful eccentric action. These powerful actions increase as running velocity increases (Higashihara et al., 2010b).

Modifiable Risk Factors

Modifiable risk factors can be modified under certain interventions. In the case of hamstrings risk factors, these include player load, warm-up preparations, lumbo-pelvic hip stability, motor patterns (e.g., "Groucho" position), cardiovascular fitness, fatigue, mobility, low back pain, recovery strategies, strength, asymmetry, nutrition and psychosocial factors (Liu et al., 2012; Mendiguchia et al., 2012; Schuermans et al., 2014; Buckthorpe et al., 2019; Huygaerts et al., 2020). Using proper exercise-based programs, establishing a balance between load and recovery, carefully structuring of the intervention sessions, and providing adequate physical preparation, can readily tackle most of these risk factors, which is valid for nearly all injuries. We do not aim to provide an in-depth discussion of modifiable risk factors, as it would escape the main goals of our work. However, we feel it is relevant to briefly discuss four topics that should be more widely acknowledged (i.e., fatigue) or seen under a more critical perspective (i.e., warm-up, flexibility and functional asymmetry).

Fatigue

Fatigue was associated with decreased eccentric hamstrings strength and an altered neuromuscular coordination, suggesting a higher risk of developing an injury (Jones et al., 2015; Buckthorpe et al., 2019; Huygaerts et al., 2020). In the review of Danielsson et al. (2020), fatigue was associated to HSI, underlining that, aside from strength and flexibility, endurance was a relevant risk factor (Watson et al., 2017; Farley et al., 2020). Eccentric strength endurance of the hamstrings was reported to be significantly lower in male soccer players with previous hamstrings injuries (Schuermans et al., 2014). A study with elite footballers (n = 50) presented contradictory findings when comparing the previously injured limbs (n = 11) with non-injured limbs (n = 89) (Freitas et al., 2021), but the athletes acquired hamstrings injury in the previous 2 years, possibly providing enough time and exercise intervention for the hamstrings to recover their endurance levels. Previous injuries are non-modifiable risk factors, but improvements in endurance and increased tolerance to fatigue can be achieved with exercise training (Delextrat et al., 2018). In this sense, both detraining and hamstrings damage (secondary to a previous injury) may be considered partially modifiable factors.

The Warm-Up

The role of warm-up in reducing injury risk is not clear (Fradkin et al., 2006; McCrary et al., 2015), and adherence to warm-up protocols may interfere with its effectiveness (Owoeye et al., 2020), i.e., the frequency and degree of compliance to the warm-up protocol may largely dictate how effective the protocol is. It is unclear whether different types of warm-ups induce distinct acute effects on modifiable injury risk factors (Niederer et al., 2020). A prospective two-season study registered posterior thigh injuries in 83 Australian rules football players, of which 62 were confirmed to have hamstrings injury through magnetic resonance imaging (MRI) (Verrall et al., 2003). In this study, 15% of hamstrings injuries occurred during the warm-up period and 85% after the warm-up (Verrall et al., 2003). Considering that the main part of an exercise session is longer than the warm-up, these percentages of distribution provide insufficient information. Harking back to the discussion surrounding fatigue (Delextrat et al., 2018; Danielsson et al., 2020), it could be speculated that overly long and/or intense warm-ups may generate excessive fatigue for the remainder of the training session. Warm-up protocols aiming to produce performance potentiation usually generate potentiation and fatigue, and this balance varies from individual to individual (Afonso et al., 2019; Blazevich and Babault, 2019). Regarding hamstrings injury prevention, individually monitoring responses to warm-up is suggested.

The Role of Flexibility

A clear relationship between hamstrings flexibility and injury risk has also not been established (Worrell and Perrin, 1992; Christopher et al., 2019). de la Motte et al. (2019) assessed 27 studies and explicitly stated they considered studies showing *association* between flexibility and other factors and musculoskeletal injury in military and civilian populations. The authors showed there was moderate yet conflicting evidence associating hamstrings flexibility and musculoskeletal injury risk. It is easy for readers to interpret this association using a causative framework, but caution should be used, as previous hamstrings injuries reduce flexibility until 20–30 days post-injury (Maniar et al., 2016), but reduced flexibility does not necessarily increase injury risk. In this context, the role of stretching in reducing injury risk is controversial (Herbert and Gabriel, 2002; Small et al., 2008; Behm et al., 2015), specifically in the case of hamstrings injuries (Liu et al., 2012; Rogan et al., 2013).

Functional Asymmetry

Some degree of interlimb asymmetries in hamstrings strength is the norm (Boccia et al., 2018; Kulas et al., 2018; Cuthbert et al., 2021) and, if not excessive, are beneficial for performance of tasks involving change of direction and sprinting, without impaired jumping (Coratella et al., 2018). Injured soccer players exhibit a more symmetrical recruitment pattern between ST, SM and BF (corresponding to a less economic hamstrings muscle activation), as opposed to the more asymmetric pattern of subjects without previous injuries (Schuermans et al., 2014). Athletes with previous hamstrings injuries also demonstrated a decrease of ST metabolic activity, compensated by a greater involvement of the BF (Schuermans et al., 2014). The mechanisms underlying the symmetrical muscle activation possibly involve a compensatory increase of BF activation and a maladaptation neuromuscular coordination behavior, resulting in a less efficient hamstrings contraction. This condition, combined with peripheral metabolic changes (e.g., earlier onset of pH changes), leads to muscle fatigue and may explain the reduced endurance capacity, associated to a re-injury of hamstrings (Allen et al., 2008; Schuermans et al., 2016; Suarez-Arrones et al., 2021).

Exercise-Based Strategies for Mitigating Injury Risk

Studies assessing the effectiveness of exercise-based programs in mitigating hamstrings injury risk proliferated. The focus has relied on injury prevention, although we prefer terms such as "risk mitigation," since there is always an inherent injury risk. A systematic review with meta-analysis (SRMA) including 17 studies showed that exercise-based interventions reduced hamstrings injury risk in ∼50% (Vatovec et al., 2020). However, the quality of assessment through the PEDro (Physiotherapy Evidence Database) scale showed major problems in many studies, with nine having a classification ≤4 (i.e., low methodological quality), and none presenting a classification above six points in the PEDro scale (i.e., no study with high quality). Another SRMA assessed 15 articles studying the effects of including the Nordic Hamstrings exercise (NHE) into wider exercise-based programs in hamstrings injury rate (van Dyk et al., 2019), which resulted in ∼50% risk reduction, but the authors showed that half of analyzed articles had high risk of bias in randomization and allocation concealment, and ∼80% had high risk of bias in blinding of outcome assessments, raising concerns regarding the trustworthiness of the findings. In their inclusion criteria (van Dyk et al., 2019), the intervention could be solely focused on the NHE *or* any wider program including the NHE compared to usual training or alternative programs; it is unclear whether the effects were attributable to the NHE, or to the wider program in which it was included.

Similar results were reported in another SRMA concerning the NHE (Al Attar et al., 2017), although a reasonable risk of bias has been reported elsewhere (Gérard et al., 2020). These two SRMA (Al Attar et al., 2017; Gérard et al., 2020) were alluding to *relative risks*, reducing the trustworthiness on the potential beneficial effect of the NHE on injury prevention. The feasibility of implementing certain protocols into real-world contexts is questionable since the evidence-based interventions are not always effective, i.e., translating into practical applications may be problematic (McCall et al., 2020). Beyond the intrinsic characteristics of each exercise-based program, its effectiveness depends on the buy-in, i.e., how well participants adhere to, and comply with the program (Buckthorpe et al., 2019). Therefore, the belief of coaches and athletes in the programs will interfere with how well they work. Indeed, the placebo and nocebo effects have been observed in sports science (Hurst et al., 2020; Raglin et al., 2020).

Weekly frequency of exercise-based programs to mitigate injury risk is an important parameter: interventions performed ≥2 times per week were more effective in reducing hamstrings injury risk than interventions performed <2 times per week, even though the differences were not large (Vatovec et al., 2020). Previous research suggested that the compliance to the program (i.e., adhering to and performing the sessions) is an important prognostic factor regarding injury risk (Goode et al., 2015; Chebbi et al., 2020).

While prevention programs are biased toward eccentric strength training of the hamstrings, interventions focused on improving endurance (Danielsson et al., 2020), motor control (Ertelt and Gronwald, 2017), lumbo-pelvic dynamics (Shield and Bourne, 2018), using video and technical feedback to improve biomechanical parameters of movement (Monajati et al., 2016), and isometric strength training (Macdonald et al., 2019) should not be neglected. Sprint training is possibly the most common mechanism to simulate the high load and high velocity eccentric actions of the hamstrings (van den Tillaar et al., 2017). A multifactorial, individualized approach should be included in the specific programs aiming to reduce injury risk (Mendiguchia et al., 2012; Lahti et al., 2020; Suarez-Arrones et al., 2021).

Rehabilitation and Return-to-Play

Rehabilitation and RTP after a hamstrings injury are challenging (Hickey et al., 2017; Taberner et al., 2020). The RTP relies on numerous determinants, such as injury mechanism, level of sport participation, and time to first consultation and pain (Fournier-Farley et al., 2016). It is common for deficits in strength, range of motion (ROM) and muscle morphology alterations to persist after RTP (Sanfilippo et al., 2013; Maniar et al., 2016). Criteria to support safe and appropriate RTP after hamstrings injuries are varied and lack validation (van der Horst et al., 2016). Some exercise-based strategies such as stretching have showed a decreased RTP time, but not reduced the risk of re-injury (Pas et al., 2015), suggesting a misconception in injury management.

The rehabilitation process depends on factors such as the extent and severity of injury, precise anatomic location and the tissue involved (e.g., fascia, muscle and/or tendon). The degree of hamstrings injuries will determine whether surgery is required or not (Ayuob et al., 2020; Sheean et al., 2021), although there is debate regarding the criteria for choosing conservative treatment vs. surgical management (Metcalf et al., 2019; Blakeney, 2020).

Modifiable Versus Non-modifiable Risk Factors

Despite the potential of exercise-based interventions for mitigating hamstrings injury risk, non-modifiable risk factors should be acknowledged, such as age and previous injury (Mendiguchia et al., 2012). A SRMA involving 71,324 athletes from 78 studies analyzed 8,319 HSIs, of which 967 were recurrences (Green et al., 2020). The stronger factors associated with an increased risk of HSI were older age, previous history of HSI, recent HSI, previous calf strain injury, and previous anterior cruciate ligament (ACL) injury, which are non-modifiable factors (Green et al., 2020). In contrast, modifiable risk factors, such as reductions in strength, strength endurance, power, and motor control, were weakly associated to increased risk for his, and flexibility, mobility or ROM were not associated with risk of HSI (Green et al., 2020).

Non-modifiable risk factors for HSI, such as age and previous injury, were consistently associated with an increased risk of injury in a previous SRMA that included 34 articles (Freckleton and Pizzari, 2013). In this SRMA, the only modifiable factor consistently associated with the risk of hamstrings injuries was the quadriceps concentric peak torque. In a prospective study with Australian football league players (n = 125), hamstrings injuries were not associated with NHE strength (Smith et al., 2021). In this study (Smith et al., 2021), player age greater than 25 years and having a previous hamstring, groin or calf injury increased the risk for hamstrings injury. Attention has been devoted to the modifiable risk factors, but non-modifiable factors should be further explored, as well as the mechanisms whereby some non-modifiable factors increase injury risk.

Although it is not possible to erase the existence or previous injuries, or change the age of the player, a better understanding of the hamstrings' architecture, anatomy and mechanisms may help to deliver a better-individualized exercise-based approach. It was previously established that previous injuries impair ROM (Maniar et al., 2016) and endurance (Farley et al., 2020), both of which can be improved through well-designed exercise intervention protocols. While some architectural features might be modifiable [e.g., cross-sectional area (CSA)], others may not (e.g., variations in insertions), and an understanding of such features may afford a better-individualized exercise prescription. Sex- and age-related risk factors will be explored further in part 2, as they are closely linked to anatomic and physiologic variations. Even when certain factors are non-modifiable, the mechanisms that contribute to the increased injury risk could be individually targeted with better exercise-based interventions, to avoid exacerbating predisposing factors.

Synopsis of the First Part

The hamstrings are highly relevant for major movements of daily life and sports (e.g., standing, walking, sprinting, cutting), and play an especially important role in decelerating knee

BOX 1 | Special box – hamstrings' strength evaluation.

Measuring muscle strength allows evaluating and comparing muscle function and performance to obtain a pattern of intramuscular synergistic recruitment between the BF, ST, and SM muscles (Mjølsnes et al., 2004; Schuermans et al., 2014; Wiesinger et al., 2020). Improvement of hamstrings strength is an important strategy to mitigate injury incidence and recurrence, and one of the RTP criteria (Maniar et al., 2016). Early identification and management of excessive strength asymmetries and muscular imbalances assist in the elaboration of a more effective intervention plan to counteract a potential injury risk scenario (Schuermans et al., 2014; Wiesinger et al., 2020). The stationary isokinetic dynamometry (IKD) is a suitable system for assessment of torque during concentric and eccentric knee flexor action (Aagaard et al., 1998; van Dyk et al., 2018; Wollin et al., 2018), although its use is scarce compared to the Nordic hamstrings device (NHD) (Mjølsnes et al., 2004). The NHD detects strength deficits or side-to-side imbalances (Timmins et al., 2016), exercise-related strength progress (Delahunt et al., 2016), and may aid predicting recovery time after injury (McCall et al., 2016). However, van Dyk et al. (2018) reported a weak within-subject correlation (r = 0.35), demonstrating a systematic bias toward lower strength values with the NHE. Several methodological specificities were not considered when comparing both methods in previous studies, e.g., IKD and NHD tests were performed under different conditions of joint velocity and hip position (Higashihara et al., 2010a; Guex et al., 2016).

To understand these issues, Wiesinger et al. (2020) compared the mechanical output of hamstrings assessed by using both IKD and NHD methods of 25 healthy male athletes in a counterbalanced repeated-measures protocol. Higher total eccentric work, peak torque at greater knee extension angles, greater side-to-side strength difference, and lower eccentric peak torque were observed in IKD compared to NHD, whereas bilateral strength difference was lower in NHD. The electromyographic analysis showed no difference in the activation of BF and ST during IKD and NHD (Wiesinger et al., 2020). These findings suggest that IKD and NHD methods measured distinct hamstrings muscle activation characteristics. Some aspects were difficult to assess, such as intraindividual strength differences, angle of peak torque and side-to-side differences in eccentric knee flexor strength. It should be highlighted that muscles such as the sartorius, gracilis and gastrocnemius also contribute to knee flexion (Standring, 2015).

Surface electromyography (EMG) allow the assessment of muscle activation patterns (Higashihara et al., 2010a). The EMG activity shows that hamstrings muscles have functional differences, as the SM and BF work harder (i.e., greater EMG) during the initial phase of knee flexion, and the ST at deep flexion angles to complement the decrease in EMG of the other two muscles during open kinetic chain exercise (Hirose and Tsuruike, 2018). Miller et al. (2000) found that during isokinetic movements activation, the BF EMG increased with increasing movement velocity, while the ST and SM EMG activation remained constant during six continuous isokinetic knee extension and flexion movements at 60°, 180°, and 300° s^{-1}. In opposition, functional MRI evaluates the intramuscular and intermuscular recruitment patterns with a very high spatial accuracy and detects the magnitude of metabolic activity in muscle tissue although with no real-time information about the amount and timing of the underlying muscle activity (Segal, 2007). Using functional MRI, Schuermans et al. (2014) demonstrated that the more symmetrical and less dissociated the hamstrings muscles work together, the higher the physiological changes would be inside the recruited muscle fibers. Thus, more intramuscular variability can be associated with a reduced metabolic turnover and more economic muscle functioning. MRI-based methods have high costs, risk of injury during assessment, lack of portability and long duration of assessment. Rapid, non-invasive alternative measures of concentric and eccentric hamstrings strength are warranted.

An adapted aneroid sphygmomanometer test (Mondin et al., 2018), a rapid and non-invasive tool, was proposed to assess hamstrings strength. In 14 rugby players, Mondin et al. (2018) found an association between the sphygmomanometer derived pressures at 30 and 90° of knee flexion and isokinetic strength measures, suggesting that this method was reliable to assess hamstrings strength, but not to identify strength asymmetries between dominant and non-dominant legs or knee flexors-to-knee extensors ratios.

(Continued)

BOX 1 | Continued
The limitation of knee flexors-to-knee extensors ratios was explained by the inability of aneroid sphygmomanometer test to measure quadriceps strength at 30° knee flexion with consistency, due to many participants exceeding the readings on the sphygmomanometer scale. This method has a great potential, but requires further studies to be useful as a muscle strength assessment and injury risk screening procedure.

extension and hip flexion in high-velocity actions, common in athletic scenarios, such as sprinting. Hamstrings are prone to injuries and so it is important to understand the risk factors involved. Exercise-based interventions for mitigating hamstrings injury risk have emphasized the role of eccentrically biased strength training, although lumbo-pelvic dynamics, technical and motor pattern focused work, sprinting, isometric strength training and general strength and endurance training should not be neglected. Weekly frequency and compliance with the interventions constitutes a relevant factor mediating the effectiveness of exercise-based interventions in mitigating injury risk. However, research suggests that non-modifiable risk factors, namely age and previous injuries, may play a more relevant role in hamstrings injury risk than modifiable risk factors. In this context, relevant anatomic variations may alter the risk of hamstrings injury. Acknowledging the relevant anatomic variations may provide relevant information for prescribing exercise interventions, which will be the focus of the second part of this commentary. **Box 1** briefly explores hamstrings' strength evaluation.

PART 2: ANATOMIC AND PHYSIOLOGIC VARIATIONS OF THE HAMSTRINGS AND POTENTIAL IMPLICATIONS FOR INJURY RISK

In this second part, we address the main variations or variants of the hamstrings' anatomy and physiology, which imply that there is a "normal" or "usual" state of affairs. We start by presenting the commonly described anatomic features of the hamstrings, before engaging in an exploration of their variations.

Mainstream Anatomic Description of the Hamstrings

We overview the basic hamstrings anatomy as described in the 41st Edition of Gray's Anatomy (Standring, 2015). Throughout this section, the information derives from this source, unless otherwise stated. The hamstrings, or ischiocrural muscles, comprise the muscles of the posterior compartment of the thigh, and include the ST, SM and BF (long head – BFlh; short head – BFsh), which attach proximally to the ischial tuberosity (except the BFsh). The ST, SM and BFlh are biarticular muscles, and act in extending the coxofemoral joint (hip), as well as flexing and rotating (medially and laterally) the knee. Distally, the ST and SM attach to the tibia, while both heads of the BF attach to the fibula. The hamstrings present relevant interindividual variation in length. The hamstrings are innervated by the sciatic nerve, emerging at the level of S1 vertebra, although with interindividual variations.

Functionally, the hamstrings play an important role in transferring load between the trunk and the lower limbs, and affect the tension of the thoracolumbar fascia. They affect the normal lumbar lordosis, as their length and strength interferes with the position of the innominate bone. When raising the trunk, the hamstrings act in conjunction with the gluteus maximus. It is highlighted that the hamstrings strongly contract in actions involving the need to extend or control the rate of flexion at the hip, while the gluteus maximus contracts when powerful extension of the hip joint is required. The adductor magnus also performs hip extension, has attachments to the ischial tuberosity, and the ischial portion of the adductor magnus shares innervation from the tibial division of the sciatic nerve. The adductor magnus can be considered to have a proper hamstrings portion (Broski et al., 2016; Jeno and Schindler, 2020). Pathology or diminished functional capacity of the adductor magnus and/or the gluteus maximus may result in a greater demand upon the hamstrings. The coordination of the hamstrings with other muscles of the lumbopelvic region may be relevant in understanding hamstrings injuries (Thelen et al., 2006; Shield and Bourne, 2018). **Figure 1** presents an overview of the hamstrings muscles.

Semitendinosus

The ST has a posteromedial position in the thigh and has a long tendon. Proximally, its tendon is shared with the BFlh, but then these muscles diverge. In the first ±7.5 cm of their path, they share an aponeurosis. The belly of the ST usually spans only until the mid-thigh, after which its long tendon runs until its attachment in the upper part of the medial surface of the tibia, behind the attachment of the sartorius muscle and distal to the attachment of gracilis muscle. The terminal portion the ST tendon is united with the tendon of gracilis (further reinforcing the functional connections between the hamstrings and the adductor group), provides an expansion to the deep fascia of the leg and to the medial head of the gastrocnemius, and anatomically (and perhaps functionally) links the ST with the medial gastrocnemius. It is open to speculation whether pathology or dysfunction of the medial gastrocnemius interfere with the action of the ST. Usually, the midpoint of the ST receives a muscular slip from the BFlh, denoting a role in lateral force transfer.

Innervation to the ST is provided by the sciatic nerve (L5, S1, S2), through its tibial division. This pattern is shared with the SM, which lies deep to the ST, and by the BFlh, but not by the BFsh. Beyond the actions common to all hamstrings, the ST (as well as the SM) can medially rotate the knee when this joint is semi-flexed. With the hip extended, ST (and SM) can act to produce medial rotation of the thigh.

Semimembranosus

The SM also lies posteromedially in the thigh, deep to the ST. Proximally, it exhibits a long and flat tendon attached to the ischial tuberosity, and it receives fibrous expansions that flank the adductor magnus. Distally, the SM has a ramified pattern,

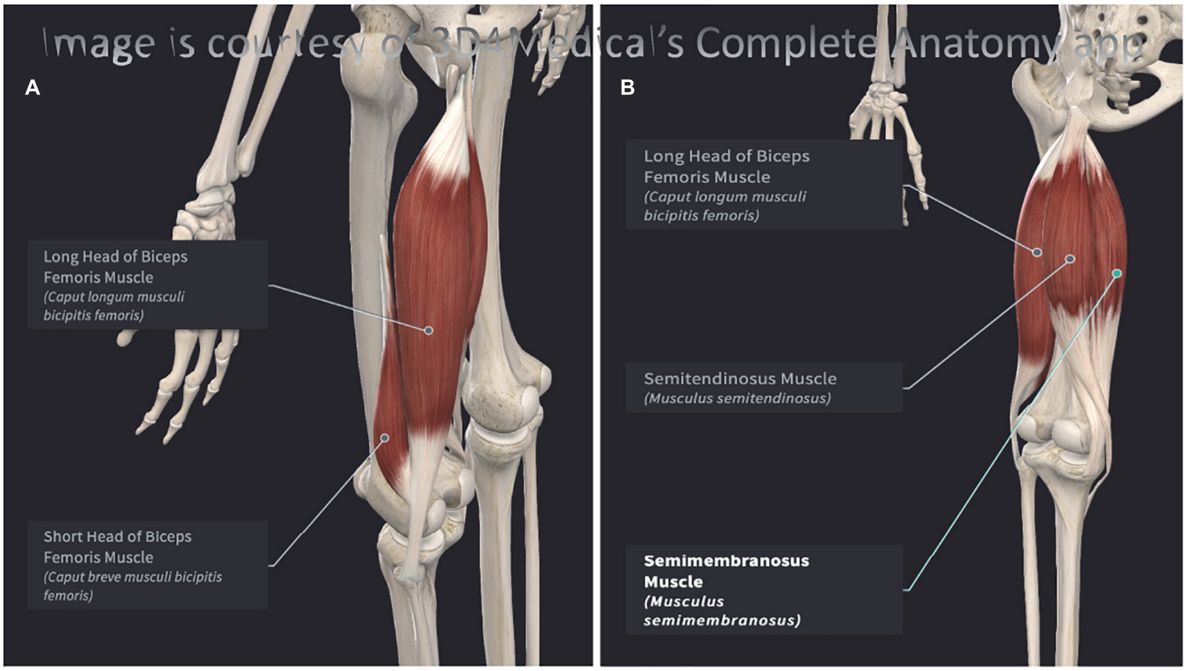

FIGURE 1 | (A) Posterolateral view of the left hamstrings. **(B)** Posteromedial view of the left hamstrings. Both images were elaborated with Complete Anatomy 2021, version 7.0.0. (desktop version for Mac OS) and reproduced here with permission (3D4Medical, Elsevier).

sharing fibers with both ST and BFlh. Circa the mid-thigh, the SM gives off muscle fibers that converge to a second aponeurosis that attaches distally over the terminal tendon. The SM distal tendon divides into five components: anterior, direct, capsular, inferior and the oblique popliteal ligament (Beltran et al., 2003). The SM has a close anatomic relationship with the medial gastrocnemius, usually separated by a bursa.

Gray's Anatomy describes that the SM can vary considerably in size and may be absent (which could potentially overload the ST) or double (which could potentially underload the ST). Proximally, the SM can arise from the sacrotuberous ligament instead of the ischial tuberosity. It can have muscular slips to the femur or to the adductor magnus. Although myotendinous and avulsion injuries are common in the SM, complete tears are seldom reported in the literature (Beltran et al., 2003).

Biceps Femoris

Unlike the ST and SM, the BF occupies a posterolateral position in the thigh. Proximally, the BFlh attaches to the ischial tuberosity, through a common tendon with the ST, but it can also insert into the sacrotuberous ligament. The BFlh has a fusiform belly, and its fibers terminate in an aponeurosis that also receives fibers from the BFsh. Pennation angle of the BFlh fibers is non-uniform, being greater in the proximal-middle sections, in comparison to the distal and extreme proximal sections (Huygaerts et al., 2021). The BFsh has its proximal attachment in the lateral lip of the *linea aspera* and this attachment may extend upward to almost the level of gluteus maximus. Distally, these muscles share a common tendon inserting into the head of the fibula and send expansions to the fibular collateral ligament and to the lateral condyle of the tibia. A variation noted in Gray's Anatomy description is the absence of the BFsh.

While the BFlh shares innervation with the ST and SM, arising from the tibial division of the sciatic nerve (L5, S1, S2), the BFsh is innervated by nerves emerging from the same levels, but traveling in the common fibular division of the sciatic nerve. This differential innervation of the two heads of the BF may result in asynchrony and impaired coordination (Beltran et al., 2012; Huygaerts et al., 2021), potentially placing the BF at greater risk of injury (Burkett, 1975; Entwisle et al., 2017). Theoretical models have proposed that BF may be at increased injury risk in comparison with ST and SM (Dolman et al., 2014), owing to an additional BF compensation induced by a poor ST endurance, placing the BF at higher injury risk (Schuermans et al., 2014). In a retrospective study with 275 men soccer players who had sustained hamstrings injuries, the BFlh was the most commonly affected (56.5%), followed by the ST (24.4%), SM (13.7%), and BFsh (5.6%) (Crema et al., 2016). In Australian rules football players, injury of the BF represented 78% of all hamstrings injuries (Verrall et al., 2003). Beyond the actions common to all hamstrings, the BF can laterally rotate the knee when this joint is semi-flexed. With the hip extended, BF can act to produce lateral rotation of the thigh.

Anatomic and Physiologic Variations of the Hamstrings

The hamstrings integrate the posterior compartment of the thigh, considered to be the most variable compartment (Tubbs et al., 2016). We explore known anatomic variations to unfold their implications for injury risk. It is unlikely that any single risk factor allows establishing a strong relationship with injury risk, but recognizing their existence may provide a more thorough understanding of individualized injury risk. Technological developments may translate that enhanced knowledge into practical applications. **Table 1** summarizes the articles exploring anatomic variations of the hamstrings.

Muscle Bellies

Cadaveric analysis showed that the extension of BFsh attachments to the femur vary substantially between individuals, leading to the hypothesis that individuals with more extensive attachments may apply more force, potentially increasing the risk of a strain injury (Burkett, 1975). The opposite interpretation is also possible, as attachments that are more extensive would allow the BFsh muscle belly to be more firmly attached to the femur, and therefore may be at less risk of a strain injury. The two heads of the BF have different proximal attachments and innervation, possibly representing muscles that were independent, but fused during the human evolutionary process (Tubbs et al., 2016). In some individuals the BFlh and BFsh remain separate, and occasionally the BFsh is absent (Tubbs et al., 2016). From 29 cadaveric specimens with a median age of 71.5 (range from 45 to 98), BFlh and BFsh extended approximately 42.0 and 29.8 cm, respectively (van der Made et al., 2015).

The ST can be partly fused with the SM (Tubbs et al., 2016) or the BFlh (Schmuter et al., 2021), and may present accessory slips to the coccyx, sacrotuberous ligament or to the ischial tuberosity (Fraser et al., 2013). Supernumerary ST bellies have been observed (Gray, 1945). Although ST and BFlh usually share a common proximal tendon attachment and aponeurosis, complete separation of these two muscles has been reported (Tubbs et al., 2016). Of note, the tendon of the ST may receive muscle slips from the quadratus femoris (Tubbs et al., 2016).

The SM can be doubled, absent, or split, and up to four bellies have been described inserting into the adductor magnus, planum popliteum and tibia (Tubbs et al., 2016). The ST, SM and BFlh may originate from a common tendon, which can be continuous with the intermuscular septum and, occasionally, envelop the piriformis muscle (Tubbs et al., 2016), providing another example of the connections between the hamstrings and other muscles of the lumbo-pelvic region.

Muscle Fiber Type

The muscle fibers vary in their morphology and histochemical properties (Galpin et al., 2012). Classifications of fiber muscle types have evolved through the years and certain classifications are still debated (Talbot and Maves, 2016). Here, we use the original classification reported by the authors being cited. There are important functional differences between different muscle fiber types, such as distinct metabolic properties (Mishra et al., 2015). There may be a bidirectional relationship, whereby metabolic environment also influences the characteristics of the muscle fibers (Julien et al., 2018). Type II muscle fibers are more easily fatigued than type I fibers (Lievens et al., 2020), and more susceptible to plyometric- (Macaluso et al., 2012) as well as eccentric-induced muscle damage (Fridén et al., 1983). Despite this information, eccentric actions have been emphasized by many programs for reducing injury risk of the hamstrings (van der Horst et al., 2015; Almeida et al., 2018; Elerian et al., 2019). Perhaps athletes with greater percentage of type II fibers should follow a more careful monitoring of the eccentric work they perform.

A previous cadaveric study with seven men and three women (37 to 76 years upon death) showed that type II fibers composed 48.5 to 59.5% of the proximal BFlh, 51.0 to 58% of the distal BFlh, 45.5 to 69.0% of the BFsh, 51.0 to 57.5% of the proximal ST, 50.0 to 69.5% of the distal ST, 47.5 to 54.5% of the proximal SM, and 44.0 to 55.5% of the distal ST (Garrett et al., 1984). In a study with 15 sedentary men aged 17 to 40 years old (Dahmane et al., 2006), the BF had a mixed fiber composition of ∼50% type 1 and ∼50% type 2 (25.2 ± 1.3% type 2a, 20.7 ± 1.4% type 2x and 5.7 ± 0.7% type 2c), meaning that BF has a considerable amount of fast-twitch fibers. Biopsies of the BFlh performed in 31 healthy, young men, showed a balanced distribution of myosin heavy chains (MHC), with 47.1 ± 9.1% MHC-I, 35.5 ± 8.5% MHC-IIA, and 17.4 ± 9.1% MHC-IIX (Evangelidis et al., 2017). For the ST, a composition of 50 ± 13% type 1 fibers, 26 ± 8% type 2A, 23 ± 19% type 2B and 1 ± 1% type 2C was found on biopsies from 16 patients 7–11 months after ACL surgery (Shalabi et al., 2002). In this reported ST composition (Shalabi et al., 2002), the standard-deviations of the reported ST muscle composition were relevant, especially in type 2B fibers, denoting interindividual variation.

It has been suggested that high-level sprinters are sharply different than the average person, with a percentage of up to ∼70% of type 2 fibers in the lower limb muscles, but this was derived from analysis of the vastus lateralis (Trappe et al., 2015), and should not be generalized to other lower limb muscles. Differences in fiber type composition of the lower limbs have been observed between endurance runners, power-trained individuals, and strength-trained individuals (Methenitis et al., 2019), but again these findings were extrapolated from observations of the vastus lateralis. *In vivo* knowledge of hamstrings muscle composition in humans remains largely unknown (Evangelidis et al., 2017).

As Lievens et al. (2020) pointed out, athletes with distinct muscle typology should train differently, and the individualization of training on the basis of this information is important to optimize performance and lowering the risk of potential injury. Unfortunately, knowledge of muscle fiber composition of the human hamstrings is scarce for building a broad picture. Furthermore, feasibility may require the development of more readily available, non-invasive, non-expensive technologies.

Pennation Angles

A study analyzed the pennation angles of BFlh and ST muscles from six legs derived from three male cadavers (Kellis et al., 2009). The BFlh and ST exhibited very similar pennation angles through cadaveric dissection (13.52 ± 2.35° versus 13.39 ± 3.31°) and ultrasound (13.88 ± 2.76° versus 13.34 ± 2.61°). The small standard deviation was suggestive of non-relevant intra- and interindividual variations. A posterior study of the same group

TABLE 1 | Summary of studies cited in Section "Anatomic and Physiologic Variations of the Hamstrings."

References	Population	Anatomic part assessed	Assessment method	Main findings
An et al. (2010)	50 lower extremity cadavers from 15 males and 12 females (age: 71 years)	Innervation patterns of HT	Dissection	BFsh and SM show one innervation entry, and BFlh and ST show two innervation entries. In those with two entries, for BFlh, the incidence of type I innervation was 82% (18% of type II) while for ST, the incidence of type I innervation was 14% (86% of type II).
Blazevich et al. (2006)	16 women and 15 men physically active (age: 19.9–20.6 years)	Architecture of quadriceps	Ultrasound	Description of the architecture of the quadriceps. Changes in the relative activation of individual muscles determines alterations in force, velocity movement range and contraction mode. Intramuscular activation changes with the movement requirements. Training should alter the pattern of activations to improve force transmission between muscles with different architecture, make these variations in activations efficient, and promote region-specific hypertrophic responses. There are little differences between sex in these adaptations.
Burkett (1975)	Review	Anatomical variation of HT	–	Extensive biceps femoris-femur attachment coupled with strength imbalances between HT increase the risk of strains.
Dahmane et al. (2006)	15 healthy sedentary males (age: 17–40 years) and 15 male sprinters (age: 23.2 ± 3.1 years)	Muscle fiber types of BF	Mechanomyography	Strong potential for the BF muscle to transform from slow to faster contracting muscle fibers after long-term sprint training.
Evangelidis et al. (2015)	30 healthy recreationally active individuals (age: 20.7 ± 2.6 years)	Aponeurosis of BFlh	MRI	The proximal aponeurosis size is highly variable between individuals, and it is not associated with to muscle size or knee flexor maximal isometric or eccentric strength. This disproportion may predispose those individuals with relatively small aponeurosis to hamstring strain injuries, as they could be subjected to greater mechanical strain in the muscle tissue surrounding the aponeurosis.
Evangelidis et al. (2017)	31 healthy, recreationally active participants (age: 21 ± 3 years)	Muscle fiber types of HT	MRI	HT muscles exhibited a balanced myosin heavy chain isoform distribution comparable to that of vastus lateralis, so that the predominance of fast-twitch fibers in HT muscle increasing the risk of strains is not supported.
Fiorentino and Blemker (2014)	12 male track and field athletes	Musculotendon of BFlh	MRI	A larger muscle and/or narrower proximal aponeurosis of the BFlh could predispose an athlete to an increased risk of injury by increasing peak local muscle tissue strain.
Fraser et al. (2013)	1 female cadaver (age: 87 years)	Anatomical variation of ST	Dissection	Two-part origin of the ST, with the variant portion being originated along the medial border of the ischial tuberosity. Could predispose to HT injury, chronic pain and pelvic floor discomfort.
Freitas et al. (2020)	40 male professional football players (age: 24.5 ± 4.9 years)	Aponeurosis of BFlh	MRI	There were no significant differences for size aponeurosis of the BFlh between players with and without previous BFlh injury.
Fridén et al. (1983)	12 male physical education students (age: 25 ± 7 years)	Muscle fiber types of vastus lateralis	Muscle biopsy	Type II fibers were more extensively damaged than type I fibers after 30 min of pedaling at a frequency of 60 rpm, at an intensity of 80–100% of VO_2max, in a bicycle ergometer modified for use in eccentric work.
Galpin et al. (2012)	Description muscle fiber types	–	–	–
Garrett et al. (1984)	7 male and 3 female cadavers (age: 60 years)	Muscle fiber types of lower limb muscles	ATPase histochemical reaction	Relatively higher percentage of type II fibers in HT compared to other thigh and leg muscles.
Gérard et al. (2020)	SR: 10 RCTs evaluating 346 healthy adults (age: 18.3–29.6 years)	Architecture of BF	MRI and ultrasound	Eccentric strength training associated with increased fascicle length and muscle thickness, and decreased pennation angle, as well as eccentric strength of the HT.
Gray (1945)	2 male cadavers (age: 44 and 84 years)	Anatomical variation of HT	Dissection	All HT muscles originated from a common tendon in case 1. A muscle from the linea aspera and passing medially to the capsule of the knee joint, homologous with the ST, is presented in case 2.
Huygaerts et al. (2021)	Critical review	Muscle-tendon unit of HT	–	Fiber-fascicle lengthening is greater, and architectural structure non-uniform in the BFlh, with pennation being greater in the proximal-middle section compared to the distal and extreme proximal sections, that in addition to the inter-individual differences in BFlh structural features may predispose to this muscle to higher risk of HT injury.

(Continued)

TABLE 1 | Continued

References	Population	Anatomic part assessed	Assessment method	Main findings
Julien et al. (2018)	Review	Muscle fiber types	–	The changing environment could elucidate changes in muscle fiber characteristics, which could have implications in metabolism-related muscular atrophies.
Kellis (2018)	Narrative review	Architecture of HT	–	BFlh, compared to other HT, may be at higher increase of injury due to inter-muscular differences in HT architecture. Targeting the specific muscle-tendon region, instead of HT as a whole, could be beneficial in rehabilitation programs.
Kellis et al. (2009)	3 cadavers (age: 68.3 years)	Architectural parameters of BFlh and ST	Ultrasound dissection	High level of agreement in BFlh and ST architectural parameters measured through ultrasound compared to direct dissection.
Kellis et al. (2012)	8 cadavers (age: 67.8 years)	Architecture of HT	Dissection	The four hamstring components showed low to moderate architectural dissimilarity. Pennation angles were similar between BFlh, BFsh and SM, but higher than for ST.
Lievens et al. (2020)	32 male recreational athletes	Muscle fiber types of gastrocnemius	Magnetic resonance spectroscopy	Participants with predominantly fast typology fibers fatigues more markedly in repeated Wingate tests than participants with slow typology fibers.
Macaluso et al. (2012)	8 healthy, untrained individuals (age: 22 ± 1 years)	Muscle fiber types of vastus lateralis	Muscle biopsy	An acute bout of plyometric exercise preferentially affects type II fibers
Marušič et al. (2020)	40 healthy adults (age: 23.7 ± 2.5 years)	Architecture of BFlh	Ultrasound	A 6-week progressive eccentric HT training in a lengthened position showed positive effects for fascicle length (increase) and pennation angle (decrease), but not for muscle thickness.
Medeiros et al. (2020)	32 adult football players (age: 18–23 years)	Architecture of BFlh	Ultrasonography	An 8-week Nordic hamstring exercise training program was not effective at elucidating any improvement in muscle thickness, pennation angle, or fascicle length.
Mendiguchia et al. (2020)	32 adult football players	Architecture of BFlh	Ultrasound	The sprint training group showed moderate increase in fascicle length, and the Nordic group a small increase. The Nordic group presented a small increase at pennation angle.
Methenitis et al. (2019)	10 sedentary, 9 endurance runners, 10 power-trained and 9 strength-trained individuals	Muscle fiber composition of vastus lateralis	Muscle biopsy	Muscle fiber composition and rate of force development is affected by systematic training among different athletes. Type IIx fibers better correlated to rate of force development.
Rehorn and Blemker (2010)	1 healthy male individual	Aponeurosis of BFlh	MRI and 3D modeling	The fact that proximal aponeurosis is narrower than distal aponeurosis in BFlh could explain the prevalence of injuries near the proximal myotendinous junction in this muscle, and relative aponeurosis dimensions could also explain the more prevalence of injuries in BFlh compared to other HT muscles.
Schmuter et al. (2021)	1 female cadaver (age: 59 years)	Anatomical variations of BFlh and ST	Dissection	BFlh and ST were fused near their origin at the ischial tuberosity.
Shalabi et al. (2002)	14 males and 2 females (age: 26 years)	Muscle fiber types of ST	Muscle biopsy (percutaneous ultrasound-guided biopsy)	Patients following ACL reconstruction show a composition of 50 ± 13% type 1 fibers, 26 ± 8% type 2A, 23 ± 19% type 2B and 1 ± 1% type 2C. Muscle biopsy technique is adequate to identify muscle composition.
Solomon and Stevenson (2008)	1 male (age: 40 years)	Anatomical variation of BF tendon insertion	MRI	Case of a bilateral tibial insertion of the BF tendon in a previously asymptomatic patient, which could have implications on lateral knee stability.
Trappe et al. (2015)	1 world champion sprinter	Muscle fiber types of vastus lateralis	Muscle biopsy	Large proportion of vastus lateralis type IIx fibers in the sprinter, and power output from type IIa and IIx fibers was higher than any human values reported to date.
van der Made et al. (2015)	29 human cadaveric specimens (age: 71.5 years)	Anatomical variations of HT (origin dimensions, muscle length, tendon length, MTJ length and width, length of tendinous of ST (raphe).	Dissection	Overlapping proximal and distal tendons and muscle architecture may lead to a force not in line with the tendon and predispose to muscle injury. Protective effect of the presence of a raphe.

(Continued)

TABLE 1 | Continued

References	Population	Anatomic part assessed	Assessment method	Main findings
Vieira et al. (2007)	41 male and 56 female asymptomatic patients (age: 52.8 years)	Innervation patterns of BF	MRI	Description of the normal anatomy of the distal BF and the relationship with the peroneal nerve. The peroneal nerve can pass downward posterior to the BFsh and superficial to the lateral head of the gastrocnemius, but also in a tunnel between the two muscles (23%). An unusual relationship between the nerve and the distal BF could predispose to peroneal neuropathy.
Woodley and Mercer (2005)	3 female and 3 male cadavers (age: 69–88 years)	Architecture of HT	Dissection	ST, SM and BF showed anatomical partitioning defined by architecture and/or pattern of innervation. There was high degree of variation from subject to subject in most of the architectural features of the HT, such as, fascicular length, volume, physiological cross-sectional area or tendon characteristics.
Yang et al. (2018)	93 patients (age: 40.2 years)	Innervation patterns of BF	MRI	There were less participants with type I (38.7%) than type II innervation (61.3%), in which the thickness was lower. The course of common peroneal neuropathy through the "popliteal tunnel" formed between the BFsh and the lateral gastrocnemius muscle was approximately 40%.
Yasin et al. (2010)	25 patients undergoing ACL reconstruction with HT tendon autograph (age: 28 years)	Anatomy of accessory bands	Dissection	Gracilis and ST tendons showed a variable pattern of accessory bands, all of them occurring more than 11-cm proximal to the insertion of the tendons onto the tibial crest.

HT, hamstrings; MTJ, musculotendinous junction; ST, semitendinosus; BFlh, biceps femoris long head; VO_2max, maximal oxygen consumption; ATPase, adenosine triphosphatase; BF, biceps femoris; MRI, magnetic resonance imaging; ACL, anterior cruciate ligament; BFsh, biceps femoris short head; SM, semimembranosus; RCT, randomized controlled trial.

dissected eight cadavers (age: 67.8 ± 4.3 years) (Kellis et al., 2012) and demonstrated that pennation angles of the BFlh, BFsh and SM were similar (13.46 ± 2.88°, 13.17 ± 2.60°, and 15.95 ± 2.39°, respectively), but substantially different from the pennation angle of the ST (9.14 ± 3.54°), which differs from the results found in their previous work (Kellis et al., 2009). The authors used a combination of mean fiber length, sarcomere length, physiological CSA, and pennation angle to calculate a similarity index (δ) between pairs of muscles, with lower values denoting greater similarity. While the BFlh and BFsh had a δ = 0.54, and BFlh and SM had a δ = 0.35, denoting a moderate similarity, there was low similarity between SM and ST (δ = 0.98) and between BFlh and ST (δ = 1.17). These findings underline that although the hamstrings are usually treated as a muscle group, there are relevant inter-muscular architectural differences between its individual muscles (Kellis et al., 2012).

A SRMA showed a limited to moderate confidence in evidence that eccentric training performed for a minimum of 4 weeks decreased the BFlh pennation angle (Gérard et al., 2020), which was also shown in a recent study (Marušič et al., 2020). However, another study with 32 soccer players (age, 18–23 years) performing an 8-week NHE program once a week versus twice a week showed no between-group differences in BFlh pennation angles (Medeiros et al., 2020). A prospective controlled study with soccer players analyzed the NHE and sprint interventions, showing that only the NHE induced small increases in the BFlh pennation angle (Mendiguchia et al., 2020), contradicting the decreases seen in previous studies (Gérard et al., 2020; Marušič et al., 2020). Results are conflicting, largely limited to the BFlh and involve a narrow set of exercise modalities. Further research is warranted to understand how different exercise modalities affect pennation angles.

Tendons

There is variation in the accessory bands of the hamstrings tendons (Yasin et al., 2010), as well as in tendon length. One study showed that the muscle belly of the SM originated at varying distances from the ischial tuberosity in different subjects, ranging from 8.6 to 14.5 cm (Woodley and Mercer, 2005). Interindividual variations in tendon-to-fiber length ratios may play a role in injury risk, as in the case of tendons of similar structure, tendon length critically influences compliance (Huygaerts et al., 2021). If two athletes have different ratios for the ST, it is possible that one will be at increased risk of injury than the other. It was hypothesized that bigger tendon-to-fiber length ratios provide a greater buffer to the muscle belly, potentially affording increased protection from injury, but this requires confirmation (Kellis, 2018; Huygaerts et al., 2021).

Aponeurosis

The aponeurosis may affect different muscle regions distinctly, demanding greater elongation of fibers in certain regions and less so in others (Blazevich et al., 2006; Huygaerts et al., 2021). A modeling study suggested that smaller ratios of aponeurosis to muscle width generated larger maximum peak local strain, postulating those larger muscles and/or narrower aponeurosis increased risk of injury (Fiorentino and Blemker, 2014). Interindividual variation in anatomic structure may put individuals with distinct aponeurosis to muscle width ratio at different levels of injury risk. However, an MRI study conducted in 30 healthy young men performing maximum voluntary actions of knee flexion showed interindividual variability in the area of the BFlh proximal aponeurosis, ranging from 7.5 to 33.5 cm (Evangelidis et al., 2015), and this area was not correlated with BFlh maximal anatomic CSA. The aponeurosis-to-muscle area ratio exhibited six-fold variability, with an interindividual ratio variation of 83%, and aponeurosis size was not related to isometric or eccentric knee flexion strength (Evangelidis et al., 2015). The authors stated that "individuals with a relatively small aponeurosis may be at increased risk of HSI" (Evangelidis et al., 2015, p. 1383), but this was not demonstrated.

A study using MRI to compare 80 thighs from 40 professional soccer players with (n = 9) or without previous (n = 71) BFlh injury in the preceding 3 years, suggested that proximal aponeurosis size of the BFlh was not an independent risk factor for HSI (Freitas et al., 2020). Notwithstanding, another computational modeling study suggested that varying the width, length and thickness of the BFlh aponeurosis had an impact on the location and magnitude of peak stretches within the muscle (Rehorn and Blemker, 2010). The authors found that location and magnitude of peak stretch could be explained by the difference in widths between the proximal and distal aponeurosis of the BFlh (Rehorn and Blemker, 2010). Aponeurosis characteristics may not represent an independent risk factor, but their interaction with factors such as tendon length, muscle belly anatomic CSA, among others, may provide further cues to better understand its relationship with hamstrings injury.

Innervation Patterns

Interindividual differences in innervation patterns of the hamstrings should be acknowledged, and their relationship with injury explored (Huygaerts et al., 2021). In some persons, the BFsh has two distinct regions, each innervated by a separate nerve (Woodley and Mercer, 2005). In the same study, two of the six subjects had a common trunk for nerve supply to SM and an inferior compartment for ST (Woodley and Mercer, 2005). The nerve to SM had branches to the adductor magnus muscle, highlighting the functional connections between the adductor magnus and the hamstrings. Woodley and Mercer (2005) showed that the primary branch of the nerve to SM, that supplied its distal region, had varied entry points (i.e., the location where the nerve branch pierces the muscle belly), from 22.5 cm distal to the ischial tuberosity in one, and 34.5 cm in another. Similar variations were found for both ST and BF (Woodley and Mercer, 2005). In a dissection of 50 cadaveric lower limbs, motor entry points and intramuscular nerve endings of the hamstrings were examined (An et al., 2010). In ST and BFlh, two distinct branching nerve patterns were found, classified into type I (only one primary motor branch emerging from the sciatic nerve) and type II (two primary motor branches) (An et al., 2010). In the BFlh, 82% of lower limbs presented a type I innervation, while in ST 86% had a type II innervation (An et al., 2010). Whether (and to what extent) subjects with different pattern type present alterations of motor coordination or how they affect functional performance is currently unknown.

In one study, two observers retrospectively reviewed one hundred 1.5-T knee MRI studies in 97 asymptomatic subjects (41 men and 56 women) for assessing anatomy of the distal BF (Vieira et al., 2007). The posterior extent of the BFsh was ≤1 cm in 50% of subjects, between 1–2 cm in 34%, between 2–3 cm in 15%, and ≥3 cm in one subject. The distal extent of the muscle belly of BFsh from the joint space varied from −2 cm in 5% of subjects to +2 cm in 2%, with 51% presenting 0 cm, and ∼42% showed +1 or −1 cm. The length and path of the BFsh showed interindividual variation in asymptomatic subjects. In this distal MRI assessment (Vieira et al., 2007), the muscle belly of the BFlh was identified in 40% of the subjects, and not visible in 60% of the subjects, suggesting that interindividual differences exist for muscle belly-to-distal tendon length ratios for the BFlh. In 77% of subjects the common peroneal nerve was situated superficial to the lateral head of the gastrocnemius and posterior to the BFsh; in the other 23% of subjects, a narrow tunnel between the lateral head of the gastrocnemius and the BFsh enveloped this nerve, but this tunneling effect did not result in neuropathy (Vieira et al., 2007).

A similar study retrospectively analyzed 1.5-T knee MRI scans of 93 Korean subjects, divided into types according to the course of the common peroneal nerve: type I (no tunnel) and type II (tunnel) (Yang et al., 2018): ∼40% of subjects were classified as type II, which is superior to the percentage observed in the aforementioned study (Vieira et al., 2007). This suggests that the prevalence of certain anatomic features may vary geographically (possibly related to genetic, environmental, and historical factors). In this study (Yang et al., 2018), type II subjects had significantly greater BFsh thickness. The functional relevance of these anatomic variations is not straightforward.

Attachment Sites

Despite traditional textbook description of the BF distal tendon as attaching to the fibular head (Standring, 2015), at least one case is described where the distal tendon of the BF inserted on the lateral aspect of the tibia, posterior to the iliotibial band and above the level of the fibular head (Solomon and Stevenson, 2008). In this case, the BF did have attachments to the fibular head, but these were muscular in nature. Whether this feature interferes with the mechanics of knee lateral rotation, implicates in the proximal actions (i.e., at hip joint level), or changes the coordination with other hip lateral rotators muscles (e.g., gluteus maximus, piriformis), remains speculative.

Sex and Age-Related Differences in Hamstrings' Anatomy and Physiology

This section addressed sex and age-related differences in hamstrings' features, and **Table 2** summarizes the articles consulted in this section.

Sex-Related Differences in Hamstrings' Anatomy and Physiology

A study with recreational athletes (11 men and 10 women) assessed the relationship between hamstrings optimal length and hamstrings flexibility and isokinetic strength (Wan et al., 2017). While hamstrings muscle optimal length correlated with hamstrings flexibility, these factors did not demonstrate a relationship with hamstrings strength. For the same flexibility score, women had shorter hamstrings optimal muscle length than

TABLE 2 | Summary of studies cited in Section "Sex- and Age-Related Differences in Hamstrings' Anatomy and Physiology."

Reference	Population	Anatomic part assessed	Assessment method	Main findings
Behan et al. (2018)	32 males (age: 20.6 years) and 34 females (age: 20.9 years) healthy, young, individuals with a low-moderate level of physical activity	Knee joint muscle morphology	MRI	Sex differences in muscle morphology that may predispose females to greater risk of ACL injury, primarily, a smaller knee flexors to knee extensors size ratio, but also a proportionately small sartorius and gracilis and a proportionately large vastus lateralis. Females have a larger BFlh as a proportion of the knee flexors than males, which may contribute to the higher risk of HSI in males.
Blackburn et al. (2009)	20 male (age: 20.7 years) and 20 females (age: 20.4 years) physically active individuals	Musculotendinous stiffness	Ultrasound	Musculotendinous stiffness was greater in males than in females, elastic modulus did not differ significantly across sex. Hamstring muscle size predicted 16% of the variance in hamstring musculotendinous stiffness.
Ebben et al. (2010)	12 male (age: 21.0) and 12 female (age: 19.9) university students	Magnitude and timing of hamstring activation, knee flexors-to-knee extensors ratio activation ratios, and knee flexors-to-knee extensors timing ratios of a variety of hamstring and quadriceps muscles.	EMG	In the precontact phase of jump landings and cutting: men and women are similar with respect to degree of activation of the hamstring In postcontact phase of the cut: men showed a trend toward higher knee flexors-to-knee extensors activation ratio than women
Hepple and Rice (2016)	Review	–	–	Aging conducts to changes in quantity and quality of motor unit, namely caused by motor neuron loss, neuromuscular joint instability, and repeating cycles of denervation and reinnervation leading to fiber type grouping.
Kirk et al. (2018)	11 healthy (age: 26) and 10 old (age: 80) male individuals		Intramuscular and surface EMG	Voluntary strength, evoked contractility, and MU discharge rates were diminished in old compared with young adult men No difference in relative surface EMG concurrent with significantly lower MU discharge rates may indicate that graded force generation in the hamstrings of old men is more dependent on MU recruitment. MU discharge rates of the SM and ST had a greater age-related effect compared to BF
Kong and Burns (2010)	25 males (age: 26.2) and 15 females (age: 24.2) healthy recreationally active individuals.	Hamstrings and quadriceps strength across multiple knee angles and angular velocities between the dominant and non-dominant legs	Isokinetic and isometric analysis (Biodex)	Knee flexors-to-knee extensors ratio was higher in the dominant leg than the non-dominant leg for both isometric and isokinetic measurements No difference in knee flexors-to-knee extensors ratio was found between males and females.
Lim et al. (2017)	10 young adults (24.2 ± 2.7)	Gluteus maximus, gluteus medius, vastus medialis, lateral hamstring, medial gastrocnemius, and soleus	Kinematic, force-plate and EMG	Increases in step length and frequency increases the contribution from the forces developed by gluteus maximus, gluteus medius, vastus medialis, medial gastrocnemius and soleus to both vertical support and forward progression. However, increase in step length results in greater differences in the contributions of vastus medialis and gluteus maximus and limb posture to vertical support.
Martín-San Agustín et al. (2019)	36 female (age: 21.1) and 34 male (age: 21.6) physically active individuals	Velocity contraction of BF and ST	TMG	Both male and female individuals had a similar pattern among the velocity of contraction
Narici et al. (2008)	Review	–	–	Training process apparently had no effect estimated relative length-tension properties of the muscle. Possibly, tendon stiffness and fascicle length increases canceled out each other.

(Continued)

TABLE 2 | Continued

Reference	Population	Anatomic part assessed	Assessment method	Main findings
Overend et al. (1992)	13 young (age: 24.5 years) and 12 old (age: 70.7 years) male individuals	Quadriceps and hamstring CSA	CT	Old male individuals had smaller quadriceps muscles and were weaker (22–32%) in knee flexion and knee extension at both angular velocities vs. young male Strength to CSA ratios were similar at 0 degree/s, but elderly had decreased ratios for both extensors and flexors at 120 degree/s. Correlations of knee extensor and flexor strength with muscle CSA were significant at both velocities in elderly men, but not at either velocity for the knee flexors in young men.
Roos et al. (1999)	13 young adults (26.2 ± 4.1) and 12 (80.0 ± 5.3) moderately active men	Quadriceps	Isometric dynamometer and EMG	Difference in age was observed on the voluntary and stimulated forces, while modest differences were found in contractible speed (slowest in older) and no change in the mean steady-state firing rates at any force level.
Ruas et al. (2019)	Review	Alternative methods of determining the knee flexors-to-knee extensors ratio as a measure of knee muscle strength balance.	–	There is not sufficient evidence to recommend any of the alternative methods of determining knee flexors-to-knee extensors ratio The higher reliability was found for rate for torque development knee flexors-to-knee extensors ratio
Smith et al. (2021)	125 football players	Association between hamstring strength, age and lower limb soft tissue injury history and subsequent hamstring injury		Increased age and previous hamstring, groin and calf injury are all associated with an elevated risk of subsequent hamstring injury in football players.
Vaughan et al. (2016)	Mice (11–13 months; and 15–21 months)	Extensor digitorum longus and soleus	Dissection	Significant increases of the number of Ia afferents in young compared to older mice. Fewer II afferents were also found in mice of middle and older age. However, intrafusal muscle fibers had no significant changes across the age. Thus, proprioceptive sensory neurons seem to degenerate prior to atrophy of intrafusal muscle fibers during aging.
Wan et al. (2017)	11 males (age: 23.6) and 10 females (age: 24.7 years) college students	Length, flexibility, and strength of hamstrings	3D modeling	Hamstring muscle optimal lengths were significantly correlated to hamstring flexibility score but not to hamstring strength The optimal knee flexion angle for maximal knee flexion moment decreased as hamstring flexibility score increased, which indicate that hamstring muscle optimal lengths may be affected by hamstring flexibility.
Webber et al. (2009)	Mathematical model	Quadriceps	Mathematical model	Changes in the Heckman-Binder motoneuron model for human data improved the frequency-current, and muscle unit force-frequency relationships. This adjustment resulted in lower firing frequencies in older and reduction in maximal force output.
Yoshiko et al. (2017)	15 young (21.0 ± 0.4 years old) and 15 old (70.7 ± 3.8)	Quadriceps femoris, hamstring and adductor	MRI	Age-related increase the intramuscular fat content, namely in the thigh areas, possibly explained by the loss of skeletal muscle cross sectional area in older.

HT, hamstrings; ST, semitendinosus; BFlh, biceps femoris long head; BF, biceps femoris; MRI, magnetic resonance imaging; ACL, anterior cruciate ligament; BFsh, biceps femoris short head; SM, semimembranosus; TMG, tensiomyography; EMG, electromyography; CSA, cross-sectional area.

men. Thus, at any given ROM during a movement, hamstrings muscle maximal strain may differ between sexes. Another study used tensiomyography to assess normalized response velocity in BF, ST, rectus femoris, vastus medialis and vastus lateralis muscles of recreationally active young adult women (n = 36) and men (n = 34) (Martín-San Agustín et al., 2019). Comparisons between women and men were adjusted by height and mass. Sex-related differences were observed in velocity of action, with women having >15% differences between BF-to-quadriceps ratio, as well as ratios in the hamstrings, in comparison to men. These ratios should probably be termed knee flexors-to-knee extensors ratios, as more muscles than merely the hamstrings and the quadriceps are involved in the regulation of knee flexion and extension (Standring, 2015).

Sex-based differences in magnitude and timing of hamstrings and quadriceps activation during drop jump, sprint, and cutting (45°) tasks were assessed in 24 young adults (12 men, 12 women) (Ebben et al., 2010). In the post-contact phase of the cutting movement, men showed greater activation of all the hamstrings in comparison to women, while women produced longer bursts of the rectus femoris and vastus medialis activation. Hamstrings' stiffness was shown to be greater in men than in women, but without differences in stress, strain, and elastic modulus (Blackburn et al., 2009). Blackburn et al. (2009) speculated that the smaller hamstrings stiffness observed in women could compromise their ability to resist changes in length associated with joint perturbation. However, the authors recognized that the sex-related differences in hamstrings stiffness could be partly attributed to anatomic CSA (Blackburn et al., 2009). It has been suggested that age or training level are more relevant than sex to explain differences in knee flexors-to-knee extensor ratios (Kong and Burns, 2010). It is also possible that the specific method used to determine knee flexors-to-knee extensors ratios (e.g., ratios calculated by angle-specific torque, rate of torque development) provide different results [for more information, see Ruas et al. (2019)].

Other evidence suggests that sex-related anatomic differences play a prominent role in explaining the differences in knee flexors-to-knee extensors ratios between men and women. A study with 1.5T MRI of healthy, but untrained young men (n = 32) and women (n = 34), showed sex-related differences in the maximal anatomic CSA of knee flexors and extensors (Behan et al., 2018). Women had a smaller ratio of knee flexors to knee extensor anatomic CSA. Although the hamstrings are not the only knee flexors, they play a prominent role in this action. In comparison with men, women showed a greater proportion of vastus lateralis, BFlh and SM in relation to their respective muscle groups. Conversely, women showed a lesser proportion in sartorius, gracilis and BFsh.

Effects of Aging in Hamstrings' Structure and Function

There is limited evidence regarding the effect of aging in hamstrings' anatomy, although ages >25 years have been associated with increased risk of hamstrings injury (Smith et al., 2021). Kirk et al. (2018) found that voluntary strength, evoked contractility, and motor unit (MU) discharge rates were diminished in a group of 10 elderly (mean age: 80 ± 5 years) compared to a group of 11 young men (26 ± 4 years). These findings could be due to the infiltration of non-contractile tissue in aged hamstrings (Overend et al., 1992; Yoshiko et al., 2017), altered MU facilitation, age-related remodeling (Webber et al., 2009; Hepple and Rice, 2016), muscle fiber membrane dysfunction, decreased sarcoplasmic reticulum function, altered calcium ion kinetics, and changes in connective tissue elements (Narici et al., 2008; Hepple and Rice, 2016). Hamstrings have a significantly lower MU discharge rates in the elderly, as opposite to findings in the vastus medialis portion of the quadriceps (Roos et al., 1999). *In vitro* and animal studies suggest that the loss of proprioceptive sensory neurons and innervation differences occur at different rates between flexors and extensors during the aging process (Vaughan et al., 2016).

Additionally, ST and SM could be more affected by aging than BF, providing evidence that musculature and neuromuscular system could be differently impacted by aging (Kirk et al., 2018). As a longer step length may require higher contributions from the hip and knee extensor musculature (Lim et al., 2017), hamstrings modifications with aging, and the decreased muscle strength, may explain why elderly people choose shorter length steps and walk more slowly (Lim et al., 2017). These findings warrant further investigation.

The Consequences of Previous Knee Surgeries

Knee surgeries often harvest hamstrings tendons, potentially increasing the risk for future hamstrings injuries. When harvesting hamstrings tendons for surgical purpose, such as patellar tendon (Friedman et al., 2020) or ACL reconstruction (Keyhani et al., 2020), iatrogenic nerve injuries can occur (Colombet and Graveleau, 2016; Ruffilli et al., 2017). For example, the common fibular nerve emerges posterior to the BF distal tendon, to which it adheres (Standring, 2015); collecting part of the BF tendon for knee surgeries presents considerable risks. Consequently, the ST has been a common choice (comparable to gracilis in terms of effects) for transfer in surgical treatments (Yasin et al., 2010; Colombet and Graveleau, 2016), including those for the ACL (Horteur et al., 2020; Keyhani et al., 2020), medial collateral ligament (Cao et al., 2016) and patellar tendon (Jain et al., 2014; Friedman et al., 2020).

In a study of female soccer players (n = 90) that had undergone ACL reconstruction, with data available for a minimum of a 2-year follow-up, the outcomes for hamstrings autograft and bone-patellar tendon-bone autografts were very similar (Britt et al., 2020). Notwithstanding, a meta-analysis of 5,561 patients undergoing ACL reconstruction found that hamstrings autografts were less likely than bone-patellar tendon-bone autografts to incur in a contralateral ACL rupture (Zhou et al., 2020). The safety of surgical procedures and its effects on the specific injury addressed should not be disregarded (Jain et al., 2014). Nonetheless, these autografts may increase the fragility of the hamstrings and put them at increased risk of sport-related injuries. One study assessed recreationally

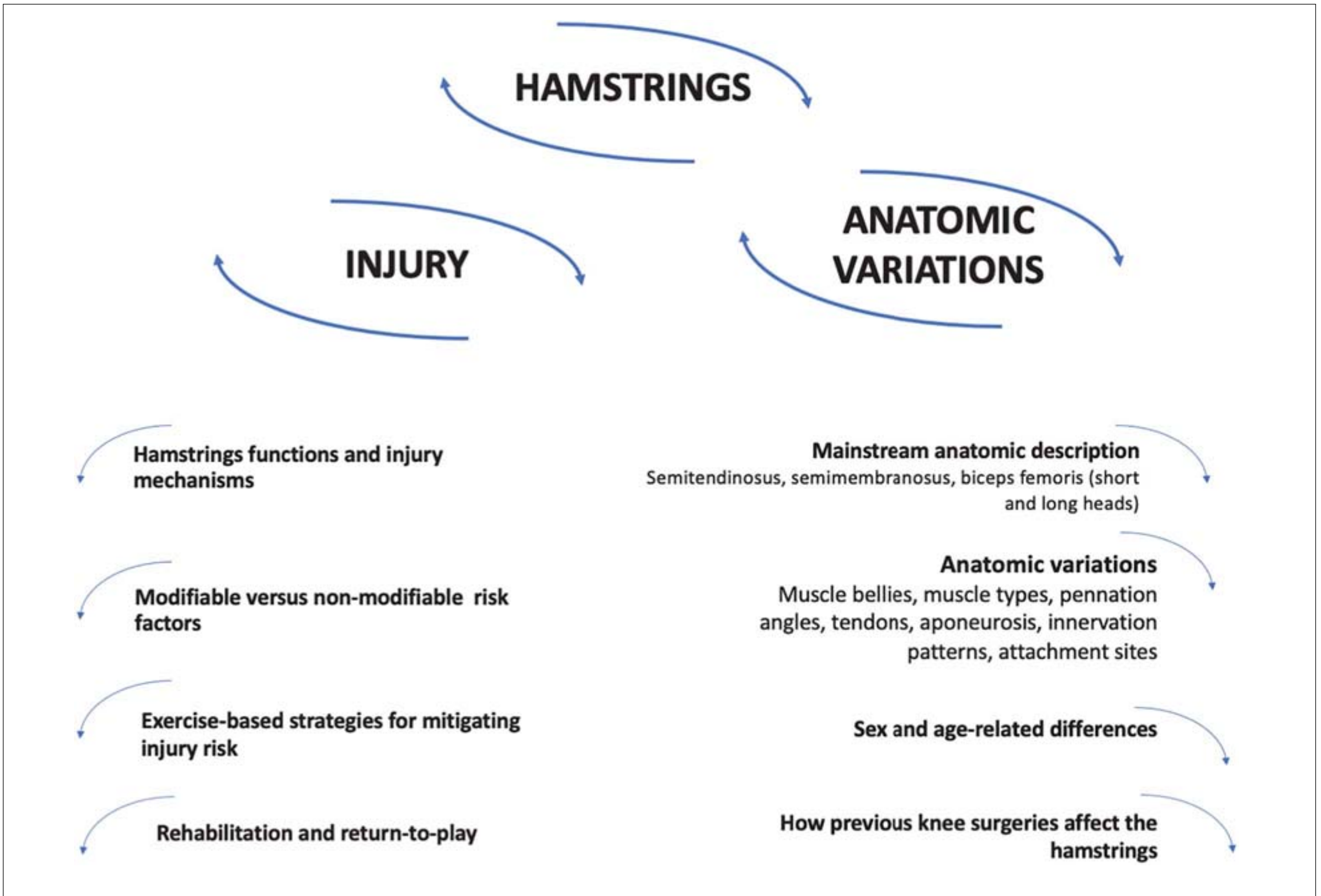

FIGURE 2 | Overview of concepts and hamstrings anatomic and physiologic variations.

active participants (five men and nine women) that had returned to sport after unilateral ACL reconstruction using ST tendon autografts (Messer et al., 2020). The athletes had undergone surgery between 12 to 78 months prior to the study and MRI was used to compare the surgical limb to the other limb. The surgically treated limbs' STs had significantly smaller anatomic CSA and muscle volume than non-surgical limbs, and the surgically treated limbs also exhibited a lower exercise-induced transverse relaxation time. Surgically treated limbs also exhibited *higher* volumes of the SM and BFsh, perhaps to compensate for the lower volumes of the ST (Messer et al., 2020).

Using ST autografts for ACL reconstruction has long-term consequences for the hamstrings of the surgically treated limb, such as ST and gracilis hypotrophy (Sherman et al., 2021). Although alternatives such as peroneus longus autografts are being explored for ACL reconstruction (Rhatomy et al., 2019), it is possible that could bring some problems for the ankle and foot, since peroneus longus (a.k.a., fibularis longus) acts in eversion and plantar flexion of the foot and also provides support to the longitudinal and transverse arches of the foot (Standring, 2015). Trade-offs are unavoidable, and their consequences should be acknowledged and addressed by rehabilitation programs, requiring multifactorial approaches including different modalities of strength training, variation in the exercises, balance knee- and hip-dominant exercises, and careful managing of loading (Buckthorpe et al., 2020).

Albeit inadvertently, surgical procedures that extract tissue from the ST may compromise the endurance of this muscle (Vairo, 2014; Lee and Lee, 2020). Consequently, the BF will produce greater force and for a longer period to compensate for this reduced capacity of the ST (Schuermans et al., 2014). This could lead to the BF fatiguing earlier and/or being exposed to loads that exceed its capacity. Paradoxically, this may expose the "healthier" muscle (i.e., BF) to increased injury risk, and may explain why the majority of acute HSI occur in the BFlh (Freitas et al., 2021; Huygaerts et al., 2021). Still, a study with healthy subjects performing an indoor running task showed that the largest peak strain was achieved by the BF, while the highest peak force and the most power and work were generated by the SM (Schache et al., 2012), so this issue is open to debate.

Synopsis of the Second Part

The hamstrings complex comprises the posterior compartment of the thigh, consisting of the ST, SM, and BF (BFlh and BFsh) muscles. They are biarticular muscles that act as hip extensors and knee flexors and rotators and play a critical role in several daily and sports activities. Regarding the main hamstrings anatomic and physiological variations discussed in the previous sections, it was shown that a high degree of variability exists in many of the architecture variables addressed. Lower tendons and aponeurosis to muscle ratios, higher number of type II fibers, variations in attachment sites and innervation patterns, and knee surgeries in which portions of the ST are extracted, could place the hamstrings complex musculature, especially the BF muscle, at increased risk of injury. Additionally, neuromuscular variations in women and the aging process affect the hamstrings musculature, further increasing the risk of hamstrings injury in women and elderly people.

The high level of anatomic variability from subject to subject and the variability of the study designs used in the literature, makes it difficult to draw general recommendations, and advises against adopting one-size-fits-all exercise programs. Since most sports-related injuries have a clear multifactorial nature (Monajati et al., 2016; Green et al., 2020; Lahti et al., 2020), studies may fail to find a clear relationship between the injury and any given risk factor. This does not imply that a particular risk factor is irrelevant in the probability of suffering a hamstrings injury. Although the study of resting muscle architecture of the hamstrings may provide relevant insights, it is unclear whether this provides valid information for dynamic architecture and gearing during dynamic movements (Huygaerts et al., 2021).

CONCLUDING REMARKS

Hamstrings injuries present a challenge in sports science and practice, and many exercise-based programs have been proposed to mitigate injury risk, focusing on modifiable injury risk factors. But scientifically reported data shows that non-modifiable risk factors potentially play a more relevant role than modifiable risk factors. This suggests that greater efforts should be implemented to understand and identify the non-modifiable risk factors, such as relevant anatomic variations (e.g., tendons, aponeurosis, fiber types). The possession of this enhanced knowledge may help designing better-individualized exercise interventions, considering the non-modifiable particularities of the athlete. If a certain anatomic variation puts the athlete at greater risk of injury (e.g., nerve entrapments or tunneling), training programs can design better-individualized stimuli instead of delivering one-size-fits-all solutions. The recognition and assessment of non-modifiable risk factors allows coaches to act upon them, i.e., to prescribe exercise programs that are well-suited for those identified variations. As an example: if athletes with a greater percentage of type II fibers incur in greater damage induced by eccentric exercise, coaches can reduce the weekly volume, intensity and/or frequency of eccentrically biased exercises. **Figure 2** synthesizes the concepts and domains approached in our work.

The literature has identified the need for prospective studies evaluating the influence or architectural variables in hamstrings injury, as well as the importance of targeting architectural changes in injury reduction programs, highlighting that ultrasound is potentially the most reliable technique to detect such changes (Behan et al., 2019). In the case of non-modifiable anatomic variations (e.g., number of muscular insertions into bone), it would be important to explore differential responses to exercise protocols, providing coaches with the tools for better individualizing training prescription. Of course, it is quite complicated to carry out the "gold-standard" study designs required to detect risk factors and validate screening tools (Bahr, 2016), as large-scale prospective, long-term, randomized studies assessing multiple outcomes at multiple time points are very difficult to implement. Still, this should not discourage researchers from progressively better and more complete attempts to address this complex, yet fascinating topic. We therefore encourage researchers to develop more large-scale prospective studies, including some of the mentioned architecture variables, with reliable yet non-invasive techniques such as ultrasound, to widen the body of knowledge regarding this subject matter.

As limitations of this work, we did not perform a systematic review of the literature, given the scope and goals. It is possible that relevant information has eluded our searches, although we tried our best to provide and thorough and balanced account. Where controversies existed, we attempted to explore its complexities and provide contradictory accounts.

PRACTICAL IMPLICATIONS

Improved knowledge of interindividual variations in hamstrings anatomy and physiology will help coaches designing better individualized exercise programs. As an example, athletes with greater percentage of type II muscle fibers in the hamstrings should probably be allowed greater recovery times between sessions of eccentrically biased exercise. Muscle bellies with more extensive attachments to the femur may provide some degree of injury protection when producing forces of great magnitude, but this requires further exploration. Pennation angles, tendon-to-fiber length ratios, attachment sites and features of the aponeurosis, as well as the interaction between these factors, have unclear relationships with injury risk, with further research required before practical implications are promoted. The extensive anatomic and neurologic connections between the hamstrings and the gluteal and adductor muscles suggests that training programs should include exercises that demand the interaction of these three muscle groups, instead of relying solely on more hamstring-dominant exercises (e.g., the NHE).

While coaches tend to focus on modifiable risk factors, such as strength and endurance, non-modifiable risk factors may be acted upon, by designing exercise interventions that better comply with the identified characteristics. For example, in the case of athletes with previous knee injuries using ST autografts (non-modifiable factor), coaches should recognize that hamstrings endurance may be compromised, designing exercise interventions that gradually improve their endurance, but also recognizing that those athletes may benefit from shorter training sessions and/or shorter durations of play in matches. Improvements in the quality, availability and costs of imaging techniques will expand the assessment of interindividual anatomic variations, better individualizing exercise prescription.

AUTHOR CONTRIBUTIONS

JA, SR-R, and FMC contributed to conception and design of the review. MA, PN, HS, AF, JO-J, and RR-C wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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OPINION
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Time to Move From Mandatory Stretching? We Need to Differentiate "Can I?" From "Do I Have To?"

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INTRODUCTION

Flexibility is the ability to move through full joint range of motion (ROM), while stretching is an intervention to improve flexibility and achieve other goals (e.g., post-exercise relaxation) (ACSM, 2021). Stretching has been promoted as mandatory in exercise programs (Behm, 2019; American Heart Association, 2020; ACSM, 2021), although this is changing toward an optional feature (Bull et al., 2020). There are different types of stretching, including active static stretching (SS—active lengthening of a muscle until the feeling of stretch or to the point of discomfort), passive static stretching (PS—where an external force is applied, e.g., by a coach or a colleague), dynamic stretching (DS –controlled movements through the joint ROM) and proprioceptive neuromuscular facilitation (PNF—combining PS with isometric contractions) (Behm, 2019). We will focus on SS and PS, since these methods are at the heart of most debates, with the pendulum swinging across the years (Behm et al., 2021b). The answer to "Can I perform a given exercise intervention?" is straightforward: when the benefits of an intervention outweigh its adverse effects or contra-indications, the answer is "yes." Let us take the example of a study with 15 University students (Bengtsson et al., 2018), to illustrate the difference between the two questions: the negative acute effects of SS during a warm-up were restored if followed by isokinetic contractions, suggesting that SS can be included in a comprehensive warm-up protocol. University students are not representative of athletes, and a small sample does not warrant generalizations, but our point is that answering the first question ("Can I?") does not answer the second question ("Do I have to?"). Focusing the research question and applicability on "Can I?" may be short-sighted. To date, we feel that research has focused more strongly on answering what stretching can do, while more information is required as to how stretching compares to alternative interventions. We will explore the differences between "Can I?" and "Do I have to?" stretch and their implications to warm-up, cool-down, ROM, and injury risk.

"CAN I" VS. "DO I HAVE" TO STRETCH IN THE WARM-UP?

Arguments for including stretching during the warm-up comprise acute improvements in ROM (Behm et al., 2016), improved proprioception (Walsh, 2017), psychological preparedness (Blazevich et al., 2018) and reduction of injury risk (Behm et al., 2021b), none of which is exclusive to stretching (Blazevich and Babault, 2019; Prieske et al., 2020). The evidence suggests that SS and PS may impair acute performance in power-, strength-, and speed-related activities, but these effects are minor and can be mitigated if SS lasts 30 to 60 s per muscle group and is followed by dynamic warm-up activities (Behm and Chaouachi, 2011; Kay and Blazevich, 2012; Behm et al., 2016, 2021b). But if multiple, <60 s stretches are compounded, what are their accumulated effects? And how does stretching intensity affect the outcomes? Performing unilateral PS induces improvements in passive ROM in non-local and non-stretched joints (Behm et al., 2021a), and consecutive sets of SS provoke increases of parasympathetic activity that remain for 5 min post-SS (Farinatti et al., 2011; Inami et al., 2014). Further research is warranted.

A comprehensive warm-up including a pre-stretching warm-up, followed by SS/PS, and then followed by dynamic exercises (Reid et al., 2018; Behm et al., 2021b) demands a longer than necessary warm-up duration. Alternative warm-up protocols focusing on post-activation potentiation (PAP) or performance enhancement (PAPE) are based on high-intensity, dynamic actions (Blazevich and Babault, 2019; Prieske et al., 2020), dismissing the need for SS/PS. Another argument for including SS/PS in the warm-up concerns the *acute* improvements in ROM, but these tend to last up to 30 min (Behm et al., 2016), while training sessions last longer, especially when considering athletes (not so much in the recreational- or health-related contexts), for which repeated bouts of SS/PS during the training session would be required. Interventions such as DS (Behm et al., 2016), foam rolling (Wilke et al., 2020) and high-intensity resistance training (Moreno-Pérez et al., 2021) also acutely improve ROM. The issue of *chronic* improvements in ROM is more complex and will be addressed in a separate section. For now, the answer to "Can I stretch in the warm-up?" is "probably yes"; but to the question "Do I have to stretch in the warm-up?" the answer is "maybe not."

"CAN I" VS. "DO I HAVE" TO STRETCH IN THE COOL-DOWN PHASE?

Post-exercise stretching is prescribed under the belief that it enhances recovery (American Heart Association, 2020; ACSM, 2021), but reviews do not support these claims (Herbert and Gabriel, 2002; Henschke and Lin, 2011; Herbert et al., 2011; Torres et al., 2012; Baxter et al., 2017; Van Hooren and Peake, 2018). A systematic review with meta-analysis including 11 randomized controlled trials (RCTs) assessed the effects of post-exercise stretching on short-term (≤1 h after exercise) and delayed (24, 48, 72 h) recovery markers including delayed onset muscular soreness (DOMS), strength and ROM (Afonso et al., 2021a). Comparing with passive recovery (i.e., rest) or other recovery methods (e.g., low-intensity cycling), SS and PS showed no additional benefits in any of the outcomes, at any time point, in contrast with existing guidelines (American Heart Association, 2020; ACSM, 2021).

The argument that some athletes feel better when stretching (Judge et al., 2020) is misleading, as the subjective sensation of feeling better may not translate into objective measurable improvements (Afonso et al., 2021a). And it is a dangerous argument, as it could easily be looked from the other side, whereby athletes that do not like to stretch would be evidence against stretching—so, we feel that these lines of argumentation should be avoided. The argument that stretching provides team bonding (Behm et al., 2021b) also misses the mark: other activities can promote team bonding. In sum: "Can I stretch in the cool-down?" Probably yes, but when performed, post-exercise stretching should eschew high intensities (Behm, 2019). "Do I have to stretch in the cool-down?" Probably not, as evidence suggests it does not enhance recovery.

"CAN I" VS. "DO I HAVE TO" STRETCH TO CHRONICALLY IMPROVE RANGE OF MOTION?

Stretching can chronically improve ROM (Blazevich, 2018) by increasing fascicle length, improving stretch tolerance, altering pennation angles and reducing tonic reflex activity (Guissard and Duchateau, 2004; Blazevich et al., 2014), but it is not the only method capable of doing so (Saraiva et al., 2014; Afonso et al., 2021b). Stretching may be required for sports that demand extreme ROM, but for most sports and general population, there are alternative interventions available, such as resistance training (Saraiva et al., 2014; Nuzzo, 2020) and foam rolling exercises (Aune et al., 2019). A systematic review with meta-analysis of 11 RCTs comparing strength training to stretching for ROM gains has found that for interventions lasting 5 and 16 weeks, the strength training and stretching protocols did not differ in their effects on ROM (Afonso et al., 2021b). To different degrees, eccentric and concentric strength training with full ROM, as well as plyometric training, induce changes in muscle fascicle length and pennation angle, and tendon extensibility, resulting in ROM gains (Reeves et al., 2009; Kubo et al., 2017; Valamatos et al., 2018; Gérard et al., 2020; Marušič et al., 2020). However, the studies included in the review of Afonso et al. (2021b) had considerable heterogeneity in design, populations and protocols. The answer to the question "Can I stretch to improve ROM?" is Yes. But do I have to stretch to improve ROM? Possibly not, but more comparative research is required, and sports requiring extreme ROM should be considered separately.

"CAN I" VS. "DO I HAVE TO" STRETCH TO REDUCE INJURY RISK?

Several exercise types and sports require wide use of stretch-shortening cycles (Witvrouw et al., 2004), with muscles alternating between shortening and stretching phases. It has been proposed that stretching could thus reduce injury risk (Behm et al., 2021b), but this is contentious (Witvrouw et al., 2004; Nuzzo, 2020). The systematic review of Behm et al. (2016) showed no clear effect of SS or PNF stretching on all-cause or overuse injuries, and there was insufficient data available for DS. Other systematic reviews have failed to find an association between stretching and injury risk (Herbert and Gabriel, 2002; Weldon and Hill, 2003; Thacker et al., 2004; Small et al., 2008; Lauersen et al., 2014; Leppänen et al., 2014; Lewis, 2014; Dijksma et al., 2020). Based on one of the largest RCT with military individuals (Pope et al., 2000), a total of 337 individuals would need to undergo a stretching intervention to prevent a single lower-limb injury. The argument that stretching does not reduce overall risk of injury but might reduce risk of musculoskeletal injury (Reid et al., 2018) should consider a trade-off: if overall risk is the same but risk of musculoskeletal injury is reduced, other risks were possibly aggravated. Since injury mechanisms are usually multifactorial, isolating one factor (e.g., stretching) is difficult, and so definitive claims that stretching reduces (or not) injury risk should be avoided.

The link between flexibility and injury risk is unclear (Green et al., 2020). Some evidence shows association between flexibility and injury risk (de la Motte et al., 2019), but this should not be misinterpreted as a causal effect. There is evidence that an injury impairs ROM (Maniar et al., 2016), but the inverse may not be true—although it is reasonable to assume that ROM levels that are insufficient for the movement demands of a certain exercise or sport may expose the athlete to increased injury risk. Establishing a causal relationship between insufficient flexibility and increased injury risk would not support the mandatory utilization of stretching, as there are alternative interventions to improve flexibility, including strength training (Saraiva et al., 2014; Nuzzo, 2020; Afonso et al., 2021b), but more long-term comparative studies are required. The relationships between flexibility and technique also warrant more research. For now, the relationship between stretching and injury risk remains controversial (Green et al., 2020). The answer to "can I stretch?" is yes—it probably will not increase injury risk. But the answer to "do I have to stretch?" is "possibly no"—as the likelihood of decreasing the injury risk is contentious.

DISCUSSION

Establishing that stretching *can* be performed in different contexts is not the same as establishing that stretching *must* be performed. (1) SS and PS can be performed during the warm-up, especially if using durations <30 to 60 s per muscle group, but at the cost of longer warm-up routines. Alternative warm-up protocols do not require SS or PS, seem equally effective and require less time to implement. (2) SS and PS can be performed during the cool-down, but there are apparently no benefits in short-term or delayed recovery of strength and ROM and does not decrease the magnitude of DOMS. (3) SS and PS improve ROM if performed chronically, but so does strength training, which also has multiple health-related benefits (Bull et al., 2020). However, the scarcity of comparative studies and their heterogeneity advise against stronger conclusions. Sports requiring extreme ROM may require specific approaches. (4) There is no clear relationship between stretching and injury risk, which is partly expected as most injuries are multifactorial in nature. Associations between flexibility and injury risk do not necessarily support the utilization of stretching because: (i) association does not mean causation; and (ii) flexibility can be improved through other methods (although more long-term comparative research is needed). We should adopt nuanced approaches when recommending stretching. "Can I stretch?": probably, yes. "Do I have to stretch?": possibly, no. The answer may depend on the goals, population, timing of the year or season, and individual characteristics.

AUTHOR CONTRIBUTIONS

JA conceived the original draft. All authors had equally relevant contributions for writing and revising the manuscript.

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The Differentiate Effects of Resistance Training With or Without External Load on Young Soccer Players' Performance and Body Composition

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Purpose: The purpose of this study was to examine the effects of 15 weeks (2/week) of two different resistance training (RT) programs [the self-load group (SG) vs. the overload group (OG)] on selected measures of physical performance in young male soccer players.

Methods: The countermovement jump (CMJ), aerobic endurance (VO_2 max), and body composition [body mass (BM), height (H), body fat percentage (% BF), and lean mass (LM)] were measured before and after the 15-week RT interventions. Subjects were randomized to treatments: 1. SG [age = 15.34 ± 1.34 years]; 2. OG [age = 16.28 ± 1.21 years].

Results: The level of significance set for the study ($p \leq 0.05$). Within-group analysis did report significant differences in all variables for the SG (p = 0.008 to 0.001; ES = −0.33 to 1.41, small to large) as in the OG (p = 0.001; ES = 0.82 to 1.30, large). Between-groups analysis reported differences in CMJ (F = 4.32; p = 0.004) for the OG.

Conclusion: The main findings of this study indicated that RT with and without external load was effective in improving the measures of physical performance in young soccer players, with special attention to jumping ability, where the OG group was more effective. Furthermore, there is no interference to aerobic endurance. It is recommended that soccer coaches implement RT without external load in the early stages of training or in players with late maturation development and in those soccer clubs with limited material resources.

Keywords: strength, VO_2 max, performance, football, lean mass

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INTRODUCTION

Resistance training is considered a key strategy to improve in-field soccer performance due to the proved relationship between the strength level and high-intensity actions (e.g., sprint or jump) (Swinton et al., 2014; Núñez et al., 2019). In fact, the importance of resistance training (RT) has been increased in soccer training last year due to the relevance of this in the periodization (Lesinski et al., 2016). Concerning young athletes, it has shown RT to be important in preadolescence, highlighting neural plasticity associated with prepubertal players that support muscular strength development in these years through gains in neuromuscular adaptations as intra- and intermuscular coordination (Peña-González et al., 2019). Although different RT methodologies have been used to improve physical performance in soccer, such as programs bases on traditional exercises (Spineti et al., 2016), eccentric-overload training (Suárez-Arrones et al., 2019), plyometric training (Haghighi et al., 2012; Falces-Prieto et al., 2021), ballistic exercises (Loturco et al., 2020), Olympic exercises (Hori et al., 2008), electrostimulation training (Billot et al., 2010), and a combination of different methods (Raya-González and Sánchez-Sánchez, 2018). Most of these methods need expensive materials and equipment that preclude its applicability for most athletes and thus its implementation in most soccer training facilities; so strength and conditioning coaches are advised to find valid, simple, and economic resources for this purpose (Raya-González et al., 2020). Many coaches and physical trainers have taken this into consideration, and, accordingly, with the literature and their needs, professionals on soccer often choose RT based on self-loading (own body mass) as an interesting method that could be massively implemented in soccer training programs, especially in young soccer players (Peña-González et al., 2019). In fact, the effects of this RT based on self-loading have been previously analyzed in primary education students (Conde-Corbitante, 2016), adolescent basketball players (Kosmatos et al., 2008), prepubertal athletes (Faigenbaum and Myer, 2010), and elderly people (Kanda et al., 2018). However, to the best of our knowledge, there is a lack of evidence on the use of this RT programs in soccer players. Therefore, this highlights the need for studies that consider the effect of RT based on self-loading on young soccer players.

It is well-known that soccer is an intermittent exercise and involves activities with different high intensities, such as change of direction, high-intensity running, sprinting, jumps, and among others (Sáez de Villarreal et al., 2015; Raya-González and Sánchez-Sánchez, 2018). In this sense and considering the specific requirements of soccer in muscular strength terms, especially in the lower body to perform previous types of high-intensity actions mentioned (Michailidis et al., 2013), it should be mentioned that athletes need familiarization and adaptation with strength work (Peña-González et al., 2019), mainly due to the enormous importance of technical execution and time required for the proper implementation (Blais and Trilles, 2006). On the contrary, RT based on self-loading has shown that it is easier to apply in practice (Ferrete et al., 2014; Peña-González et al., 2019; Falces-Prieto et al., 2020). In addition, this methodology is seen as being more flexible, cheaper, quicker, and easier to implement on the day-to-day basis (Falces-Prieto et al., 2020). Notwithstanding, this methodology requires a great effort and a level of technical execution; otherwise, movements can be made with less control during execution (Falces-Prieto et al., 2020).

A review of the literature reveals that acute RT based on self-loading improves strength performance (Vanderka et al., 2016; Marín-Pagán et al., 2020). Consequently, if RT based on self-loading were repeated, it would also produce acute and chronical physiological changes. Therefore, the improvement can be related to the chronic adaptations of RT over long periods of time (Sander et al., 2013; Ferrete et al., 2014; Di Giminiani and Visca, 2017; Suárez-Arrones et al., 2018; Peña-González et al., 2019). Therefore, the direction and the magnitude of these phyisiological changes are due to work stimulus required and not the instrument applied.

In this first empirical study of the effects of two different RT programs on selected measures of physical performance in young male soccer players, we take a exploratory aproach to the study of efficiency of RT based on self-loading.

Countermovement jump (CMJ) was a valid test to observe the adaptation in training (Di Giminiani and Visca, 2017). Traditionally, the CMJ test is a standard measure of lower body power (Liebermann and Katz, 2003). In addition, it has been demonstrated a relationship between RT and CMJ improvement in young soccer players (Quagliarella et al., 2011; Comfort et al., 2014) with overloads (Comfort et al., 2014; De Hoyo et al., 2016; Falces-Prieto et al., 2021) and self-loads (Falces-Prieto et al., 2020; Ferrete et al., 2014; Peña-González et al., 2019). It is for this reason that RT has become crucial for young and adult soccer players (Moran et al., 2017).

Regarding the relationship between maximum oxygen consumption (VO_2 max) and performance in soccer (Ziogas et al., 2011), the improvement of this variable could be a key strategy in the individual physical conditioning (Silva et al., 2011). Soccer players with higher VO_2 max values present greater activity in high-intensity actions and sprinting and have a better recovery between high-intensity efforts (Nobari et al., 2021a). In recent years, a series of field tests have been designed in the evaluation of the VO_2 max (Sánchez-Oliva et al., 2014). One of the tests commonly used in young soccer players is the 30–15 Intermittent Fitness Test (30–15 IFT) (Buchheit, 2008). Although endurance training inhibits or interferes with the development of RT and *vice versa* (Hennessy and Watson, 1994), previous studies have reported substantial improvements in VO_2 max after RT programs in young soccer players (Ferrete et al., 2014; Ruivo et al., 2016; Marzouki et al., 2021). Even so, there are few studies that have examined the impact and adaptations of RT over VO_2 max in soccer (Grieco et al., 2012), and, therefore, future research is needed.

Findings regarding anthropometric characteristics and body composition (BC) are of crucial importance for complex sports games such as soccer (Suárez-Arrones et al., 2019; Gardasevic et al., 2020). In addition, nonoptimal BC may adversely influence football performance and the risk of injury (Suárez-Arrones et al., 2019). There are studies that reflect a strong relationship between BC [high levels of lean mass (LM) and low-fat mass (FM)] with vertical jump performance and repeated sprint ability in both elite and youth soccer players (Rebelo et al., 2013; Brocherie et al., 2014; Nobari et al., 2021b). Regarding RT and its effects on BC in young soccer players, reflected increases in LM (Pérez-Gómez et al., 2008; Suárez-Arrones et al., 2019) and decrease in FM % (Suárez-Arrones et al., 2019; Falces-Prieto et al., 2021) after RT. Therefore, knowing the effects of different RT programs seems essential for their effective application and, consequently, improving the BC of young players.

In sum, the current empirical study was conceived to examine the effects of two different RT programs [the self-load group (SG) vs. the overload group (OG)] on selected measures of physical performance (i.e., jumping, aerobic endurance, and body composition) in young male soccer players. On the basis of the previous research on RT based on self-loading, we hypothesized that OG would induce larger adaptations on some measures of physical performance compared with SG in young male soccer players.

MATERIALS AND METHODS

Experimental Approach to the Problem

To examine the effects of 15 weeks (2/week) of two different RTs [SG vs. OG] on selected measures of physical performance in young male soccer players [Under 16 (U16) and Under 19 (U19)] players participated in this study (**Figure 1**) were randomly assigned in two groups: SG (n = 69; soccer training program + RT program with self-load) and OG (n = 75; soccer training program + RT program with overload). Both groups were made up of players from both categories (U16 and U19). Before and after the RT, countermovement jump (CMJ), aerobic endurance (30–15 IFT) and body composition analysis (BC) [Weight (W, kg), height (H), body fat percentage (% BF), and lean mass (LM, kg) evaluated by bio impedance] were assessed.

Participants

Initially, 150 young male soccer players belonging to the same high-performance academy agreed to participate in the study. The following inclusion criteria were applied to select subjects: (i) a background of $\geq$5 years of systematic soccer training and competitive experience, (ii) continuous soccer training for the previous 3 months with no musculoskeletal injuries, (iii) absence of potential medical problems, (iv) absence of any lower-extremity reconstructive surgery in the past 2 years, and (v) belongingness in the academy a full season. Subjects were required to attend $\geq$ 80% of all training sessions and attend all assessment sessions.

One hundred and forty-four young soccer players fulfilled the inclusion criteria and were randomly assigned to SG or OG. This study was conducted between the September and December of 2018/2019 season and consisted in a weekly resistance training session on Day 4 (Wednesday), allowing a rest of 72 h prior to a match and within the usual training hours (15:30–18:00 hours). The assessments were carried under weather conditions ($\sim$ 29°C and $\sim$ 60% humidity) in September and ($\sim$19°C and $\sim$ 50% humidity) in December. Only six subjects were excluded from the study because they were injured or were absent from the post-testing session. The subjects were randomized to treatments: 1. SG [age = 15.34 $\pm$ 1.34 years; height = 172.54 $\pm$ 7.18 cm; body mass = 62.69 $\pm$ 9.12 kg; % fat = 14.13 $\pm$ 3.78; lean mass = 53.85 $\pm$ 6.54 kg]; 2. OG [age = 16.28 $\pm$ 1.21 years; height = 174.18 $\pm$ 6.79 cm; body mass = 65.15 $\pm$ 8.21 kg; % body fat = 14.30 $\pm$ 3.52; lean mass = 56.10 $\pm$ 5.97 kg]. All participants were familiar with the training methods used and previous RT experience. Furthermore, completed 9 h of soccer training plus 1 competitive match per week. All parents and participants were informed about the purpose of the study and signed consent detailing their possible benefits and risks and giving the signed consent before the beginning of the study. The participants were fully debriefed about the purpose of the study at the end of the experiments. All players participated in 30 proposed sessions (100%). The participants were treated according to American Psychological Association (APA) guidelines, which ensured the anonymity of responses of the participants. In addition, the study was conducted in accordance with the ethical principles of the 1964 Helsinki declaration for human research and was approved by the Research Ethics Committee of the Pontifical University of Comillas (internal project No. 2021/65).

Testing Procedures

Countermovement Jump Performance

The evaluation system was carried with a contact platform Chronojump-Boscosystem® (Barcelona, Spain) (De Blas et al., 2012; Pueo et al., 2018). Three CMJ jumps were performed, with a recovery time of 20 s between jumps and the average of the three jumps for analysis (Falces-Prieto et al., 2021). The measurement was carried out with Chronopic and recorded with the Chronojump software version 1.4.7.0. Both for the pre- and post-evaluation of the CMJ, the subjects first performed a 10-min warm-up based on free joint and muscle mobility (3 min), skipping (2 × 30 s), gluteal heel (2 × 30 s), squats with extended arms (2 × 10 repetitions), and continuous vertical jumps (six jumps with the CMJ execution technique).

1 RM Test

For the evaluation of the 1 RM for bench press and squat in OG and the subsequent programming of the training with these values, a linear encoder ChronojumpBoscoSystem® (Barcelona, Spain) was used. Is an isoinertial dynamometer that consists of a cable extension linear position transducer attached to the barbell interfaced with a personal computer at a sampling rate of 1,000 Hz (Pérez-Castilla et al., 2019). It was measured to schedule training, but the effects on this variable were not assessed.

Aerobic Endurance

The 30–15 IFT, which consists of 30-s shuttle runs, interspersed with 15-s passive recovery periods. Velocity was set at 8 km/h^{-1} for the first 30-s run and was increased by 0.5 km/h^{-1} every 45-s stage thereafter (Buchheit, 2008). The test methodology served as a progressive warm-up of the test. The subjects had to run back and forth between two lines set 40 m apart at a pace governed by a prerecorded beep at appropriate intervals that helped them adjust their running speed by entering into 3-m zones at each extremity

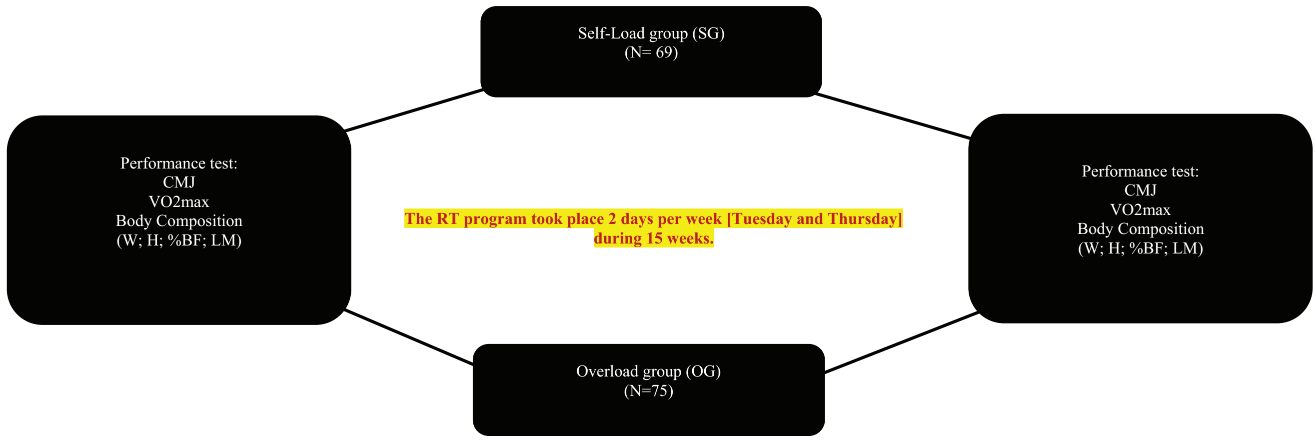

FIGURE 1 | Schematic presentation of the study design. CMJ, countermovement jump; VO_2 max, maximal oxygen consumption; U, under; RT, resistance training; SG, self-load group; OG, overload group; W, weight; H, height; % BF, % body fat; LM, lean mass.

and in the middle of the field while the short beep sounds. It was established that the subject should stop the test when, for three consecutive times, he or she does not reach the established line at the rhythm of the prerecorded sound. When the subjects could not follow the speed stipulated in the test, they should raise their hands to signal their cessation and thus note the previous speed at which the player stopped. For the estimate of VO_2 max, the following formula has been used (Buchheit, 2008):

$$VO_2max = 28.3 - (2.15 \times G) - (0.741 \times A) - (0.0357 \times W)$$

$$+(.0586 \times A \times vIFT) + (1.03 \times vIFT).$$

Variables: G: Gender (one man; two women); A: age; W: weight; vIFT: final speed reached.

Body Composition

Anthropometric measurements were taken before the physical testing. The stature of soccer players was measured with a stadiometer (Seca® 206, Hamburg, Germany). The BC was evaluated in the morning (8:00 am) at the beginning of the competitive period (September) and at the end of the treatments (December). The variables BM, % BF, and LM were analyzed with the Bioelectrical Impedance Analysis method (BIA) using a TANITA® (MC-980MA PLUS, Arlington Heights, IL, United States), where the subjects go up without footwear, without breakfast and wore only shorts and removed any metal and jewelry prior to assessment (Suárez-Arrones et al., 2019). BIA is a widely used method for estimating LM (Sun et al., 2005; Böhm and Heitmann, 2013) and offers a method that is economic and noninvasively assesses the fluid distribution and BC of young soccer players (Lozano-Berges et al., 2017).

Training Program

The subjects completed an ST program for 15 weeks. Cross the season, players had five to six training sessions a week, with an average duration of 80 min (from 45-min sessions to 100- to 120-min sessions where the ST training sessions were included before field training). During the intervention (15 weeks), the subjects performed five normal training sessions (soccer-specific trainings in the field) plus two RT sessions per week. In both groups, Day 1 was for the upper body and Day 2 for the lower body. It was carried out in a training circuit format. In SG, the intensity used was the body weight or body weight plus light resistance of the player (Ferrete et al., 2014; Peña-González et al., 2019). The training was performed on the artificial grass (the same as competition), with the subjects using appropriated soccer-equipped boots and clothes. Sets (4) and established repetitions (×12 × 10 × 8 × 8) were established. The OG performed RT in gym with overloads. The external overloads for the bench press and squat exercise were between 50 and 65% of the 1RM (Rodríguez-Rosell et al., 2017). The weight of the bar was taken into account (it was not Olympic; 11 kg). With respect to the rest of the exercises with overloads, the subjects used free weight by means of which they could complete the sets and prescribed repetitions and with the correct execution technique (Peña et al., 2016). According to the exercise to be performed, sets (4) and repetitions (×15 × 12 × 10 × 8 × 8) were established, with maximum execution speed. The resting period between each set in both treatments was 1 min. The RT program followed by the groups is outlined in **Tables 1**, **2**.

Statistical Procedures

Data are presented as mean ± standard deviations (SD). The ICC was used to determine the reliability of the measurements. To prove the normality of data distribution and the homogeneity of variances, the Kolmogorov-Smirnov and Levene tests were conducted. Since all analyzed variables had a normal distribution, parametric techniques were applied. A paired-samples t-test was used to evaluate within-group differences, and an analysis of covariance (ANCOVA) was performed to detect possible between-group differences, assuming baseline values as covariates. To examine practical significance, Cohen's

TABLE 1 | Phase 1(A): Self-load treatment.

Weeks	W1		W2		W3		W4		W5		W6		W7		W8	
Exercises/Sessions	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**
								Day 1 upper body – Day 2 lower body								
Front shoulders with EB	4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12	
Lateral shoulders with EB	4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12	
Normal Push-Ups	4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Triceps Dips	4×8		4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Biceps with EB	4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Row with EB	4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12	
Squat with pica		4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12
Bipodal glute bridge		4×8		4×8		4×8		4×8		4×8		4×8		4×8		4×8
Calf lift		4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12
Quadriceps isometric 90°		4×30′′		4×30′′		4×30′′		4×30′′		4×30′′		4×30′′		4×30′′		4×30′′
Static Lunges		4×12		4×12		4×12		4×12		4×12		4×12		4×12		4×12
Monster Walk		4×8		4×8		4×8		4×8		4×8		4×8		4×8		4×8

EB, elastic band.

TABLE 1 | Phase 1(B): Self-load treatment.

Weeks	W9		W10		W11		W12		W13		W14		W15	
Exercises/Sessions	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**	**S26**	**S27**	**S28**	**S29**	**S30**
							Day 1 upper body – Day 2 lower body							
Decline push up	4×12		4×12		4×12		4×12		4×12		4×12		4×12	
Chest TRX	4×12		4×12		4×12		4×12		4×12		4×12		4×12	
Triceps TRX	4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Row TRX	4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Biceps TRX	4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Throw medicine ball (4 Kg)	4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Bipodal Squat TRX		4×8		4×8		4×8		4×8		4×8		4×8		4×8
Unipodal Squat TRX		4×8		4×8		4×8		4×8		4×8		4×8		4×8
Hamstrings TRX		4×10		4×10		4×10		4×10		4×10		4×10		4×10
Squat Quádriceps with strap		4x12		4x12		4x12		4x12		4x12		4x12		4x12
Hamstrings with strap		4×10		4×10		4×10		4×10		4×10		4×10		4x10
Nordic Hamstring		4×8		4×8		4×8		4×8		4×8		4×8		4×8
Hamstring kick with EB		4x8		4×8		4×8		4×8		4×8		4×8		4×8

EB, elastic band; Kg, kilograms.

effect size was calculated (Cohen, 1988), and the obtained results were interpreted as follows: trivial (lower than 0.2), small (between 0.2 and 0.5), moderate (between 0.5 and 0.8), and large (above 0.8). These data were analyzed using the Statistical Package for Social Sciences (SPSS 25.0, SPSS Inc., Chicago, IL, United States), and the statistical significance was set at ($p < 0.05$).

RESULTS

In **Table 3** are the presented changes in physical performance for both groups after the intervention period. Within-group analysis did report significant differences in all variables for the SG (p = 0.008 to 0.001; ES = −0.33 to 1.41, small to large) as in the OG (p = 0.001; ES = 0.82 to 1.30, large). Between-groups analysis reported differences in CMJ (F = 4.32; p = 0.004) for the OG.

DISCUSSION

The aim of this study was to examine the effects of two different RT programs [the self-load group (SG) vs. the overload group (OG)] in physical performance of young male soccer players. The main findings of this study indicated that RT with and without external load was effective in improving jumping, aerobic endurance, and body composition in young soccer players, with

TABLE 2 | Phase 1(A): Overload treatment.

Weeks	**W1**		**W2**		**W3**		**W4**		**W5**		**W6**		**W7**		**W8**	
Exercises/Sessions	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**
								Day 1 upper body – Day 2 lower body								
Bench Press	4×15 (BW)		4×15 (BW)		4×15 50% RM		4×15 50% RM		4×15 50% RM		4×15 55% RM		4×15 55% RM		4×15 55% RM	
Curl Biceps	4×10		4×10		4×10		4×10		4×10		4×8		4×8		4×8	
Triceps Pulley	4×8		4×8		4×8		4×8		4×8		4×8		4×10		4×10	
Shoulders 30°	4x8		4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Unilateral Row Machine	4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Squat		4×15 (BW)		4×15 (BW)		4×15 50% RM		4×15 50% RM		4×15 50% RM		4×15 55% RM		4×15 55% RM		4×15 55% RM
Leg Curl		4×10		4×10		4×10		4×10		4×10		4×8		4×8		4×8
Hip Thrust		4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10
Adductor machine		4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10
Gluteus medius machine		4×10		4×10		4×10		4×10		4×10		4×10		4×10		4×10

BW, bar weight (11 kg); Kg, kilograms; RM, repetition maximum.

TABLE 2 | Phase 1(B): Overload treatment.

Weeks	**W9**		**W10**		**W11**		**W12**		**W13**		**W14**		**W15**	
Exercises/Sessions	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	**S23**	**S24**	**S25**	**S26**	**S27**	**S28**	**S29**	**S30**
							Day 1 upper body – Day 2 lower body							
Bench Press	4×15 55% RM		4×15 60% RM		4×15 60% RM		4×15 60% RM		4×15 60% RM		4×12 65% RM		4×12 65% RM	
Concentric curl	4×12		4×12		4×12		4×10		4×10		4×10		4×10	
Dumbbell triceps extension	4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Press militar	4×8		4×8		4×8		4×8		4×8		4×8		4×8	
Assisted chin-ups	4×10		4×10		4×10		4×10		4×10		4×10		4×10	
Unipodal Squat		4×15 55% RM		4×15 60% RM		4×15 60% RM		4×15 60% RM		4×15 60% RM		4×12 65% RM		4×12 65% RM
Flexo-extensión fitball unipodal		4×12		4×12		4×12		4×12		4×12		4×12		4×12
Hip thrust		4×12		4×12		4×12		4×10		4×10		4×10		4×10
Eccentric adductors		4×12		4×12		4×12		4×12		4×12		4×12		4×12
Gluteus medius machine		4×10		4×10		4×10		4×10		4×10		4×10		4×10

RM, repetition maximum.

special attention to jumping ability, where the OG group was more effective. To our knowledge, it is the first research that compares the two RT methods for young soccer players and shows a general benefit in the variables evaluated for young soccer players and could help professionals and coaches of preadult soccer players.

The demands of physical performance in soccer are related to actions of maximum and explosive strength (Sáez de Villarreal et al., 2015). In terms of CMJ performance, our findings showed significant performance improvements for the CMJ in both groups [SG: $p < 0.001$; OG: $p < 0.001$)] most effectively for the OG ($p < 0.004$). Little research had been conducted regarding the effects of RT without external load compared with external load in CMJ performance in male soccer players; therefore, current results are difficult to discuss. Regarding the use of RT without external loads, our results in SG ($p < 0.001$) after 15 weeks, coincide with different studies. Ferrete et al. (2014) trained 2 days a week for 26 weeks in young soccer players using the body weight of the player (or body weight plus light resistance) as external resistance and found improvements in CMJ in the experimental group ($p \leq 0.05$). Falces-Prieto et al. (2020) also showed improvements in CMJ ($p < 0.01$)

TABLE 3 | Physical performance before (baseline) and after (post-training) the 15-week intervention period in both groups.

Variable	SG (n = 69)					OG (n = 75)					Between-group differences	
	Baseline Mean ± SD	Post training Mean ± SD	Δ (%)	*p*	ES	Baseline Mean ± SD	Post training Mean ± SD	Δ (%)	*p*	ES	*F*	*p*
CMJ (cm)	31.35 ± 5.24	33.69 ± 4.93	7.46	**0.001**	1.22	31.15 ± 3.76	34.24 ± 4.10	9.91	**0.001**	1.30	4.32	**0.004**
VO_{2max} (ml/k/min)	49.01 ± 3.13	50.74 ± 2.67	3.53	**0.001**	0.85	48.67 ± 2.92	50.18 ± 2.86	3.10	**0.001**	0.82	1.20	0.275
Weight (kg)	62.38 ± 9.01	64.63 ± 8.46	3.61	**0.001**	0.82	64.51 ± 8.33	66.75 ± 7.73	3.47	**0.001**	0.91	0.30	0.586
Height (cm)	172.57 ± 7.40	173.57 ± 7.21	0.58	**0.001**	0.91	173.37 ± 6.65	174.08 ± 6.56	0.41	**0.001**	0.93	1.68	0.095
Body fat (%)	13.35 ± 3.01	12.89 ± 2.66	−3.45	**0.008**	−0.33	14.61 ± 3.71	13.64 ± 3.18	−6.64	**0.001**	−0.95	2.37	0.126
Lean mass (kg)	53.88 ± 6.99	55.99 ± 6.49	3.92	**0.001**	1.41	55.52 ± 5.73	57.15 ± 5.59	2.94	**0.001**	1.30	2.76	0.099

SG, self-load group; OG, overload group; SD, standard deviation; Δ (%), percentage of change between pre and post conditions; p, a level of significance ($p \leq 0.05$); ES, effect size; CMJ, countermovement jump.

in U19 soccer players after performing RT with self-loading for 2 days a week for 8 weeks. In accordance with our results in OG ($p < 0.001$) after 15 weeks, previous studies have also reported similar increases in jump ability (Franco-Márquez et al., 2015; Rodríguez-Rosell et al., 2017) after RT programs with similar duration (6–8 weeks) load (45–60% 1 RM) and training frequency (2 days per week) among young soccer players. Therefore, our results reinforce the validity of both RT methods in young soccer players to improve jumping ability.

The published training programs advise soccer players to simultaneously train strength and endurance qualities, since they are two of the most important physical abilities to develop in soccer (Hennessy and Watson, 1994). Therefore, the combination of both qualities can activate different anabolic or catabolic processes that are modulated by endocrine responses to exercise and training, producing positive adaptations in the body (Sporiš et al., 2011). That is why the results obtained in this study ($p < 0.001$) in both groups are in line with those of the researchers who justify the positive effect of RT on endurance capacity in young soccer players (Sáez de Villarreal et al., 2015; Ruivo et al., 2016; Di Giminiani and Visca, 2017). Ferrete et al. (2014) also evidenced significant increases ($p \leq 0.05$) in the VO_2 max after RT for 26 weeks using the body weight of the player (or body weight plus light resistance) as external resistance in young soccer players. Regarding the RT with external load, Ruivo et al. (2016) showed improvements in VO_2 max ($p \leq 0.05$) in young soccer players after RT (∼ 65% 1 RM; 3 days a week; 16 weeks). It should be noted that there are few studies that have examined the impact and adaptations of both positive and negative RTs over endurance capacity in young soccer players; we cannot reinforce our data, and, therefore, future research is necessary (Grieco et al., 2012). However, our results can be considered advantageous in young soccer players, because it is observed that, after RT, there is no interference of aerobic endurance.

The BC of young people undergoes rapid changes during their growth spurts, with substantial changes in H and W (Suárez-Arrones et al., 2019). Also interesting is the fact that young soccer players show a high percentage of BF due to absolute low levels of LM and not high levels of BF *per se* (Suárez-Arrones et al., 2019) so that our results show that both ST treatments have been shown to be effective in improving the BC parameters evaluated (SG: $p < 0.001$; OG: $p < 0.001$). Our data on W are in accordance with the study of Erdem-Cigerci and Genc (2020), which examined the effect of calisthenics strength exercises performed 3 days a week for 8 weeks and found significant W increases ($p < 0.032$) in the experimental group. Ferrete et al. (2014) also found significant increases in W ($p \leq 0.05$) after performing 2-day RT a week for 26 weeks in young soccer players using the body weight of the player (or body weight plus light resistance) as external resistance. Regarding the RT with external load, Ruivo et al. (2016) showed significant increase in W ($p \leq 0.05$) in young soccer players after RT.

There is a popular belief that RT when an individual has not yet fully developed negatively affects his or her growth or modifies his or her final H (Faigenbaum et al., 2009; Faigenbaum and Myer, 2010). However, no scientific evidence has been found on growth in young athletes who performed RT programs under qualified supervision and appropriate prescription (Faigenbaum et al., 2009; Faigenbaum and Myer, 2010; Peña et al., 2016). Regarding the improvement in the H variable with both treatments, our data are in accordance with the study by Sander et al. (2013), who evaluated the influence of an RT program for 2 years in young soccer players (n = 134) divided into three age groups (A: 17 years; B: 15 years; C: 13 years), and they also found significant improvements in growth in the three categories ($p < 0.05$). Ferrete et al. (2014) also showed significant increases in the H ($p \leq 0.05$) in young soccer players.

Another benefit of the RT is associated with the lowering of the % BF, which can be beneficial for a football player (Ruivo et al., 2016). We can indicate that treatments without external overload ($p < 0.008$) and with external overload ($p < 0.001$) have been shown to be effective for the decrease of % BF. Equally, Falces-Prieto et al. (2020) also showed decreases in the % BF in U17 and U19 ($p < 0.001$) young soccer players after performing RT with self-loading during 8 weeks. In addition, Suárez-Arrones et al. (2019) showed a significant decrease in % BF (ES = −0.99) after the RT program (i, RT in the gym combining free weights with flywheel inertial devices; ii, specific RT on the field; iii, individual training) organized as circuit training in young soccer players during 26 weeks. Finally, with respect to LM, both RT treatments showed significant improvements ($p < 0.001$), confirming our hypothesis. This coincides with the proposed by Milsom et al. (2015), which suggested that training should be more focused on the gain of LM and not the reduction of BM. In addition, having high LM levels allows the player to avoid traumatic injuries derived from contact and a decrease in the probabilities of muscle injuries (Keiner et al., 2014; Perroni et al., 2015). Our results are in agreement with Pérez-Gómez et al. (2008), where they analyzed the effects of an RT program, consisting of weight lifting, combined with plyometric exercises, followed a period of 6 weeks with 3 sessions/week in U16 soccer players, with an increase in significance in LM ($p \leq 0.05$). We can indicate that the benefits of the treatments proposed in this study are similar to the results obtained with other treatments such as eccentric overload (Suárez-Arrones et al., 2018) and self-loading (Falces-Prieto et al., 2020) among others in young soccer players.

It has been clearly shown that young soccer players can improve jumping, aerobic endurance, and body composition through two different RT programs (with and without external loads) performed 2 days a week for 15 weeks during the season. Furthermore, there is no apparent interference between the development of RT and the other qualities evaluated. Such benefits can be realized from only two RT training sessions per week in season. The performance improvements shown in this study are of great interest for soccer coaches and are directly applicable to prepubertal, young, and professional soccer players. In addition, the present study confirms that the RTs without and with external loads are some valid methods to produce changes at the neuromuscular, cardiovascular level and modification of BC in young soccer players. Previous authors have found similar benefits of RT in this sport, but this is the first study to our knowledge that proposes a self-loading RT methodology and its benefits on different physical qualities on young soccer players. Therefore, it should be considered during the prescription of RT by coaches and fitness coaches of soccer. The outcomes may help soccer coaches and sport scientists formulate better guidelines and recommendations for assessment and selection, training prescription and monitoring, and preparation for competition of young soccer players.

This study had some limitations. One of the main limitations was the absence of a control group, which is mainly needed to isolate the effects of resistance training from those due to growth and maturation. Second limitation was the control of the soccer-specific training loads. The subjects attended their usual soccer training with their usual teams, and differences between the soccer-specific training loads may appear. Third was that nutritional parameters were not taken into account. Nevertheless, the findings of the present research are an important strength. In fact, the intervention applied for 15 weeks (2/week) represents an innovative line of work that coaches should be considerate in their training seasons planning. Future studies may analyze if the load control and nutritional advice may induce more favorable effects. Irrespective of this, the methodology used in this research can be a good initiation treatment to ST in young soccer players, being very useful. It is worth noting that the players reported positive feelings and enjoyment regarding the training intervention as well as the technical staff, indicating the desire to maintain the strength and conditioning program during the season.

CONCLUSION

The findings of this study demonstrate that young soccer players can enhance muscle strength, aerobic endurance, and body composition by undertaking a 15-week in-season program with RT with and without external loads with special attention to training with external load is more effective to improve the jump ability. These results have no apparent interference between the development of RT and aerobic endurance and height in young soccer players. For this reason, the finding encountered in terms of performance suggests that RTs are crucial for young soccer players. Furthermore, this information could be useful for soccer coaches and technical staff due to its potential applicability to soccer performance. In fact, performance on soccer relies greatly on the specific on-field vertical jump, aerobic endurance, and high levels of lean mass. Previous authors have found a similar benefit of strength and power training in others and this sport, but this is the first study, to our knowledge, involving RT with and without external loads and its relationship with improvements in different performance parameters in soccer. It is recommended that soccer coaches implement strength training without external load in the early stages of training or in players with late maturation development and in those soccer clubs with limited material resources. The outcomes may help coaches and sport scientists formulate better guidelines and recommendations for athlete assessment and selection, training prescription and monitoring, and preparation for competition.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All procedures were approved by the local ethics committee for the use of human participants in accordance with the latest version of the Declaration of Helsinki. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MF-P and ES led the project, established the protocol, drafted the initial manuscript, and reviewed and revised the manuscript. JR-G, FG-F, and FC wrote and revised the manuscript. GB and EM-C wrote and reviewed the final version. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Relationship Between Drop Jump Training–Induced Changes in Passive Plantar Flexor Stiffness and Explosive Performance

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Passive muscle stiffness is positively associated with explosive performance. Drop jump training may be a strategy to increase passive muscle stiffness in the lower limb muscles. Therefore, the purpose of this study was to examine the effect of 8-week drop jump training on the passive stiffness in the plantar flexor muscles and the association between training-induced changes in passive muscle stiffness and explosive performance. This study was a randomized controlled trial. Twenty-four healthy young men were divided into two groups, control and training. The participants in the training group performed drop jumps (five sets of 20 repetitions each) 3 days per week for 8 weeks. As an index of passive muscle stiffness, the shear moduli of the medial gastrocnemius and soleus were measured by shear wave elastography before and after the intervention. The participants performed maximal voluntary isometric plantar flexion at an ankle joint angle of 0° and maximal drop jumps from a 15 cm high box. The rate of torque development during isometric contraction was calculated. The shear modulus of the medial gastrocnemius decreased for the training group (before: 13.5 ± 2.1 kPa, after: 10.6 ± 2.1 kPa); however, such a reduction was not observed in the control group. There was no significant group (control and training groups) × time (before and after the intervention) interaction for the shear modulus of the soleus. The drop jump performance for the training group improved, while the rate of torque development did not change. Relative changes in these measurements were not correlated with each other in the training group. These results suggest that drop jump training decreases the passive stiffness in the medial gastrocnemius, and training-induced improvement in explosive performance cannot be attributed to change in passive muscle stiffness.

Keywords: passive muscle stiffness, gastrocnemius, soleus, rate of torque development, drop jump, elastography

INTRODUCTION

Passive muscle stiffness (muscle stiffness at rest) could be an important determinant in sports performance because it has been found to be associated with explosive performance (Takahashi et al., 2018; Ando and Suzuki, 2019; Miyamoto et al., 2019; Ando et al., 2021). For example, we previously showed positive correlations of passive medial gastrocnemius (MG) stiffness with the rate of torque development (RTD) during maximal isometric plantar flexion (Ando and Suzuki, 2019) and with drop jump performance (Ando et al., 2021). In addition, Miyamoto et al. (2019) showed that passive vastus lateralis stiffness positively related to the sprinting ability of sprinters. These results suggest that passive muscle stiffness is not a negligible influencing factor for considering the stretch-shortening cycle in the muscle-tendon unit, even though it is generally known that tendon stiffness is the major factor.

To the best of our knowledge, a training regimen that increases passive muscle stiffness has not yet been well developed. In an animal study, an increase in the passive stiffness of the extensor digitorum longus and rectus femoris was demonstrated after 15-week jump training (Ducomps et al., 2003). In a human study, increased passive stiffness was demonstrated in type IIa/IIx fibers of the vastus lateralis after 8 weeks of various jump trainings (Malisoux et al., 2006). Fouré et al. (2009, 2011) also indicated a possibility that 8 weeks of jump training increases the passive stiffness in the gastrocnemius of humans. However, they regarded the ankle joint stiffness calculated from the passive plantar flexor torque during dorsiflexion as passive muscle stiffness. Because joint stiffness is affected not only by the muscle but also the joint capsule, tendon, skin, and so on (Johns and Wright, 1962), the effect of long-term jump training on passive stiffness in the plantar flexor muscles is still unclear. Recently, ultrasound shear wave elastography was used to directly measure the longitudinal elasticity of muscle *in vivo*, and the results showed similar relationships between passive muscle stiffness and explosive performance as described above (Ando and Suzuki, 2019; Miyamoto et al., 2019; Ando et al., 2021). Therefore, ultrasound shear wave elastography must be employed to clarify the effect of jump training on muscle stiffness.

Furthermore, previous studies (Malisoux et al., 2006; Fouré et al., 2009, 2011) have not focused on the association between training-induced changes in passive muscle stiffness and explosive performance, even though passive muscle stiffness is considered to influence explosive performance. We clarified that the passive stiffness of the MG, not soleus (SOL), is positively related to RTD during isometric contraction (Ando and Suzuki, 2019) and drop jump performance (Ando et al., 2021). Thus, increased passive MG stiffness could be advantageous to explosive performance. In many types of jump training, drop jumping imposes considerable stress on the plantar flexor muscles because greater plantar flexor torque is produced in the contact phase of a drop jump than in the contact phase of a counter-movement jump (Bobbert et al., 1987a). Considering the necessity of sufficient mechanical stress for changing the tissue mechanical property (Kjaer, 2004), long-term drop jump training can be one of the best interventions to stiffen the passive plantar flexor muscles, thus enhancing the explosive performance.

Therefore, the purpose of this study was to examine the effect of 8-week drop jump training on the passive stiffness in the plantar flexor muscles *via* ultrasound shear wave elastography and to determine the relationship between training-induced changes in passive muscle stiffness and explosive performance. We hypothesized that the passive stiffness in the plantar flexor muscles increased after 8-week drop jump training and training-induced change in passive MG stiffness associated with change in explosive performance.

MATERIALS AND METHODS

Participants

Twenty-four healthy young men participated in an open, parallel group, randomized controlled intervention study. This number was determined by a sample size estimation using the data of a previous study that examined the effect of 8 weeks of jump training on the stiffness of plantar flexor muscles (Fouré et al., 2009). Based on an α-level of 0.05 and a power $(1-\beta)$ of 0.80, and an expected 33% change in passive muscle stiffness, the analysis indicated that at least 11 participants were required in each group. The intervention period of 8 weeks was determined based on previous studies that suggested increased passive muscle stiffness in the vastus lateralis and gastrocnemius after 8 weeks of jump training (Malisoux et al., 2006; Fouré et al., 2009). Participants were excluded from the present study if they were particularly well-trained in running, jumping, ball games, or any other form of athletics that could potentially impact the passive stiffness of their plantar flexor muscles or if they had any history of orthopedic surgery on the lower limb. They were randomly divided into a control group ($n = 12$, age: 22 ± 1 years, height: 170.3 ± 3.7 cm, body mass: 64.8 ± 12.6 kg) and training group ($n = 12$, age: 22 ± 1 years, height: 176.1 ± 7.5 cm, body mass: 66.9 ± 5.6 kg) using block randomization through a random number table. Before proceeding with the experiment, the purpose of the study, its procedures, and associated risks were explained to all the participants and written informed consent was obtained from them. The experimental protocols were approved by the ethics committees of the Japan Institute of Sports Sciences (No. 027) and the Shibaura Institute of Technology (No. 18-009). This study was conducted in accordance with the Declaration of Helsinki.

Experimental Design

The participants in the training group underwent 8-week drop jump training and maintained their habitual daily physical activity. The participants in the control group were asked to refrain from any resistance or plyometric training during the control period. Before and after the 8-week control and training periods, the muscle shear moduli of the MG and SOL, isometric plantar flexor torque, RTD, electromechanical delay (EMD), rate of electromyogram rise (RER), and drop jump performance were determined. Because plantar flexor muscles produce greater torque than knee and hip extensor muscles during the drop jump (Bobbert et al., 1987a), we evaluated the functional and mechanical properties of plantar flexor muscles. All the participants visited the laboratory at least 1 week prior to testing for a familiarization trial.

Drop Jump Training

The participants in the training group performed drop jumps 3 days per week for 8 weeks. The training consisted of five sets of 20 drop jumps each with a 1-min rest between the sets. The participants dropped from a 15 cm high box in the first 4 weeks and a 30 cm high box in the next 4 weeks. They were instructed to jump as high as possible with a short contact time for each jump. While jumping, they kept their hands on their hips. They were instructed to avoid deep flexion of the knee and hip joints to ensure a short contact time, and this was practiced during a familiarization trial (described later).

Muscle Shear Modulus

The shear wave propagation speed of the muscle was measured using ultrasound shear wave elastography (ACUSON S2000; Siemens Medical Solutions, United States), which was used to calculate the shear modulus (as described below) and thereby evaluate the passive muscle stiffness. The participants lay prone on an examination bed with their knees fully extended and their right foot secured to the footplate of an electrical dynamometer (CON-TREX MJ; Physiomed, Germany). The ankle joint was fixed at 0° (neutral position, 90° between the foot and tibia), and the participants were asked to relax their leg during measurement. The shear wave propagation speed through the MG was measured at the proximal 30% position of the leg length from the popliteal crease to the lateral malleolus and at the 40% position of the girth from the boundary between the MG and tibia to that between the MG and lateral gastrocnemius. The shear wave propagation speed through the SOL, on the other hand, was measured at the proximal 30% position of the leg length and at the 20% position of the girth from the boundary between the MG and lateral gastrocnemius to that between the MG and tibia. After determining the measurement sites, the ankle joint was plantar flexed to 40° from the neutral position. This position was sustained with relaxation for 5 min to eliminate the effects of static stretching at an ankle joint angle of 0°. The ankle joint was then returned to the neutral position; furthermore, the shear wave propagation speed was immediately measured thrice for each muscle in a random order. A linear array probe (9 L4 Transducer, 4–9 MHz; Siemens Medical Solutions) was longitudinally placed at each measurement site with sufficient water-soluble transmission gel, and its direction was adjusted to the orientation of muscle fascicles. The coefficient of variance for the three measurements was 3.6 ± 2.0% for the MG and 2.7 ± 1.7% for the SOL. To ensure the relaxation of muscle during scanning, electromyogram (EMG, described below) was recorded. The root-mean-square (RMS) of the EMG signals during scanning normalized by that during maximal voluntary isometric contraction (MVC) was 1.9 ± 1.3% for the MG and 2.1 ± 1.9% for the SOL.

The shear modulus was calculated using elastography images according to a procedure described in a previous paper (Hirata et al., 2020). The elastography images were exported in the Digital Imaging and Communications in Medicine (DICOM) format from the ultrasound apparatus. The region of interest on the elastography image color map was selected to be as large as possible (**Figure 1**) using an image processing software (ImageJ; NIH, United States). Tissues other than the target muscle (subcutaneous fat, aponeurosis, fascia, etc.) were not included in the selected area. Then, the red-green-blue (RGB) value of each pixel within the region of interest was converted into shear wave speed according to the RGB value–shear wave speed relationship estimated using the color scale displayed on the elastography image. The shear modulus of each pixel was calculated by multiplying the shear wave speed and tissue density (Hug et al., 2015). In the present study, the muscle density was assumed to be 1,000 kg/m^3 (Freitas et al., 2015; Hug et al., 2015). Thereafter, the muscle shear modulus of each elastography image was calculated by averaging the shear moduli of all pixels in the region of interest.

Plantar Flexion Task

The participants performed isometric plantar flexion at an ankle joint angle of 0° following the ultrasound shear wave elastography measurements. They performed several submaximal isometric contractions as a warm-up exercise. Two MVCs were performed for 4 s, and an additional trial was performed if the peak torque values differed by >10% between two trials. A rest of 1 min was allowed between trials. Subsequently, the participants performed 10 maximal isometric plantar flexions as fast and hard as possible and sustained maximal effort for approximately 1 s with a rest of 30 s between flexions.

Signals from the dynamometer, which were recorded on a personal computer using the LabChart v8.1.5 software (ADInstruments, Australia), were sampled at 2 kHz using an analog-to-digital converter (PowerLab; ADInstruments). The torque signal was sampled and averaged over 1 s during the sustained phase of an MVC to calculate the MVC torque. Out of the two values obtained, the higher value was used as the representative value for further analyses. The normalized RTD was calculated

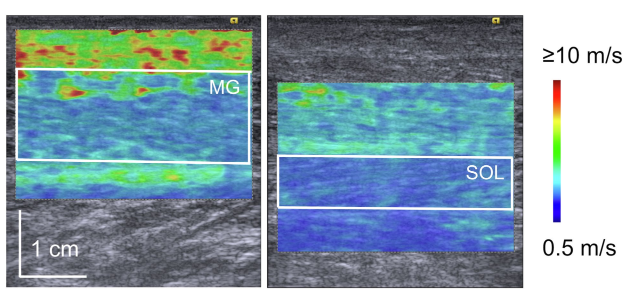

FIGURE 1 | Representative elastography images of medial gastrocnemius (MG) and soleus (SOL). The white square in the image represents the region of interest for shear wave elastography. Blue color indicates the lower speed of shear wave propagation while red indicates the higher speed of shear wave propagation.

based on previous studies (Aagaard et al., 2002; Ema et al., 2017; Ando and Suzuki, 2019). First, the onset of plantar flexion was determined as the point at which the torque exceeded the baseline by 2.5% of the MVC torque. To eliminate the effect of counter movement on RTD values, trials in which >0.5 Nm of dorsiflexion torque was observed before the onset were excluded in accordance with observations made in previous studies (Tillin et al., 2010; Balshaw et al., 2016). The RTD was then calculated as the slope of the time–torque curve over time intervals of 0–100 and 0–200 ms from the onset of plantar flexion. These RTD values were normalized by the MVC torque to exclude the effect of muscle strength. The average of the highest three RTD values was used for further analyses.

Electromyogram Recording

Surface EMG signals were acquired from the MG and SOL during the ultrasound shear wave elastography measurements and the isometric plantar flexion task. The measurement site of the EMG signals for the MG was located at the proximal 30% position of the leg length. The electrode placement for the SOL was <5 cm from the distal 40% position of the leg length, but interindividual variations were found because of the variability in the superficial region of the SOL. At these points, fascicle longitudinal directions were confirmed by B-mode ultrasonography. A single differential electrode (DE-2.1 Sensor; Delsys, United States) was used, which had an interelectrode distance of 1 cm, a contact sensor composed of two silver bars (0.1×1 cm each), an input impedance of $>10^{15}\,\Omega$ per 0.2 pF, and a common rejection ratio of 90 dB. The sensor preamplifier and main amplifier units (Bagnoli-8; Delsys) were set to 10- and 100-fold gains, respectively, which resulted in a 1,000-fold amplification of the original EMG signal and a frequency response of 20 ± 5 to 450 ± 50 Hz. The signals from the EMG system were sampled at 2 kHz using an analog-to-digital converter and synchronized with the torque data on a personal computer using LabChart v8.1.5 software.

The EMD and RER were calculated according to procedures described in a previous study (Ema et al., 2017). Briefly, EMG signals were smoothed using a moving RMS window of 50 ms during the RTD test. The onset of muscle activity was set at mean +3 standard deviations (SDs) of the EMG RMS value from the baseline (mean and SD were calculated at rest over a time window of 200 ms). The EMD was determined as the difference in time between the onset of plantar flexor torque and MG activation, which was closely related to tendon stiffness (Waugh et al., 2013). The RER of the MG (RER_{MG}) and SOL (RER_{SOL}) was determined as the slope of the smoothed time–EMG curve from EMG onset to 50 ms. The reason for selecting a time interval of 50 ms was based on the association between changes in the rate of force development and RER at a time interval of 50 ms after 10-week isokinetic training (Blazevich et al., 2008).

Drop Jump Test

The participants dropped from a 15 cm high box, landed on a jump analysis mat (DKH Co. Ltd., Japan), immediately jumped as high as possible, and landed on the mat again. The drop jump test was conducted using a 15 cm high box because relative contribution of the plantar flexors torque to drop jump was similar among different box heights in the previous study (Bobbert et al., 1987b). The instructions for the drop jump were the same as those for the drop jump training (described above). The signals from the mat, which were recorded on a personal computer using the Multi Jump Tester software (DKH Co. Ltd.), were sampled using an analog-to-digital converter. The jumping height was calculated using the following equation (Yoon et al., 2007; Miura et al., 2010):

$$\text{Jumping height} = 1/8 \times g \times t^2$$

where g and t are the gravitational acceleration (9.81 m/s^2) and flight time, respectively. The reactive strength index (RSI) was calculated by dividing the jump height (m) by the contact time (s). Drop jumps were performed twice. The highest RSI and the associated jump height and contact time were used for further analyses.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics software (version 24.0; IBM, United States). Normality was tested using the Shapiro–Wilk test, which did not indicate normality for the data of some parameters. In the present study, a two-way repeated-measures analysis of variance (ANOVA) was required to examine the group (control and training groups) and time (before and after the intervention) interaction. Therefore, all data were log transformed, which were then confirmed as the normal distribution. The two-way ANOVA with repeated measures (group and time) was used to evaluate changes in the shear moduli of the MG and SOL, MVC torque, RTD at each time interval, EMD, RER_{MG}, RER_{SOL}, RSI, jump height, and contact time before and after the intervention. When an interaction was found, an unpaired/paired t-test was performed for group/time comparison with Bonferroni's correction. For the interaction and main effect of the two-way ANOVA, η^2 was calculated as an index of the effect size. Furthermore, Cohen's d was appropriately calculated as an index of the effect size for an unpaired/paired t-test. The values of η^2 or d were interpreted as $\eta^2 < 0.01$ or $d < 0.20$ for trivial effects, $0.01 \le \eta^2 < 0.06$ or $0.20 \le d < 0.50$ for small effects, $0.06 \le \eta^2 < 0.14$ or $0.50 \le d < 0.80$ for medium effects, and $0.14 \le \eta^2$ or $0.80 \le d$ for large effects (Cohen, 1988). The relationship of the relative changes in shear moduli of the MG and SOL with RTD_{100}, RTD_{200}, and RSI from the baseline to 8 weeks later was examined for the training group using Pearson's product-moment correlation coefficient. All the data were presented as mean ± SD. The level of significance was set at $p < 0.05$ for all the analyses.

RESULTS

The data of the shear moduli of the MG and SOL, MVC torque, RTD, EMD, RER of the MG and SOL, drop jump performance, ANOVA results, and effect size results are presented in **Table 1**. A significant group × time interaction was found for the MG shear modulus. The MG shear modulus increased from the baseline to 8 weeks later for the control group ($p = 0.001$, $d = 0.86$), whereas it decreased for the training group ($p = 0.001$, $d = 1.38$). The MG shear modulus was lower for the training group than that for the control group after the intervention ($p < 0.001$, $d = 1.63$). No significant group × time interaction was found for the SOL shear modulus, and no main effect of the group on the SOL shear modulus was observed. A significant main effect of time on the SOL shear modulus was found, indicating a decreased shear modulus in both groups. A significant main effect of the group on the MVC torque was observed, but no significant group × time interaction or main effect of time on the MVC torque was found. No significant interactions or main effects were found for the RTD, EMD, and RER of the MG and SOL. A significant group × time interaction was found for the RSI. *Post-hoc* tests indicated that the RSI increased from the baseline to 8 weeks later for the training group ($p < 0.001$, $d = 1.15$), whereas it did not change for the control group ($p = 0.278$, $d = 0.14$). The RSI was greater for the training group than the control group after the intervention ($p = 0.001$, $d = 1.40$). A main effect of time on the jump height was found, indicating an increased jump height for the control group and training group. No significant group × time interaction or main effect of group on the jump height was found. A significant group × time interaction was observed for the contact time. Multiple comparisons indicated that the contact time decreased from the baseline to 8 weeks later for the training group ($p = 0.001$, $d = 1.08$), whereas it did not change for the control group ($p = 0.625$, $d = 0.20$). The contact time was lower for the training group than the control group after the intervention ($p = 0.004$, $d = 1.35$).

Figure 2 shows relationships between the relative changes in shear moduli of the MG and SOL with RTD_{100}, RTD_{200}, and RSI from the baseline to after the intervention for the training group. Shear moduli of the MG and SOL did not significantly correlate with RTD_{100}, RTD_{200}, and RSI.

DISCUSSION

This study examined the effect of 8-week drop jump training on the passive stiffness in the plantar flexor muscles and the relationship between training-induced changes in passive muscle stiffness and explosive performance. In the training group, after the intervention, the MG shear modulus decreased, the RTD did not change, and the drop jump performance improved. Furthermore, relative changes in the shear moduli of the MG and SOL did not correlate with those in the RTD and RSI in the training group. These results suggest that 8-week drop jump training decreases the passive MG stiffness; however, improvement in explosive performance cannot be attributed to change in passive muscle stiffness.

Contrary to our hypothesis, the MG shear modulus for the training group decreased with a large effect size after 8 weeks.

TABLE 1 | Shear modulus, MVC torque, RTD, EMD, RER, RSI, jump height, and contact time for control and training groups.

	Control		Training		ANOVA	Effect size
	Before	After	Before	After		
MG shear modulus (kPa)	12.7 ± 3.6	16.1 ± 4.3*	13.5 ± 2.1	10.6 ± 2.1*,†	Group: $p = 0.071$, time: $p = 0.985$, group × time: $p < 0.001$	Group: $\eta^2 = 0.19$, time: $\eta^2 < 0.01$, group × time: $\eta^2 = 0.46$
SOL shear modulus (kPa)	5.3 ± 2.2	4.9 ± 0.7	5.8 ± 1.1	4.7 ± 0.8	Group: $p = 0.597$, time: $p = 0.025$, group × time: $p = 0.168$	Group: $\eta^2 = 0.01$, time: $\eta^2 = 0.19$, group × time: $\eta^2 = 0.07$
MVC torque (Nm)	118 ± 21	115 ± 15	122 ± 21	134 ± 16	Group: $p = 0.049$, time: $p = 0.291$, group × time: $p = 0.152$	Group: $\eta^2 = 0.21$, time: $\eta^2 = 0.04$, group × time: $\eta^2 = 0.08$
RTD_{100} (%MVC/s)	353 ± 105	333 ± 44	407 ± 118	395 ± 113	Group: $p = 0.215$, time: $p = 0.529$, group × time: $p = 0.986$	Group: $\eta^2 = 0.33$, time: $\eta^2 = 0.02$, group × time: $\eta^2 < 0.01$
RTD_{200} (%MVC/s)	305 ± 51	311 ± 23	340 ± 67	335 ± 56	Group: $p = 0.202$, time: $p = 0.724$, group × time: $p = 0.497$	Group: $\eta^2 = 0.27$, time: $\eta^2 < 0.01$, group × time: $\eta^2 = 0.02$
EMD (ms)	78 ± 15	80 ± 15	69 ± 11	69 ± 14	Group: $p = 0.083$, time: $p = 0.799$, group × time: $p = 0.672$	Group: $\eta^2 = 0.43$, time: $\eta^2 < 0.01$, group × time: $\eta^2 < 0.01$
RER_{MG} (mV/s)	0.84 ± 0.37	0.72 ± 0.42	1.05 ± 0.68	0.94 ± 0.61	Group: $p = 0.399$, time: $p = 0.098$, group × time: $p = 0.538$	Group: $\eta^2 = 0.14$, time: $\eta^2 = 0.10$, group × time: $\eta^2 < 0.01$
RER_{SOL} (mV/s)	1.25 ± 0.90	1.14 ± 0.65	1.85 ± 1.23	1.77 ± 1.26	Group: $p = 0.532$, time: $p = 0.430$, group × time: $p = 0.276$	Group: $\eta^2 = 0.09$, time: $\eta^2 = 0.03$, group × time: $\eta^2 < 0.01$
RSI (m/s)	0.88 ± 0.42	0.93 ± 0.27	0.96 ± 0.42	1.48 ± 0.47*,†	Group: $p = 0.090$, time: $p < 0.001$, group × time: $p = 0.007$	Group: $\eta^2 = 0.23$, time: $\eta^2 = 0.31$, group × time: $\eta^2 = 0.13$
Jump height (cm)	17.9 ± 7.3	19.1 ± 5.7	19.8 ± 7.8	25.8 ± 6.8	Group: $p = 0.173$, time: $p = 0.005$, group × time: $p = 0.081$	Group: $\eta^2 = 0.19$, time: $\eta^2 = 0.23$, group × time: $\eta^2 < 0.08$
Contact time (ms)	214 ± 36	208 ± 22	213 ± 41	178 ± 23*,†	Group: $p = 0.124$, time: $p = 0.006$, group × time: $p = 0.030$	Group: $\eta^2 = 0.15$, time: $\eta^2 = 0.21$, group × time: $\eta^2 = 0.13$

*Values are presented as mean ± standard deviation. ANOVA, analysis of variance; MVC, maximal voluntary contraction; MG, medial gastrocnemius; SOL, soleus; RTD, rate of torque development; EMD, electromechanical delay; RER, rate of electromyography rise; RSI, reactive strength index. *p < 0.05 vs. pre by post-hoc test.*
†p < 0.05 vs. control by post-hoc test.

This could be due to lower drop jump performance-related fascicle behavior in the contact phase during drop jumping in training periods. The performance of reactive jumps such as drop jumps or rebound jumps was dramatically lower in the present study than in previous studies (Fouré et al., 2009, 2011; Laurent et al., 2020). The fascicle lengthening in the participants was speculated in the contact phase during drop jump because the MG fascicles behaved isometrically or shortened in the contact phase during drop jumping (Ishikawa et al., 2005), and the magnitude of fascicle shortening was greater in high-performance individuals (Hoffrén et al., 2007). Blazevich (2019) suggested that long-term training, including eccentric muscular contraction, decreases passive muscle stiffness. Taken together, drop jump training might impose lengthening of the MG fascicles in most of our participants, which would lead to a decrease in the MG shear modulus. Because fascicle lengthening in the contact phase during drop jump was not proven by ultrasound imaging or kinematic analysis, further studies are warranted in the future.

Because the MG shear modulus decreased in the present study, a decrease in the RTD must be expected based on the positive correlation between the MG shear modulus and RTD (Ando and Suzuki, 2019). However, no significant changes were observed in RTD_{100} and RTD_{200}, and no significant correlations were found between the relative changes in the MG shear modulus and RTD_{100} or RTD_{200}. These results were attributed to the possibility that the effect of changes in passive muscle stiffness on the RTD was masked by changes in muscle activation. Muscle activation, as assessed by surface EMG, is a major factor influencing the rate of force development (Maffiuletti et al., 2016). A strong correlation was found between the relative changes in RER_{MG} and RTD_{100} in the training group ($r = 0.735$). We previously found moderate correlations between the MG shear modulus and RTD in a cross-sectional study ($r = 0.460–0.496$; Ando and Suzuki, 2019). Therefore, influence of the change in the MG shear modulus was relatively low compared with that in muscle activation.

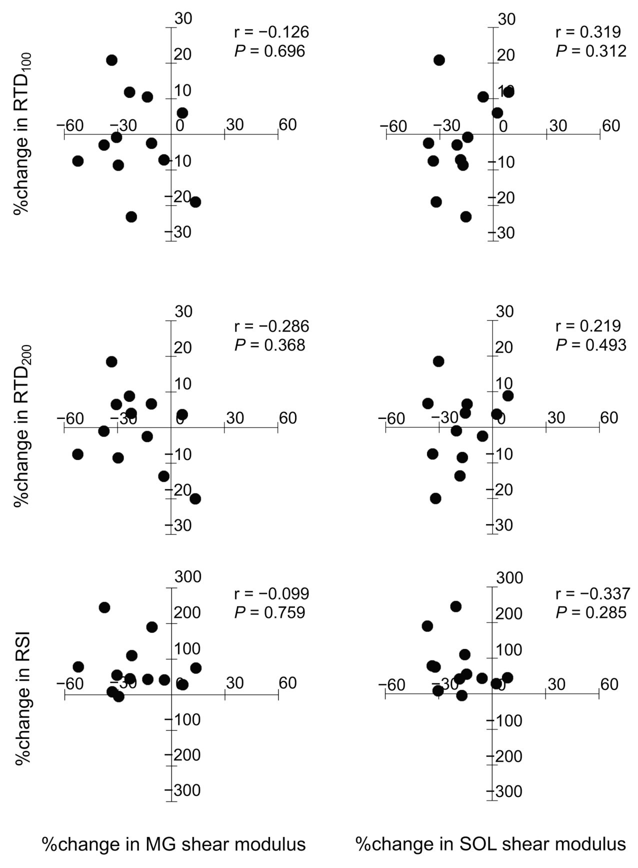

FIGURE 2 | Relationships between the relative changes in shear moduli of the medial gastrocnemius (MG) and soleus (SOL) with rate of torque development (RTD_{100}, RTD_{200}), and reactive strength index (RSI).

We expected an association between training-induced changes in the MG shear modulus and the RSI because of a positive relationship between the MG shear modulus and drop jump performance (Ando et al., 2021). However, the drop jump performance for the training group improved after the intervention, whereas the MG shear modulus decreased. Therefore, other determining factors such as muscle strength, mechanical properties of tendons, muscle fiber type, and neural factors could have contributed to the increase in the drop jump performance in the present study. Regarding muscle strength, no significant group × time interaction was found for the MVC torque. In addition, the EMD, which is an index of tendon stiffness (Waugh et al., 2013), did not change before and after the intervention in both the groups. Subsequently, the muscle fiber type composition was assumed to not change during the 8-week drop jump training. This is because no change in the fiber type composition was observed after an 8-week ballistic resistance exercise (Winchester et al., 2008), which would cause higher training stress than drop jump training. Thus, changes in neural factors would improve the drop jump performance in the present study. Hoffrén et al. (2007) showed that active joint stiffness was related to plantar flexor muscle activation in the early contact phase (i.e., braking phase) during drop jumping. Furthermore, they indicated an association between active joint stiffness and drop jump performance. Therefore, we believe that increased active joint stiffness due to muscle activity (i.e., a neural factor) strongly affects the improvement in the drop jump performance in the training group. Thus, the effect of decreased MG shear modulus might disappear.

Previous studies have reported inconsistent results regarding the effect of jump training, including drop jumping, on Achilles tendon stiffness. One study reported that Achilles tendon stiffness increased after long-term jump training (Laurent et al., 2020), whereas others found that it decreased or did not change (Kubo et al., 2007; Fouré et al., 2009, 2011; Houghton et al., 2013). In the present study, no significant change was found in the EMD, which was closely related to tendon stiffness (Waugh et al., 2013), before and after the 8-week drop jump training. This result indicates a possibility that Achilles tendon stiffness did not change. Reactive jump performance has been shown to be related to Achilles tendon stiffness (Abdelsattar et al., 2018; Laurent et al., 2020). However, drop jump performance improved without a change in the EMD in the present study. Because our participants showed lower drop jump performance (especially jump height), it is speculated that the first step toward improving drop jump performance is to increase active joint stiffness due to muscle activity and then increase Achilles tendon stiffness or other factors.

There were limitations in the present study. First, the shear moduli of the MG and SOL changed significantly in the control group after the 8-week control period. These results may be partly due to inter-days difference for measurement of the shear modulus. However, the effect size of the MG shear modulus between before and after the intervention was greater for the training group ($d = 1.38$) than for the control group ($d = 0.86$). Therefore, decreased MG shear modulus after the 8-week drop jump training would be meaningful. Second, in the present study, no muscular contraction was performed before the ultrasound scanning, although Stubbs et al. (2018) suggested that contraction at short lengths eliminates the history dependence of the muscle slack length. However, the procedures for ultrasound scanning before and after the intervention were the same for all the participants (e.g., relaxation of the muscle at the slacked length for 5 min before ultrasound scanning). Therefore, lack of muscular contraction would not have affected the interpretation of our results. Third, no measurements of kinetics and kinematics of the lower limbs were taken during drop jumping before and after the 8-week training program. Therefore, the improvement in the drop jump performance in the training group may be attributed to increased torque by the ankle, knee, and hip joints. The ankle joint produced more torque than the knee and hip joints in the contact phase during drop jumping (Bobbert et al., 1987a), resulting in higher stress imposed on the plantar flexor muscles than the knee and hip extensor muscles during the training period. In the present study, no significant increase was found in the MVC torque in the plantar flexor muscles for the training group (**Table 1**). Thus, no increase in the muscle strength of the knee and hip extensors was expected. Fourth, the height of the box was increased up to 30 cm during the intervention in the present study. Bobbert et al. (1987b) indicated that the torque produced by the plantar flexors was greater using a higher box (20–60 cm). Therefore, further training effect might be gained if the height of box was increased further during the intervention. However, because participants in the present study could not be tested for higher physical activity before recruiting for the experiment and performed poorly in the drop jump test, injury risk during training needed to be avoided. Further studies in this regard are warranted in the future. Finally, an 8-week training program was adopted in the present study. A longer training period is generally considered to induce greater morphological and functional changes. A longer intervention (e.g., 12 weeks) could improve RTD and indicate a significant group × time interaction for the SOL shear modulus. Furthermore, the relative changes in the shear moduli may be related to those in RTD and RSI.

In conclusion, the 8-week drop jump training decreased the MG shear modulus, but this was not related with any change in RSI. These results suggest that drop jump training decreases the passive stiffness in the MG, and training-induced improvement in explosive performance cannot be attributed to change in passive muscle stiffness.

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 Japan Institute of Sports Sciences and Shibaura Institute of Technology. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RA, SS, YS, KH, and RA: conceived and designed the experiments. RA, SS, NH, HT, NI, and KH: performed experiment. RA and KH: analyzed data. RA: drafted manuscript and prepared tables and figures. All authors interpreted the results of the research, edited, critically revised, and approved the final version of the manuscript, and have agreed to be accountable for all aspects of the work related to its accuracy and integrity.

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

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Gastrocnemius Muscle Architecture in Elite Basketballers and Cyclists: A Cross-Sectional Cohort Study

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Eccentric and concentric actions produce distinct mechanical stimuli and result in different adaptations in skeletal muscle architecture. Cycling predominantly involves concentric activity of the gastrocnemius muscles, while playing basketball requires both concentric and eccentric actions to support running, jumping, and landing. The aim of this study was to examine differences in the architecture of gastrocnemius medialis (GM) and gastrocnemius lateralis (GL) between elite basketballers and cyclists. A trained sonographer obtained three B-mode ultrasound images from GM and GL muscles in 44 athletes (25 basketballers and 19 cyclists; 24 ± 5 years of age). The images were digitized and average fascicle length (FL), pennation angle (θ), and muscle thickness were calculated from three images per muscle. The ratio of FL to tibial length (FL/TL) and muscle thickness to tibial length (MT/TL) was also calculated to account for the potential scaling effect of stature. In males, no significant differences were identified between the athletic groups in all parameters in the GM, but a significant difference existed in muscle thickness in the GL. In basketballers, GL was 2.5 mm thicker (95% CI: 0.7–4.3 mm, $p = 0.011$) on the left side and 2.6 mm thicker (95% CI: 0.6–5.7 mm, $p = 0.012$) on the right side; however, these differences were not significant when stature was accounted for (MT/TL). In females, significant differences existed in the GM for all parameters including FL/TL and MT/TL. Female cyclists had longer FL in both limbs (MD: 11.2 and 11.3 mm), narrower θ (MD: 2.1 and 1.8°), and thicker muscles (MD: 2.1 and 2.5 mm). For the GL, female cyclists had significantly longer FL (MD: 5.2 and 5.8 mm) and narrower θ (MD: 1.7 and 2.3°) in both limbs; no differences were observed in absolute muscle thickness or MT/TL ratio. Differences in gastrocnemius muscle architecture were observed between female cyclists and basketballers, but not between males. These findings suggest that participation in sport-specific training might influence gastrocnemius muscle architecture in elite female athletes; however, it remains unclear as to whether gastrocnemius architecture is systematically influenced by the different modes of muscle activation between these respective sports.

Keywords: ultrasound, fascicle length, pennation angle, muscle thickness, concentric exercise, eccentric exercise

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INTRODUCTION

Skeletal muscle architecture is described by the arrangement of fiber bundles, known as fascicles, to the force-generating axis of pennate muscles (Wickiewicz et al., 1983; Lieber and Friden, 2000). Fascicles insert obliquely into the superficial and deep aponeuroses of a muscle which defines the pennation angle (θ), and the distance between aponeuroses defines anatomical muscle thickness. Muscle architecture can be measured *via in vivo*, two-dimensional (2D), B-mode ultrasound imaging (Kwah et al., 1985; Scholten et al., 2003; Timmins et al., 2015; Oliveira et al., 2021). Due to lower cost and greater accessibility in a variety of settings, ultrasound is popular compared to MRI, an alternative tool that requires a technique known as diffusion tension imaging (DTI) to discern individual muscle fibers (Van Donkelaar et al., 1999; Franchi et al., 2018a; Bolsterlee et al., 2019). Ultrasound measurements obtained by appropriately trained sonographers during resting or isometric conditions have been shown to be repeatable and reliable, as well as valid against historic measures based on microdissection of whole cadaveric muscles (Kwah et al., 1985; Reeves and Narici, 1985; Ando et al., 2014). Once images are acquired by a trained sonographer, measurements can be made using manual digitization with custom-written computer software (Narici et al., 1996; Maganaris et al., 1998; Aagaard et al., 2001; Chleboun et al., 2001, 2007; Muramatsu et al., 2001; Boer et al., 2008; Aggeloussis et al., 2009; Raj et al., 2012a; Gillett et al., 2013; Lidstone et al., 2016), or automated tracking (Cronin et al., 1985; Rana et al., 2009; Zhou et al., 2014; Drazan et al., 2019).

Skeletal muscle architecture parameters have been measured in studies of muscle physiology and biomechanics. The geometric arrangement determines muscle length and the length range over which active force can be generated, which influences contraction velocity, force-generating capacity, and the functional excursion of the muscle (Wickiewicz et al., 1983; Blazevich et al., 1985; Narici et al., 1996; Fukunaga et al., 1997; Kawakami et al., 1998; Maganaris et al., 1998; Lieber and Friden, 2000, 2001; Hodges et al., 2003; Blazevich, 2006; Kruse et al., 2021). Muscle length is determined by fascicle length (FL) which is defined by the number of sarcomeres in-series, as well as by θ (Kruse et al., 2021). Recent studies have found that certain architectural characteristics relate to advantages in physical performance. Longer FL has been associated with sprint performance, as the number of sarcomeres arranged in-series is proportional to the maximum shortening velocity of the muscle (Abe et al., 2000; Kumagai et al., 2000; Blazevich, 2006). Greater θ allows a greater amount of contractile tissue to attach to a given area of tendon or aponeurosis within a cross-sectional area and predicts the maximal capacity for force production (Kawakami et al., 1993; Fukunaga et al., 1997; Bamman et al., 2000; Lieber and Friden, 2001; Blazevich and Sharp, 2005). Muscle thickness correlates to muscle cross-sectional area which is proportional to the number of sarcomeres in-parallel, influencing maximal force production (Lieber and Friden, 2000; Narici et al., 2016). Consequently, architectural adaptations following specific modes of training have become an area of interest.

Isotonic skeletal muscle actions are defined by changing muscle length whilst tension remains unchanged (Hill, 1925). There are two basic types of isotonic actions (concentric and eccentric). During concentric action, muscle tension is increased to meet resistance then remains stable as the muscle shortens, generating force *via* the tendon complex which results in joint movement (Padulo et al., 2013). Eccentric muscle action occurs when a force applied to the muscle exceeds the force produced by the muscle itself, resulting in a lengthening action of the muscle-tendon complex and absorption of mechanical energy which is either lost as heat or stored as elastic potential energy for subsequent concentric actions (Lindstedt et al., 2001; LaStayo et al., 2003). Overall, eccentric actions can produce greater muscle force (Hortobagyi and Katch, 1990) with a lower metabolic energy and oxygen cost (Abbott et al., 1952; LaStayo et al., 2003). Training interventions that selectively assigned participants to eccentric or concentric actions resulted in different morphological adaptations in muscle architecture (Franchi et al., 2014, 2015, 2017; Hoppeler, 2016; Kruse et al., 2021). Authors of a recent narrative review concluded that concentric training might increase muscle length due to the addition of sarcomeres in-parallel within the muscle fibers, whereas eccentric training may result in longer fascicles and thus longitudinal muscle growth due to the addition of sarcomeres in-series (Kruse et al., 2021). The intensity of training and the length range at which the muscle is active is also thought to influence muscle architecture adaptations, however, the mechanism and degree to which this occurs are not clear (Kruse et al., 2021). Interpretation of findings from past studies should follow the principle of specificity, meaning that the training responses described are tightly coupled to the muscle group and the muscle fibers that have been recruited during the training.

Training adaptations to gastrocnemius muscle architecture have been explored previously due to its contribution to the functional demands of locomotion as part of the triceps surae muscle group, as well as the convenience and ease of examination under ultrasound. The gastrocnemius medialis (GM) and gastrocnemius lateralis (GL), along with the soleus, are the greatest contributors to propulsion for walking and running in mid to late stance (Sasaki and Neptune, 2005; Hamner et al., 2010), ankle power release required in jumping (Bobbert et al., 1986; Kurokawa et al., 2001), and the support of body weight during landing (Farris et al., 2016). Highly trained athletes who repeat specific movement patterns might have specific muscle architecture profiles related to the physical requirement of their training (Savelberg and Meijer, 1985; Brughelli et al., 2010). Alongside the vastus medialis and vastus lateralis (VL), the GM and GL are the most activated muscles during cycling on a bicycle (Ericson et al., 1985). Athletes specialized in conventional cycling, where the gastrocnemius is predominantly activated concentrically (Ericson et al., 1985; Bijker et al., 2002), could have structural adaptations different to locomotive sports athletes. Basketball is a locomotive sport where the gastrocnemius muscle of an athlete is activated eccentrically as well as concentrically, at different joint angles and muscle lengths during running, sprinting, accelerating, sudden stopping, changing direction, jumping, and landing (Savelberg and Meijer, 1985; Vogt and Hoppeler, 1985; Ben Abdelkrim et al., 2007; Ullrich and Brueggemann, 2008). Throughout locomotion, eccentric actions of the gastrocnemius muscles support the weight of the body against gravity particularly during body deceleration, by generating force while lengthening and exerting a breaking action against downward movement (LaStayo et al., 2003; Gault and Willems, 2013; Isner-Horobeti et al., 2013). The lengthened muscle-tendon system converts absorbed mechanical energy into stored elastic recoil energy for subsequent concentric actions of the gastrocnemius during limb propulsion, which results in less muscle work and energy required in running or plyometric activities such as jumping, landing, or changing direction (Vogt and Hoppeler, 1985; LaStayo et al., 2003; Konow and Roberts, 2015; Farris et al., 2016). During running, the gastrocnemius muscles experience higher loads during a forefoot strike pattern, to attenuate the impact energy associated with eccentrically controlling the ankle dorsiflexion moment stimulus (Gruber et al., 2014; Yong et al., 2020). This eccentric phase to dissipate impact and reduce shock is absent in cycling.

Past studies have examined the effects of dedicated eccentric or concentric training on muscle architecture, but there is a paucity of studies specific to the gastrocnemius muscle which also differ in results. Two studies found that eccentric training increased FL and muscle thickness but had conflicting θ findings (Duclay et al., 2009; Geremia et al., 2019). Another found similar results to previous studies that examined the VL (Franchi et al., 2015, 2018b), reporting that eccentric or concentric training produced similar increases in muscle thickness (English et al., 2014). The comparable increases in muscle thickness between the two types of training could be due to distinct structural adaptations (Franchi et al., 2017), such as greater FL increases following eccentric training, vs. greater θ increases after concentric training (Franchi et al., 2014, 2015, 2018b). However, this adaptation has not been consistently reported in studies of GM. Other studies found no change in GM architecture at all following both types of training (Foure et al., 1985; Raj et al., 2012b). It cannot be assumed that the changes seen in the GM will be consistent with the GL. Whilst they are synergists, these muscles have differences in muscle-tendon anatomy and joint articulation. They receive different activation messages and have different force-generating capacities due to their different architectural properties (Crouzier et al., 2018; Lai et al., 2018). GL is characterized by longer FLs and smaller θs whereas the GM is characterized by shorter FLs and larger θs (Koryak, 1985; Kawakami et al., 1998). Two studies found that shorter FLs are more susceptible to muscle damage caused by eccentric training (Proske and Morgan, 2001; Baroni et al., 2013). Consequently, each muscle should be examined separately due to its specific adaptive responses.

The aim of this study was to explore the differences in muscle architecture of the GM and GL muscles at rest, between two cohorts of athletes; one group of endurance cyclists that train with primarily concentric muscle actions compared to one group of basketballers that train with a combination of eccentric and concentric muscle actions. To account for the effects of mechanical differences that might result from the unilateral forces that apply to jumping athletes who repetitively and forcibly jump off one leg, we assessed muscle architecture in both left and right limbs to ensure that any group differences were not due to habitual unilateral loading associated with limb dominance (Bohm et al., 2015; Bayliss et al., 2016). The authors hypothesized that differences in muscle architecture arrangements between the two groups of athletes would exist for both genders.

MATERIALS AND METHODS

Study Design and Participants

The study group consisted of 44 adult athletes who volunteered to participate in this study and signed written informed consent (**Table 1**).

Participants were screened for eligibility using a questionnaire that covered demographic information such as age, dominant leg, training experience, and competition experience measured in years, current training amount per week, level of competition, and previous history of lower limb injury. Athletes had on average 8.7 ± 5.5 years of experience in their sport and trained at a minimum of at least three times per week, including competition. Participants were excluded if they did not perform their sport at least three times per week; had any history of an acute or chronic lower limb injury within the previous 12 months; or if they had exercised using their calf muscles during the same day prior to the assessment, including jogging, running, sprinting, hopping, skipping, jumping, or performing heel raises. The study was approved by La Trobe University Human Ethics Committee (Application Number: S17–114) and was conducted according to the National Statement on Ethical Conduct in Human Research.

Procedures

Body mass was measured to the nearest 0.1 kg using a calibrated analog floor-scale (Model 762; Seca, Germany); stretch stature was measured to the nearest 0.1 cm using a portable stadiometer (Model 213; Seca; Germany) according to the procedures described by the International Society for the Advancement of Kinanthropometry (Marfell-Jones et al., 2012). The tibial length was estimated using validated regression equations based on stature (Saco-Ledo et al., 2019). Skeletal muscle architecture of the GL and GM muscles at rest was measured using 2-dimensional B-mode ultrasound (LOGIQ V2; GE Healthcare, Australia), with a 38 mm wide linear probe and a standardized frequency of 12–13 MHz.

Each participant lay prone on an examination table and an incline foam wedge was used to support their flexed knee up to 30° to ensure the gastrocnemius muscle was relaxed at the knee joint. A custom splint secured the ankle joint close to 90° where the sole of the foot is perpendicular to the tibia (**Figure 1**). This position was confirmed with a manual goniometer and a position within the range of 85° to 95° was accepted. The sonographer identified the probe site at one-third of the distance from the popliteal crease of the knee to the tip of the medial malleolus for the GM, and the lateral malleolus for the GL (Koryak, 1985; Kawakami et al., 1998; Kurokawa et al., 2001; Legerlotz et al., 2010; Raj et al., 2012a; Cho et al., 2014; Konig et al., 2014). The

TABLE 1 | Participant characteristics (mean ± SD).

		Females			Males		
		BB	**CYC**	**Total**	**BB**	**CYC**	**Total**
	Number	**13**	**10**	**23**	**12**	**9**	**21**
Age	(year)	27 ± 6	24 ± 4	26 ± 6	25 ± 5	24 ± 4	25 ± 5
Body mass	(kg)	76.8 ± 17.7	64.4 ± 9.3	71.4 ± 15.6	91.6 ± 13.7	76.0 ± 9.7	84.9 ± 14.3
Stature	(cm)	174.9 ± 8.1	166.5 ± 5.8	171.3 ± 8.2	192.7 ± 6.5	179.3 ± 5.4	187.0 ± 9.0
Tibial Length	(cm)	39.2 ± 2.6	37.7 ± 2.2	38.1 ± 2.8	44.2 ± 5.0	41.0 ± 2.1	42.8 ± 4.2
Training experience	(years)	11.4 ± 4.4	5.6 ± 5.7	8.9 ± 5.7	8.3 ± 5.4	8.8 ± 5.9	8.5 ± 5.5
Compete ≥ state level	(%)	100%	50%	78%	100%	55%	81%

BB, basketballer; CYC, cyclist.

FIGURE 1 | Position of the lower limb during ultrasonography.

transducer probe was positioned perpendicular to the long axis of the leg, at the midpoint of the GL and GM muscle bellies, found at the center of each muscle halfway between its medial and lateral borders (Koryak, 1985; Kawakami et al., 1998; Kurokawa et al., 2001; Legerlotz et al., 2010; Raj et al., 2012a; Cho et al., 2014; Konig et al., 2014). These sites were chosen as previous studies concluded that this level is where the anatomical cross-sectional area of the muscle is maximal (Fukunaga et al., 1992; Kawakami et al., 1998); it best aligns the image plane with muscle fascicles (Benard et al., 2009; Bolsterlee et al., 2016); there is minimal fascicle curvature at these sites with participants at rest (Raj et al., 2012a). While holding the transducer at a single site on the skin, manipulation, and rotation of the probe around the sagittal-transverse axis was performed by the investigator to ensure the superficial and deep aponeuroses were as parallel as possible and to optimize the visibility of the fascicles as continuous striations from one aponeurosis to the other (Bolsterlee et al., 2016). Care was taken to ensure that minimal compression by the ultrasound probe on the skin occurred, and transmission gel was applied to improve acoustic coupling. Three ultrasound images were captured from each GM and GL at the left and right limb within one session, totaling 12 images per participant. These images were recorded digitally and sent to an external investigator for de-identification and randomization (www.randomizer.org). The de-identified and randomized images were analyzed using novel computer software designed in LabVIEW (version 16; National Instruments, USA). One investigator performed the digitization of the blinded images, and from each image, three fascicles, three θs, and a single measure of muscle thickness were calculated. **Figure 2**, panel A shows the points of interest plotted during manual digitization. As three images were taken per muscle, this gave a total of nine FLs, nine θs, and three measures of muscle thickness for each muscle belly. From there, the average was calculated, and this final measure was used in the statistical analysis. Furthermore, the ratio between FL (cm) to tibial length (m) (FL/TL), and the ratio between muscle thickness (cm) to tibial length (m) (MT/TL), were used to explore any effects of scaling on stature differences between the two comparison groups and normalize architectural measurements to limb length (Kubo et al., 2003).

Intra-rater test-retest reliability analysis for this method of digitization using a single investigator was performed prior to the study with 100 images digitized on two separate occasions and showed excellent test-retest reliability with an ICC of 0.99 (95% CI: 0.98, 0.99, $p < 0.001$) for FL, and 0.99 (95% CI: 0.98, 0.99, $p < 0.001$) for θ (May et al., 2021).

Statistical Analysis

All data were assessed for normality using the Shapiro-Wilk test, and data were normally distributed.

To explore our first hypothesis, a mixed-model ANOVA (within factors: athletic group and limb; between factor: gender) was conducted to examine the effect of gender (male vs. female), group (basketballer vs. cyclist), and limb (left vs. right) on absolute muscle architecture values and relative to tibial length values. Where there was a significant three-way or two-way interaction effect, simple main effects were evaluated by limb, gender, and group. Pearson Product Moment correlation was

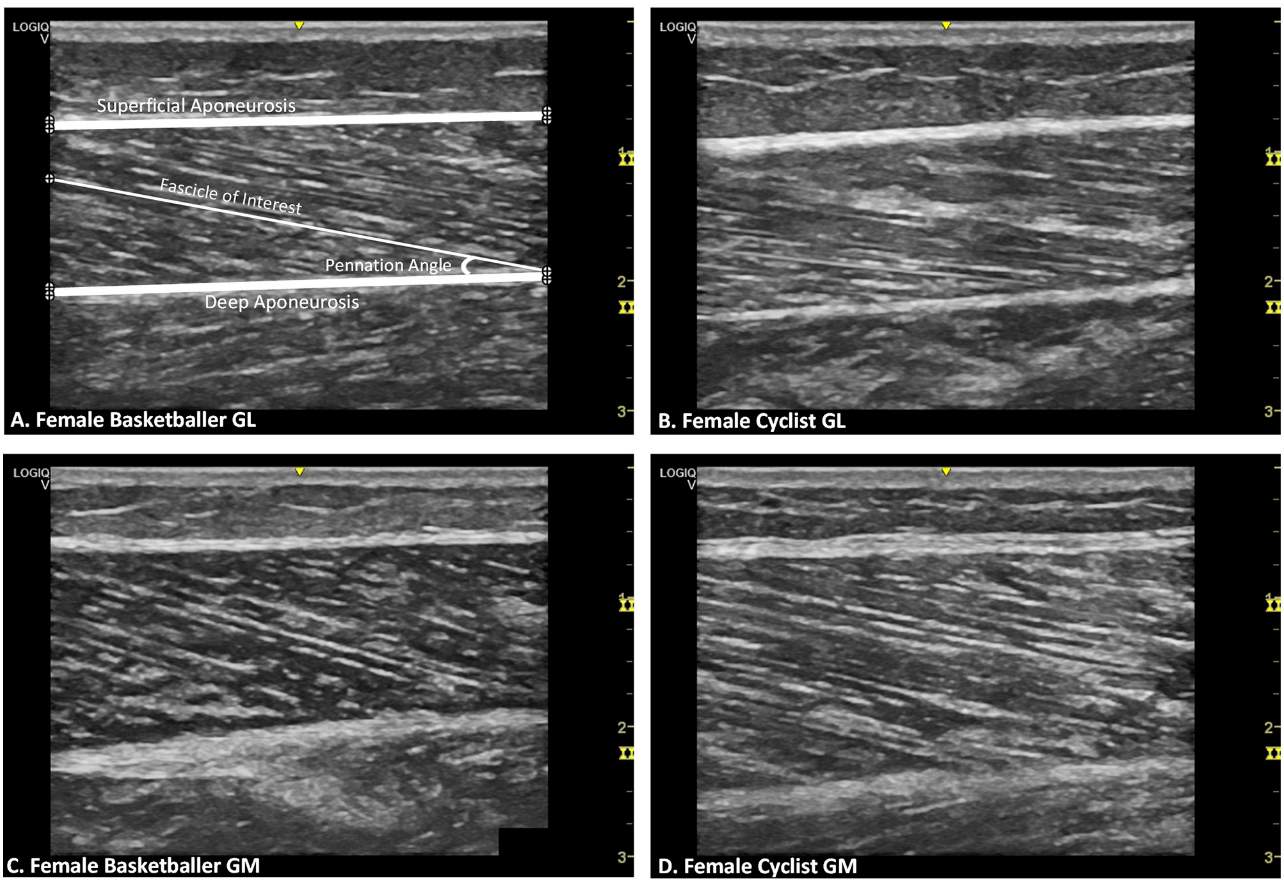

FIGURE 2 | Representative B-mode ultrasound images for female athletes by muscle and training group. **(A)** Gastrocnemius lateralis (GL) of female basketballer Points of interest plotted on the image (⊕) are for superficial aponeurosis borders, deep aponeurosis borders, and a selected fascicle length with the corresponding pennation angle. **(B)** GL of female cyclist. **(C)** Gastrocnemius medialis (GM) of female basketballer. **(D)** GM of female cyclist.

performed to explore relationships between age, body mass, stature, and training years with FL, θ, and muscle thickness, respectively. Statistical analyses were performed using IBM SPSS version 25 (Armonk, NY: IBM Corp., USA). Group data are presented as mean ± standard deviation (*SD*) and statistical significance was set at $p \leq 0.05$.

RESULTS

Table 2 displays muscle architecture data for basketballers and cyclists by gender and limb. **Figure 2** provides representative ultrasound images of female GM and GL muscles for both athlete groups.

Fascicle Length

For GL, significant two-way interaction effects existed for both absolute FL and FL/TL ($F_{(1, 40)} \geq 7.586$, $p \leq 0.009$). In the female group, cyclists had longer absolute FL compared to basketballers with a difference of 22% for both left and right limbs ($p \leq 0.019$; **Figure 3**). Relative to tibial length, the intergroup differences for the left and right limbs were 27 and 30%, respectively ($p \leq 0.015$; **Table 2**).

Two-way interactions were significant for both absolute FL and FT/TL in GM ($F_{(1, 39)} \geq 9.971$, $p \leq 0.003$). Female cyclists had 19% longer FL in the left limb ($p = 0.001$), and 20% longer FL in the right limb ($p = 0.007$; **Figure 3**). This pattern remained for FL/TL ratio comparisons, with the difference increasing to 26% on both sides ($p \leq 0.006$; **Table 2**).

There were no statistically significant differences in FL or FL/TL between groups in male athletes for either GM or GL (**Table 2**).

Pennation Angle

For GM, female basketballers had left limb θ mean ± SD values of 19.5° ± 1.5°, and right limb θs of 19.5° ± 0.8°, which were 11% and 10% greater than the cyclists who had left limb θs of 17.4° ± 1.8°, and right θs of 17.7° ± 1.3° ($p \leq 0.05$; **Table 2**). For GL, basketballers had greater θs by 16% on the left and 20% on the right ($p \leq 0.003$; **Table 2**).

There were no significant differences in θ values for both the GL and GM muscles between athletic groups in the males (**Table 2**).

Muscle Thickness

For GL and GM, significant two-way interaction effects existed ($F_{(1, 39)} \geq 4.298$, $p \leq 0.045$).

Male basketballers had a greater absolute muscle thickness in GL than cyclists by 17% for the left limb ($p = 0.011$) and 20% for the right ($p = 0.012$; **Table 2**), but no significant differences were seen for the GM. The ratio of MT/TL for GL and GM was not significantly greater in male basketballers compared to cyclists. The GM absolute muscle thickness and MT/TL ratio were significantly greater in female cyclists compared to female

TABLE 2 | Summary of gastrocnemius architectural data in basketballers and cyclists (mean ± SD).

		Gastrocnemius lateralis			Gastrocnemius medialis		
		Basketballers	**Cyclists**	**Difference MD (95% CI)**	**Basketballers**	**Cyclists**	**Difference MD (95% CI)**
Fascicle length (mm)	Male, Left	74.2 ± 7.2	65.3 ± 16.0	8.9 (−6.5–24.3)	59.6 ± 12.7	52.8 ± 7.4	6.8 (−2.5–16.1)
	Male, Right	82.1 ± 14.6	75.2 ± 16.8	6.9 (−7.4–21.3)	60.5 ± 13.6	53.2 ± 8.7	7.3 (−3.6–18.2)
	Female, Left	66.1 ± 13.0	82.5 ± 17.8	16.3 (3.0–29.6)*	52.6 ± 6.6	63.8 ± 10.5	11.2 (3.5–18.9)*
	Female, Right	64.6 ± 10.9	80.9 ± 14.2	16.3 (5.4–27.2)*	51.3 ± 1.6	62.6 ± 8.7	11.3 (4.9–17.6)*
FL/TL (ratio: cm/m)	Male, Left	16.8 ± 3.2	16.4 ± 4.2	0.8 (−2.6–4.1)	13.5 ± 2.5	12.9 ± 1.8	0.6 (−1.5–2.7)
	Male, Right	18.7 ± 3.1	18.4 ± 4.2	0.3 (−3.1–3.6)	13.7 ± 2.3	13.0 ± 2.3	0.6 (−1.5–2.8)
	Female, Left	16.8 ± 2.9	22.0 ± 5.2	5.2 (1.2–9.1)*	13.6 ± 1.6	17.6 ± 3.5	4.0 (1.4–6.6)*
	Female, Right	16.5 ± 2.6	22.3 ± 4.9	5.8 (2.1–9.5)*	13.2 ± 1.3	17.2 ± 3.1	4.0 (1.7–6.2)*
Pennation Angle (°)	Male, Left	14.5 ± 2.7	12.7 ± 3.4	1.9 (−0.9–4.7)	23.6 ± 3.8	23.2 ± 3.5	0.5 (−2.9–3.9)
	Male, Right	12.5 ± 2.3	11.8 ± 2.7	0.7 (−1.6–3.0)	21.0 ± 3.7	22.8 ± 4.0	1.8 (−1.7–5.4)
	Female, Left	11.7 ± 1.7	10.0 ± 1.6	1.7 (0.7–2.6)*	19.5 ± 0.8	17.4 ± 1.8	2.1 (0.1–4.1)*
	Female, Right	12.4 ± 0.5	10.1 ± 1.5	2.3 (0.9–3.7)*	19.5 ± 1.5	17.7 ± 1.3	1.8 (0.6–3.1)*
Muscle thickness (mm)	Male, Left	15.6 ± 2.6	13.1 ± 1.3	2.5 (0.7–4.3)*	21.0 ± 3.0	19.0 ± 2.7	2.1 (−0.6–4.7)
	Male, Right	16.2 ± 2.4	13.6 ± 1.7	2.6 (0.6–5.7)*	20.1 ± 2.5	19.5 ± 2.9	0.6 (−1.8–3.1)
	Female, Left	11.9 ± 1.8	12.4 ± 1.7	1.0 (−0.6–2.5)	15.9 ± 2.2	18.0 ± 2.8	2.1 (0.1–4.2)*
	Female, Right	12.6 ± 2.2	12.7 ± 2.2	0.0 (−1.9–1.9)	16.0 ± 2.0	18.5 ± 3.2	2.5 (0.2–4.8)*
MT/TL (ratio: cm/m)	Male, Left	3.6 ± 0.6	3.2 ± 0.4	0.3 (−0.2–0.8)	4.8 ± 0.7	4.7 ± 0.7	0.1 (−0.5–0.8)
	Male, Right	3.7 ± 0.4	3.3 ± 0.5	0.3 (−0.1–0.8)	4.6 ± 0.4	4.8 ± 0.9	0.2 (−0.5–0.9)
	Female, Left	2.9 ± 0.3	3.4 ± 0.6	0.5 (0.1–0.9)	4.1 ± 0.5	4.9 ± 0.9	0.8 (0.2–1.5)*
	Female, Right	3.2 ± 0.6	3.5 ± 0.8	0.3 (−0.3–0.8)	4.1 ± 0.5	5.1 ± 1.0	0.9 (0.2–1.7)*

*FT/TL, ratio of fascicle length (mm) and tibial length (mm) × 100; MT/TL, ratio of muscle thickness (mm) and tibial length (mm) × 100; MD (95% CI), mean difference between athletes with 95% confidence intervals; *p < 0.05, significantly different.*

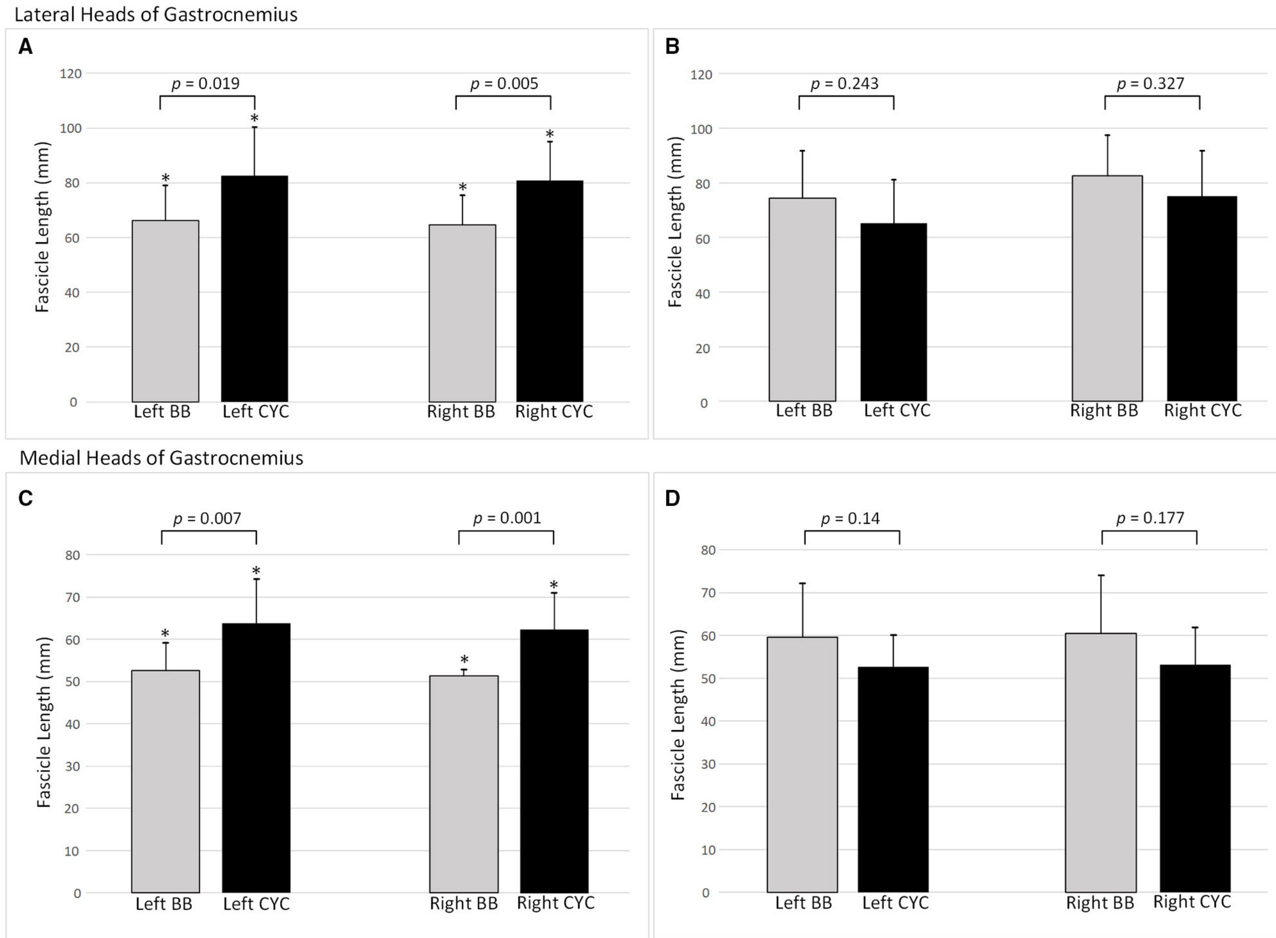

FIGURE 3 | Fascicle lengths by training group and limb side. Mean ± *SD*. **(A)** Female lateral heads of gastrocnemius fascicle lengths (FLs); **(B)** Male lateral heads of gastrocnemius FLs; **(C)** Female medial heads of gastrocnemius FLs; **(D)** Male medial heads of gastrocnemius FLs. BB, basketballer; CYC, cyclist; *p-value indicates statistical significance.

basketballers ($p \leq 0.037$; **Table 2**). No significant differences were observed in absolute muscle thickness and MT/TL ratio in the GL of female athletes.

Correlations

For all athletes, there were no significant bivariate correlations between age or training time in years, with FL, θ, or muscle thickness for the GL or the GM.

For the GL, moderate correlations existed between body mass and FL in both right and left limbs for males ($r \geq 0.531$, $p \leq 0.013$), and between body mass and muscle thickness ($r \geq 0.435$, $p \leq 0.048$), but not for θ. Moderate correlations existed between stature and muscle thickness for males ($r \geq 0.529$, $p \leq 0.014$), but not for FL or θ. No significant correlations existed for GL in the female athletes.

For the GM, moderate correlations existed between body mass and FL in both right and left limbs for males ($r \geq 0.471$, $p = 0.031$) but not for female athletes. A moderate correlation existed between body mass and muscle thickness in the right and left limbs of males ($r \geq 0.468$, $p \leq 0.032$). No significant correlations existed between body mass and θ in males and females. Between stature and FL, a moderate correlation existed for males ($r \geq 0.419$, $p \leq 0.059$) but not females, and there was no significant correlation between stature and θ, or between stature and muscle thickness.

DISCUSSION

In comparison with female basketball players, female cyclists had longer FL and smaller θ at the mid-belly in GM and GL as well as greater muscle thickness in the GM. These differences remained significant when FL and muscle thickness were normalized relative to tibial length. In males, differences in FL, θ, and muscle thickness of GM between cyclists and basketballers were not evident, and differences in GL muscle thickness were not significant when normalized relative to tibial length. Although differences can exist in the muscle architecture of GM and GL between athletic populations, the disparity in findings between genders suggests that long-term exposure to sports-specific training does not systematically influence GM or GL muscle architecture.

To our knowledge, this is the first study to compare the architecture of GM and GL in trained female and male cyclists and basketballers. The GM FL in female basketballers was similar in length to findings of a previous cross-sectional study that examined the GM architecture of female volleyball players. With an average FL of 47.4 mm (Donti et al., 2019), the volleyball players had FLs that were in similar lengths to the basketballers and shorter than the cyclists in our study. Volleyball is a sport that shares some common physical requirements as basketball as it includes a mixture of eccentric and concentric training methods, which include explosive jumping, landing, acceleration, agility, and periodic resistance training. Another cross-sectional study identified that female sprinters had FL of 74.4 ± 10.7 mm for the GL and 59.2 ± 7.7 mm for the GM, which were significantly longer than the study control group (62.6 ± 8.7 and 55.2 ± 6 mm for GL and GM, respectively) (Abe et al., 2001). Longer fascicles are thought to improve sprint performance due to increased contraction shortening velocity (Abe et al., 2000; Kumagai et al., 2000). However, the female cyclists in this study, who engaged in endurance training with the concentric activity of their gastrocnemius, had FLs that were consistently longer than the female basketballers, and these differences in FL were maintained when FL was presented as a ratio of tibial length (FL/TL). Interestingly, the female cyclists in the current study had similar anthropometric characteristics (stature: 163 ± 6 cm; tibial length: 37.9 ± 2.6 cm; body mass: 54.9 ± 4.9 kg) as the female sprinters in the aforementioned study (Abe et al., 2001).

In males, no significant differences were identified in FL or FL/TL of GM and GL between cyclists and basketballers. Lee et al. (Lee et al., 2021) explored architectural differences between sprint cyclists and endurance cyclists and found no significant differences in FL for both GM and GL. For GL, the mean FLs of the male athletes in this study were more closely matched to male sprinters from previous studies (GL: 80.4 ± 14.7 mm) than male endurance cyclists (GL: 54.7 ± 11.8 mm), male sprint cyclists (GL: 54.5 ± 19.1 mm) and male endurance runners (GL: 62.3 ± 10.7 mm) of a similar age (Abe et al., 2000; Lee et al., 2021). Although it is possible that the intermittent nature of basketball might explain the similarity in FL between basketballers and sprinters, FL in the male endurance cyclists was not different from the basketballers in this study. Furthermore, the mean FL of the GM of the male athletes was more closely matched to male endurance runners (GM: 53.6 ± 7.2 mm) (Abe et al., 2000), endurance cyclists (GM: 56.2 ± 7.7 mm), and sprint cyclists (GM: 57.2 ± 11.5 mm) (Lee et al., 2021) compared to male sprinters (GM: 66.4 ± 13.2 mm) (Abe et al., 2000). The lack of consistency in the differences between cycling and basketball athletes in FL and FL/TL across genders suggests that different muscle activation modes in select sports are unlikely to systematically influence FL.

This study found that female cyclists had thicker muscles in the GM, and there were no significant differences in the GM thickness of male athletes. Past studies have reported significant links between muscle thickness and muscle strength (Abe et al., 2001), with longitudinal studies supporting increased muscle thickness correlating to greater power output as seen in the anterior thigh and gastrocnemius muscles of sprinters (Abe et al., 2000). Specific to the gastrocnemius, Lee et al. (Lee et al., 2021) reported that GM muscle thickness was significantly greater in male sprint cyclists (GM: 20.9 ± 4.1 mm) compared to endurance cyclists (GM: 15.9 ± 3.2 mm) and that this was correlated to cycling power over 20 s. The study of Abe et al. (Abe et al., 2000) found GM thickness of male sprinters (GM: 23.9 ± 3.4 mm), was significantly greater than endurance runners (GM: 21 ± 2.4 mm). In basketball games where physical demands are intermittent and high intensity, ~10% of movements are short sprints up to 20 m (McInnes et al., 1995). The male sprinters from the previous study did have thicker gastrocnemius muscles than the male basketball players in this study, and male sprint cyclists from the previous study did have thicker gastrocnemius muscles compared to the male endurance cyclists analyzed by this study, however, we did not determine a difference in GM adaptive responses to training between male basketballers and endurance cyclists. The inconsistency in adaptive responses for the GM between genders confirms that muscle hypertrophy can be equally associated with both concentric only and concentric/eccentric training, a finding supported by previous studies (Seger et al., 1998; English et al., 2014; Franchi et al., 2014, 2015, 2017). For the GL, no significant differences in GL thickness between female basketballers and cyclists existed. For males, although basketballers had significantly thicker GL muscles, the difference in the GL normalized once standardized against tibial length, which suggests that muscle thickness can be proportional to stature. Moderate-strength correlations have been reported between stature and body mass with both FL and muscle thickness. Accounting for stature differences with scaling or attempts to match groups for stature and body mass should be performed in future studies (Kubo et al., 2003; Morse et al., 2005). Unlike the GM, the study of Lee et al. (Lee et al., 2021) found no significant differences in GL thickness between male sprint cyclists (GL: 13 ± 2.6 mm) and endurance cyclists (GL: 13.7 ± 5.2 mm). The work of Geremia et al. (Geremia et al., 2019) also found that the GL muscle showed the smallest adaptability to eccentric training compared to GM and soleus, the calf synergists. This confirms that adaptive responses to the GM and the GL are likely to be muscle-specific. Greater micro-damage to GM could occur following either type of concentric or eccentric/concentric training. The GM and GL should be separately studied when determining the effects of training (Koryak, 1985; Kawakami et al., 1998).

Regarding θ, the pattern of results in our male population supports previous findings that hypertrophied muscles have greater θ (Kawakami et al., 1993) as seen particularly in sprinters. However, the differences in θ between male basketballers and cyclists were not significant for either the GM or GL. Interestingly, the male θ for the GM was closer to sprint (20.3 ± 3.7°) than endurance (17.2 ± 2.8°) cyclists from a previous study (Abe et al., 2000), and like our study, found no difference in GL angle between the two groups. Paradoxically, this study found that female cyclists had smaller θ with thicker muscles, and this was significantly different to female basketballers. Compared to female sprinters, female basketball players had narrower muscles compared to sprinters (21.2 mm in GM and 16.9 mm in GL) but had similar θ to sprinters (21.1° in the GM and 13.1° in the GL) (Abe et al., 2001). Overall, this study found that θ does not appear to be systematically influenced by sports-specific training, nor systematically related to muscle thickness. Past longitudinal study reports of θ adaptations following short-term periods of resistance training in various muscle groups have also been conflicting. Some studies have shown greater increases in θ with concentric-only (Franchi et al., 2014, 2015, 2018b), or conventional concentric and eccentric resistance training (Reeves et al., 2009), others with eccentric-only training (Duclay et al., 2009), and some finding equally increased θs following both types of muscle actions (Blazevich et al., 1985) or no change at all (Raj et al., 2012b; Baroni et al., 2013). This may be due to the lower reliability of θ measurements using ultrasound compared to FL or muscle thickness (Klimstra et al., 2006; Baroni et al., 2013; Geremia et al., 2019).

Ultrasound is an operator-dependent tool, and potential sources of measurement error stem from probe placement or location, probe pressure, and probe orientation (Klimstra et al., 2006; Konig et al., 2014), which can lead to over- or underestimation of θ (Benard et al., 2009; Konig et al., 2014). The best practice is to place the probe parallel to the plane of the fascicle, however past studies have found that small manipulations of the orientation and rotation of the ultrasound probe can result in a 12% difference (13.6–15.5°) in the θ reported, and this measurement error is close to the reported adaptive response of θ to resistance training (Klimstra et al., 2006; Baroni et al., 2013; Geremia et al., 2019). To overcome limitations with ultrasonography use, this study used a single skilled sonographer who took care with applied pressure during probe placement to avoid potential tissue deformation (Konig et al., 2014). Probe placement was manually adjusted to the sagittal plane of the muscle to obtain the clearest possible image in the plane parallel to at least three fascicles (Loram et al., 1985; Narici et al., 1996; Morse et al., 2005; Kanehisa et al., 2006; Klimstra et al., 2006; Benard et al., 2009; Konig et al., 2014; Bolsterlee et al., 2016). Scans were obtained at an ankle angle of ∼90° at rest using a cast to passively maintain the position during scanning and to match the position in other participants. Intra-rater, between image and within-session reliability analysis, was excellent (May et al., 2021) and supports that this protocol can examine group differences for both the GM and GL.

Additional limitations of the study include the relatively small sample size, as high inter-individual variation can exist in muscle architecture. The observations of this study are only valid for gastrocnemius muscles. It may be more useful to explore adaptations in muscles that are more greatly activated, such as the quadriceps which is the primary muscle group for cycling (Ericson et al., 1985; Ryan and Gregor, 1992). As bi-articular muscles spanning two joints, the gastrocnemius medialis and lateralis length changes are dependent upon both the knee and ankle joints (Kawakami et al., 1998). Foot position and changing saddle height can result in increased ankle plantarflexion and knee extension (Ercison et al., 1988; Turpin and Watier, 2020). In this study, factors such as cyclist seat height, ankle or knee joint positions, and range, and participation in additional exercise requiring gastrocnemius activity such as walking, were not explored as covariates that are very likely to be highly variable between participants. The effects of these factors on resulting morphological adaptations cannot be separately identified in this cross-sectional study. Cross-sectional studies that compare populations with different training backgrounds are useful to evaluate the association between variables but are unable to determine cause and effect relationships. The genetic muscle morphology patterns of an athlete could have been suited to their respective sports from the outset, or muscles could have adapted over long-term training to become more suitable. Furthermore, the male and female groups of basketballers in this study exceeded the cyclists in terms of the level of competition, and training frequency per week. Unlike the males, many female basketballers had participated in other sports at a competitive level in their history, even though they had ceased well before this study. Whilst it remains possible that the results observed may reflect training history, it cannot be concluded that long-term sports-specific training systematically influences muscle morphology. Future research investigating the influence of sport-specific training on muscle architecture should compare athlete groups with similar training backgrounds, in addition to anthropometrics and age. Following the recent narrative review that concluded that the muscle length at which training is performed and intensity of training may be important factors that influence adaptations (Kruse et al., 2021), it is recommended that these be observed or directly controlled in future studies. Longitudinal studies might provide useful information in addition to cross-sectional comparisons, particularly if muscle architecture could be measured at baseline when the athlete first begins high-level training in their selected sport.

In conclusion, participation in selected competitive sports was associated with differences in gastrocnemius medialis and lateralis muscle architecture in female athletes; however, participation in concentric dominant muscle action, or a combination of concentric and eccentric muscle action, did not systematically influence gastrocnemius muscle architecture. Further research is required to improve the understanding of architectural adaptations in response to training muscle architecture in all regions of the skeletal muscle system.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Open At La Trobe; 2021. https://doi.org/10.26181/612f0b7259195.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by La Trobe University Human Research Ethics Committee. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SM, SL, and MK contributed to the conceptualization and design of the study. MK contributed to resources and software, and alongside SL contributed to supervision. SM performed the investigations and data curation. Together, SM and MK contributed to formal analysis and validation. SM prepared the original draft manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Mechanical, Cardiorespiratory, and Muscular Oxygenation Responses to Sprint Interval Exercises Under Different Hypoxic Conditions in Healthy Moderately Trained Men

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Objective: The aim of this study was to determine the effects of sprint interval exercises (SIT) conducted under different conditions (hypoxia and blood flow restriction [BFR]) on mechanical, cardiorespiratory, and muscular O_2 extraction responses.

Methods: For this purpose, 13 healthy moderately trained men completed five bouts of 30 s all-out exercises interspaced by 4 min resting periods with lower limb bilateral BFR at 60% of the femoral artery occlusive pressure (BFR_{60}) during the first 2 min of recovery, with gravity-induced BFR (pedaling in supine position; G-BFR), in a hypoxic chamber ($FiO_2 \approx 13\%$; HYP) or without additional stress (NOR). Peak and average power, time to achieve peak power, rating of perceived exertion (RPE), and a fatigue index (FI) were analyzed. Gas exchanges and muscular oxygenation were measured by metabolic cart and NIRS, respectively. Heart rate (HR) and peripheral oxygen saturation (SpO_2) were continuously recorded.

Results: Regarding mechanical responses, peak and average power decreased after each sprint ($p < 0.001$) excepting between sprints four and five. Time to reach peak power increased between the three first sprints and sprint number five ($p < 0.001$). RPE increased throughout the exercises ($p < 0.001$). Of note, peak and average power, time to achieve peak power and RPE were lower in G-BFR ($p < 0.001$). Results also showed that SpO_2 decreased in the last sprints for all the conditions and was lower for HYP ($p < 0.001$). In addition, $\Delta[O_2Hb]$ increased in the last two sprints ($p < 0.001$). Concerning cardiorespiratory parameters, BFR_{60} application induced a decrease in gas exchange rates, which increased after its release compared to the other conditions ($p < 0.001$). Moreover, muscle blood concentration was higher for BFR_{60} ($p < 0.001$). Importantly, average and peak oxygen consumption and muscular oxyhemoglobin availability during sprints decreased for HYP ($p < 0.001$). Finally, the tissue saturation index was lower in G-BFR.

Conclusions: Thus, SIT associated with G-BFR displayed lower mechanical, cardiorespiratory responses, and skeletal muscle oxygenation than the other conditions.

Exercise with BFR_{60} promotes higher blood accumulation within working muscles, suggesting that BFR_{60} may additionally affect cellular stress. In addition, HYP and G-BFR induced local hypoxia with higher levels for G-BFR when considering both exercise bouts and recovery periods.

Keywords: blood flow restriction (BFR), exhaustive exercise, hypoxia, oxygen extraction, skeletal muscle, supine exercise, gravity-induced blood flow restriction, vascular occlusion

INTRODUCTION

Physical exercise promotes the modulation of a large panel of cellular signaling pathways to promote metabolic and/or morphological changes that enhance performance (Bishop et al., 2019; Sanchez et al., 2019; Solsona et al., 2021). In recent years, repetition of short (≤30 s) all-out exercises were boosted in popularity because they can induce similar or higher gains of performance for a lower training volume compared to conventional endurance protocols (MacDougall et al., 1998; Barnett et al., 2004; Burgomaster et al., 2006, 2008; Gibala et al., 2006). Two distinct repeated sprint protocols are distinguished. Repeated sprint training (RST) consists of maximal sprint exercises of short duration (≤10 s) interspaced with short recovery periods (i.e., exercise to rest ratio < 1:4). On the other hand, sprint interval training (SIT) includes repeated long sprints (∼30 s) interspaced by longer rest periods (∼2–4 min) (Buchheit and Laursen, 2013a,b; Brocherie et al., 2017). In recent years, studies have suggested that the addition of a hypoxic stimulus to chronic SIT and RST may favor several training responses (Faiss et al., 2013; Brocherie et al., 2017; Brechbuhl et al., 2020; James and Girard, 2020). For example, systemic hypoxia (HYP) may promote higher muscle perfusion and oxygenation with greater modulations of molecular adaptations (Faiss et al., 2013; Brocherie et al., 2017). These changes include increases in HIF-1α, myoglobin, and the expression of genes involved in mitochondrial biogenesis.

In addition to HYP, blood flow restriction (BFR) training strategies can also favor several training adaptations (Preobrazenski et al., 2021). BFR results in reduced arterial and/or venous blood flow according to the level of pressure exerted, thus generating local hypoxia and an accumulation of metabolites in the working muscles (Sugaya et al., 2011; Teixeira et al., 2018; Okita et al., 2019). BFR exercise training was found to be beneficial for enhancing adaptations such as muscle hypertrophy and aerobic capacity (Preobrazenski et al., 2021). Acute exercise with BFR may increase neuromuscular activation and type II muscle fibers recruitment (Moritani et al., 1992). In addition, BFR aerobic exercise stimulates ventilatory and cardiac response through the stimulation of group III and IV afferents (Adreani and Kaufman, 1998). These acute responses result in increased energy expenditure leading to a loss of muscle efficiency during submaximal exercise (Ozaki et al., 2010; Mendonca et al., 2014; Silva et al., 2019). However, to the best of our knowledge, BFR studies were conducted with low-intensity exercises or during RST (Willis et al., 2019), and little is known about SIT protocols using BFR.

Furthermore, different BFR models, such as cuff-, pressure chamber-, and gravity-models rise in popularity, and studies are needed to highlight their specific effects and related adaptations. Recently, a gravity-induced BFR (G-BFR) aerobic protocol has been investigated in a study conducted by Preobrazenski and colleagues (Preobrazenski et al., 2020). An ergocycle titled 45° was used to generate ischemia within working muscles. In this study, muscle oxygenation was effectively found to be reduced during submaximal aerobic exercises, as previously indicated during supine exercise where faster O_2 uptake kinetics was also observed (Hughson et al., 1991, 1993). Of note, Preobrazenski et al. suggested that the G-BFR model seems favorable to enhance aerobic adaptations (Preobrazenski et al., 2020). However, nothing is known about the use of such a protocol during SIT. Importantly, to our knowledge, the acute effects of HYP and BFR models on both cardiovascular and muscular responses have never been compared during SIT protocols. It appears important to understand the acute effects of these models to identify potential training adaptations for the efficient prescription of training strategies.

Thus, the goal of this study was to assess the effects of each condition on mechanical output, cardiorespiratory responses, and muscle oxygenation in healthy moderately trained men. In the present study, it has been chosen to set maximal stress for both HYP and BFR conditions that could be tolerated in this cohort in combination with the SIT protocol proposed based on preliminary work in our laboratory. Concerning the G-BFR condition, the inclined position was maintained during the entire exercise session. The hypotheses were that we would observe different responses depending on the condition with (i) a higher power output during the successive exercise bouts in NOR since the other conditions induce additional stress that may lead to a reduction of acute muscle efficiency and subsequent performance, (ii) a greater impact of HYP on the cardiorespiratory responses because HYP causes hypoxemia, and (iii) a more pronounced impact of BFR models on muscle oxygenation because they generate local hypoxia.

MATERIALS AND METHODS

Subjects

Thirteen healthy moderately trained men (mean ± SD, age 24 ± 3 years; weight 73.8 ± 6.5 kg; height 179 ± 6 cm; body fat percentage 12.5 ± 2.1%; training frequency 8 ± 4 h per week) participants took part in this experiment. Prior to the first visit, the participants were informed about the experimental procedures and the possible discomforts and risks. The participants provided written informed consent and completed a questionnaire to exclude all potential cardiorespiratory and injury risks. The experimental protocol was approved by the local ethics committee (VD-2021-00597). All experiments were performed in accordance with the last Declaration of Helsinki.

Study Design

Participants visited the laboratory on four occasions over 4 weeks. During the 4 days of experimentation, participants performed an SIT session in different conditions, namely normal condition (NOR), with bilateral limb blood flow restriction at 60% of the total femoral artery pressure (BFR_{60}) during the first 2 min of recovery (Taylor et al., 2016; Mitchell et al., 2019), with G-BFR, and in a hypoxic room at $FiO_2 \approx 13\%$ (HYP). Exercises were performed in a random order established by an independent blinded researcher. Sessions lasted between 90 and 120 min and were separated by at least 5 days to avoid fatigue-related interferences with the exercise sessions. Anthropometric measurements were carried out on the first visit and body fat percentage was estimated with the four skinfold thickness method (Durnin and Womersley, 1974). All tests were performed at the same time of the day to minimize the effects of circadian cycles and within similar environmental conditions. The participants were asked to maintain their dietary habits without alcohol consumption 48 h before each test. Athletes did not take medication or dietary supplements during the studied period. A standardized diet (55% carbohydrate, 15% protein, and 30% fat) was proposed to the participants the day preceding each test.

Determination of Femoral Artery Occlusion Pressure

Total femoral artery occlusion pressure was measured on the day the participant followed the BFR protocol to avoid a potential effect of time and to be more accurate. The participants sat on a chair for the measurement of the total femoral artery occlusion pressure. The cuffs (SC12D, cuff size 13 cm × 85 cm) were placed around the right inferior limb proximal to the hip articulation. The occlusion pressure was progressively increased with the inflation apparatus (E20/AG101 Rapid Cuff Inflation System, D.E Hokanson Inc., Bellevue, WA, United States). The occlusion level was determined with an ultrasound linear probe (EchoWave II 3.4.4, Telemed Medical Systems, Milan, Italy) to measure blood flow. Total occlusion pressure was considered as reached when there was no detectable arterial blood flow. A total of three measurements were taken with a 1 min recovery between each evaluation. The highest-pressure value obtained was used to determine the 60% pressure applied for BFR_{60} during the exercise sessions. Importantly, the cuff pressure of 60% was used following preliminary work performed in the laboratory that highlighted it was the highest level that could be tolerated by the participants in combination with the present SIT protocol.

Exercise Sessions

All testing was performed in a controlled indoor environment with an ergocycle (Lode Excalibur Sport 911905, Lode B.V., Groningen, The Netherlands) programmed on constant torque mode (Wingate mode) with a torque factor of $0.8\,Nm.kg^{-1}$. The warm-up consisted of 10 min of cycling at 100 W (85 rpm) and two 6-s maximal sprints interspaced by a passive recovery of 54 s. Then, after 4 min recovery, the participants completed five bouts of 30 s standing start all-out exercises interspaced by 4 min rest periods. BFR was applied with inflatable cuffs during the first 2 min of recovery after each sprint. Concerning the G-BFR condition, participants maintained their inclined position during exercise bouts and recovery periods. For this condition, a structure was built to allow participants to lay horizontally on their backs as comfortably as possible. The structure also permitted handgrip to avoid body displacements during exercise. HYP was normobaric and was used during the whole session, such as the warm-up. NOR was performed below 400 m of altitude. The configuration (height and length) of both the saddle and handlebars was recorded to be reproduced in subsequent tests. Participants had to maintain saddle contact. They were encouraged energetically to complete every exercise maximally. Verbal indication of time was not provided to minimize pacing strategies during each sprint exercise.

Participants quoted their subjective perception of effort through the 6–20 rating perceived exertion (RPE) scale after each sprint. Individual measurements of peak (P_{peak}), minimal ($P_{\min}$), and average power, time to achieve peak power, and a fatigue index (FI) were collected. FI was calculated as follow for each sprint:

$$FI = \frac{P_{peak} - P_{\min}}{P_{peak}} \times 100$$

During the BFR_{60} condition, the cuffs were placed bilaterally and proximally to the hip articulation. The cuffs were inflated during the first 2 min of recovery. The laboratory where the tests took place was below 400 m of altitude.

Gas Exchanges and Peripheral Oxygen Saturation Measurements

Breath-by-breath gas exchanges and peripheral oxygen saturation (SpO_2) were continuously monitored throughout the exercises and recovery periods. Oxygen consumption ($\dot{V}O_2$), carbon dioxide ($\dot{V}CO_2$) production, and minute ventilation ($\dot{V}E$) were measured with a gas exchange analyzer (Quark CPET, COSMED, Rome, Italy). Tidal and gas volumes (ΣVT, ΣVO_2, and ΣVCO_2) were cumulated for each period (exercise and recoveries). Data were treated using a second-order Butterworth filter with a cutting frequency of 0.1 Hz. Breathing flow was measured by a bi-directional digital turbine that was calibrated using a 3-l syringe (C00600-01-11, Cosmed, Rome, Italy). A known gas mixture (O_2: 15.05%, CO_2: 5.05%) was used to calibrate O_2 and CO_2 analyzers. Heart rate was collected with a Garmin monitor (HRM3-SS, Garmin, Southampton, United Kingdom). Peak and minimal values were determined for these variables during each sprint. Delta values (Δ) were calculated as the absolute difference between peak and minimal values. SpO_2 was continuously recorded with a pulse oximeter (WristOx 3100, Nonin Medical Inc., Amsterdam, The

Netherlands) and the sensor (8000Q2Sensor, Nonin Medical Inc., Amsterdam, The Netherlands) was placed at the earlobe.

NIRS Measurements and Data Assessment

Muscular O_2 extraction measurements were monitored by an absolute near-infrared spectroscopy (NIRS) probe (OxiplexTS, ISS, Champagne, USA). The device was placed on the distal portion of the right vastus lateralis and was held by an elastic band wrapped around it to minimize extraneous light and movement. NIRS device includes four transmitters situated at 2.5, 3.0, 3.5, and 4.0 cm from the receptor. The acquisition frequency was 50 Hz and data were averaged every 1 s. Two different wavelength laser diodes provided the light source (682 and 834 nm), and the differential pathlength factor was set to 4. Oxygen extraction was estimated by the tissue saturation index (TSI) from the NIRS measurement, which also includes total hemoglobin concentration ([tHb]), and concentrations of deoxyhemoglobin ([HHb]), and oxyhemoglobin ([O_2Hb]).

Statistical Analysis

The statistical analyses were performed using Jamovi (Version 1.6.15.0). After inspecting residual plots, no obvious deviations from homoscedasticity or normality were observed. Therefore, linear mixed models were used to be more accurate with the specificity of our experimental design. Indeed, this is a longitudinal approach and linear mixed models (LMM) use mixed effect modeling to provide more precise estimates when data are hierarchized compared to repeated measures ANOVA (Gueorguieva and Krystal, 2004; Boisgontier and Cheval, 2016; Muth et al., 2016). Indeed, LMM have been developed to consider both the nested (i.e., multiple observations within a single participant in a given condition) and crossed structure (i.e., participants observed in multiple situations) of the data (Baayen et al., 2008; Boisgontier and Cheval, 2016). The flexibility of LMM makes them more appropriate for analyses of repeated measures data and when working with missing data or limited samples (Gueorguieva and Krystal, 2004; Boisgontier and Cheval, 2016; Muth et al., 2016). Conditions (i.e., NOR, BFR_{60}, G-BFR, and HYP) and sprint number were the fixed effects, and the participant was set as the random effect. *Post-hoc* comparisons with Holm's corrections for multiple comparisons were used to adjust *p*-values. The level of significance was set at 0.05 and dispersion about the mean was expressed as SD. Effect sizes (d) (Judd et al., 2017) are provided (trivial effect $d < 0.10$, small effect $0.10 \leq d < 0.50$, medium effect $0.50 \leq d < 0.80$ and large effect $d \geq 0.80$).

RESULTS

No interaction between conditions and sprint number was found for any variable ($p > 0.05$).

BFR Pressure, RPE, and SpO_2

The total occlusion pressure of the participants was 191.0 ± 13.9 mmHg and BFR_{60} pressure was 114.6 ± 8.3 mmHg. A significant main effect of the condition ($p < 0.01$) and sprint number ($p < 0.01$) was found on RPE values, with higher values observed for BFR_{60} and HYP compared to G-BFR ($p < 0.01$, $d = 0.2$ for both). No difference was found between NOR and the other conditions for RPE. RPE increased significantly throughout the sprints ($p < 0.05$), except between sprints three and four ($p > 0.05$) (**Figure 1A**). Concerning the average SpO_2, a main effect of the condition was found ($p < 0.01$). *Post-hoc* analyzes revealed that SpO_2 was lower for HYP compared to the other conditions ($p < 0.01$, d = −2.1, −2.5, and −1.9 for BFR_{60}, G-BFR, and NOR, respectively). The values were 91 ± 5, 99 ± 1, 100 ± 1, and 98 ± 2% for HYP, BFR_{60}, G-BFR, and NOR, respectively. Moreover, there was a main effect of the sprint number on SpO_2 ($p < 0.01$). SpO_2 significantly decreased ($p < 0.05$) between the first and the last sprint, and between the second and the last two sprints (**Figure 1B**). Remarkably, BFR_{60} did not induce a reduction of SpO_2 during its application compared to the other conditions ($p > 0.05$).

Peak and Average Power Output, Time to Achieve Peak Power, and Fatigue Index

A main effect of the condition was observed for peak power ($p < 0.01$) with a lower value found for G-BFR compared to NOR ($p < 0.01$, $d = -1.0$). A main effect of the sprint number was also found for peak power that decreased over time ($p < 0.01$) except between the two last sprints ($p > 0.05$). These results are presented in **Figure 2A**. The condition had a main effect on average power ($p < 0.01$) and *post-hoc* analyzes showed that values were significantly lower for G-BFR compared to the other conditions ($p < 0.05$, $d = -0.9$, −0.4, and −0.5 for NOR, HYP, and BFR_{60}, respectively) (**Figure 2B**). The sprint number also had an impact on average power as the main effect was detected ($p < 0.01$). Average power decreased over time ($p < 0.01$) except between the two last sprints ($p > 0.05$). Regarding time to achieve peak power, a main effect of the condition was observed ($p = 0.02$). *Post-hoc* analyzes revealed that the time to reach peak power was significantly greater for G-BFR compared to BFR_{60} and HYP ($p < 0.05$, $d = 1.1$, and 1.0, respectively) (**Figure 2C**). An effect of the sprint number was also detected for the time to achieve peak power ($p < 0.01$) that was longer in the last sprint compared to the first three sprints ($p < 0.01$). Finally, FI did not appear different depending on the condition or the sprint number ($p > 0.05$). These results are shown in **Figure 2D**.

Gas Exchanges

Results are presented in **Table 1**.

During sprints, there was a main effect of the condition on ΣVT ($p < 0.01$). Values were lower for G-BFR compared to the other conditions ($p < 0.01$, $d = -1.2$, −1.2, and −1.0 for NOR, HYP, and BFR_{60}, respectively), and higher in HYP compared to BFR_{60} ($p < 0.05$, $d = 0.19$). A main effect of the condition was observed for $\dot{V}E_{peak}$ ($p < 0.01$), which was lower in G-BFR compared to the other conditions ($p < 0.01$, $d = -1.1$, −1.3, and −0.9 for NOR, HYP, and BFR_{60}, respectively). Values were also lower in BFR_{60} and NOR compared to HYP ($p < 0.05$, $d = -0.4$, and −0.1, respectively).

Oxygen consumption showed a main effect of both conditions ($p < 0.01$) and sprint number ($p < 0.01$). Values were lower in G-BFR compared to the other conditions ($p < 0.01$, $d = -1.0$, −0.7,

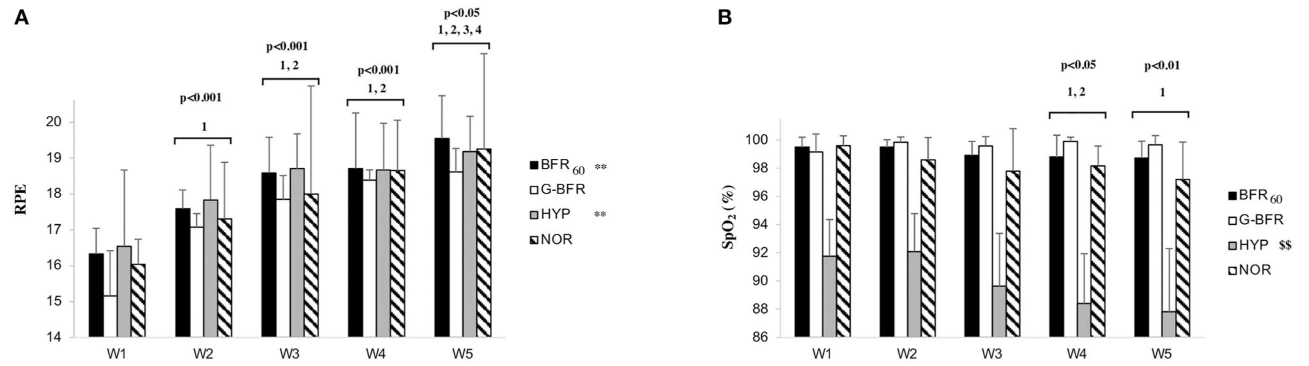

FIGURE 1 | Rating of perceived exertion (RPE) **(A)** and SpO_2 **(B)** values between the different conditions. BFR_{60}, blood flow restriction during the two first minutes of recovery; HYP, hypoxia in a chamber at 13.0% FiO_2; G-BFR, gravity-induced BFR; NOR, control. Differences between the numbers of the sprint are highlighted by numbers and symbols are used for differences between conditions. [1]Different from sprint number one; [2]different from the second sprint; [3]different from the third sprint; [4]different from the fourth sprint, these differences concern the effect of sprint number without distinguishing the exercise modality (n.b., no statistical interaction was found); $^{**}p < 0.01$ different from G-BFR; $^{\$\$}p < 0.01$ different from the other conditions.

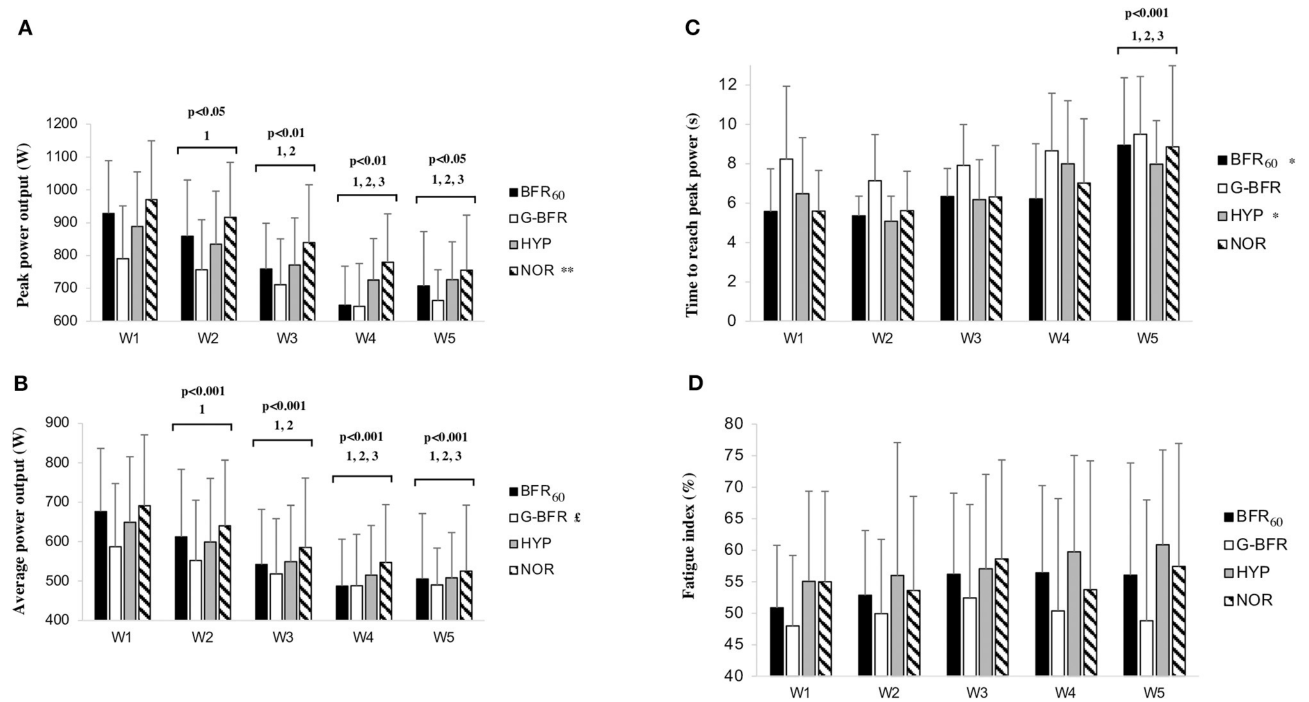

FIGURE 2 | Peak **(A)** and average **(B)** power, time to reach peak power **(C)**, and fatigue index **(D)** between the different conditions. BFR_{60}, blood flow restriction during the two first minutes of recovery; HYP, hypoxia in a chamber at 13.0% FiO_2; G-BFR, gravity-induced BFR; NOR, control. Differences between the numbers of the sprint are highlighted by numbers and symbols are used for differences between conditions. [1]Different from sprint number one; [2]different from the second sprint; [3]different from the third sprint; these differences concern the effect of sprint number without distinguishing the exercise modality (n.b., no statistical interaction was found); $^{*}p < 0.05$ different from G-BFR; $^{**}p < 0.01$ different from G-BFR; £$p < 0.05$ different from the other conditions.

and −0.8 for NOR, HYP, and BFR_{60}, respectively) and in HYP compared to NOR ($p < 0.01$, $d = -0.5$). We found main effects reflecting a difference on $\dot{V}O_{2peak}$ depending on the condition ($p < 0.01$), and the sprint number ($p < 0.01$). Values were lower in HYP compared to all conditions ($p < 0.05$, $d = -1.2$, −0.8, and −0.9 for HYP, BFR_{60}, and G-BFR, respectively), and higher in NOR compared to BFR_{60} and G-BFR ($p < 0.05$, $d = 0.3$, and 0.2, respectively). Regarding the sprint number, it was higher in the second sprint compared to the fourth and fifth ($p < 0.01$): 3932 ± 248, 3740 ± 316, and 3656 ± 298 $ml.min^{-1}$, respectively.

Finally, mean sprint heart rate (HR) presented a condition main effect ($p < 0.01$) and a sprint number main effect ($p < 0.01$). HR was lower in G-BFR ($p < 0.01$, $d = -0.8$) and in BFR_{60} ($p < 0.05$, $d = -0.3$) compared to NOR. HR was also

TABLE 1 | Cardio-respiratory responses during the interventions.

	BFR_{60}	G-BFR	HYP	NOR
Exercise				
ΣVT (L)	58.2 ± 15.2	43.7 ± 13.5‡‡	61.1 ± 15.1‡**	61.0 ± 15.9**
$\dot{V}E_{peak}$ (L.min^{-1})	150.2 ± 30.7	124.8 ± 27.4‡‡	161.9 ± 28.5‡**	157.7 ± 30.5**$
$\dot{V}E_{min}$ (L.min^{-1})	39.3 ± 11.9	29.3 ± 11.2‡‡	36.0 ± 14.3**	38.5 ± 12.4**
$\Delta\dot{V}E$ (L.min^{-1})	111.2 ± 24.3	95.5 ± 20.4‡‡	125.9 ± 24.7‡**	119.2 ± 24.2**$
ΣVO_2 (L)	1.208 ± 0.232	0.994 ± 0.297‡‡	1.154 ± 0.175**	1.243 ± 0.200**$$
$\dot{V}O_{2peak}$ (mL.min^{-1})	3872.9 ± 698.8	3895.4 ± 632.3	3422.0 ± 437.8‡*	4033.7 ± 543.1‡*$
$\dot{V}O_{2min}$ (mL.min^{-1})	663.9 ± 171.4	562.9 ± 144.3‡‡	609.1 ± 199.8	665.5 ± 179.5**
$\Delta\dot{V}O_2$ (mL.min^{-1})	3202.0 ± 637.8	3332.5 ± 589.5	2812.9 ± 388.8‡‡**	3368.2 ± 483.0‡$$
ΣVCO_2 (L)	1.106 ± 0.230	0.874 ± 0.193‡‡	1.015 ± 0.198‡‡**	1.110 ± 0.228**$$
$\dot{V}CO_{2peak}$ (mL.min^{-1})	3105.1 ± 828.8	3109.7 ± 713.5	3046.8 ± 734.8	3241.1 ± 830.0$
$\dot{V}CO_{2min}$ (mL.min^{-1})	835.3 ± 268.6	675.8 ± 215.7‡‡	659.9 ± 229.4‡‡	803.3 ± 244.2**$$
$\Delta\dot{V}CO_2$ (mL.min^{-1})	2269.8 ± 696.4	2433.9 ± 621.6‡	2386.9 ± 680.5	2437.8 ± 717.4‡
HR (bpm)	138.3 ± 14.3	131.4 ± 14.9‡‡	139.9 ± 13.6**	142.2 ± 13.5‡**
HR_{peak} (bpm)	162.1 ± 11.6	156.4 ± 15.1‡	163.0 ± 11.0**	165.8 ± 8.5‡**
HR_{min} (bpm)	113.3 ± 21.2	101.8 ± 20.7‡‡	115.8 ± 21.3**	117.7 ± 21.2**
ΔHR (bpm)	48.8 ± 20.1	54.6 ± 20.5‡‡	47.3 ± 18.3**	48.0 ± 18.7**
Recovery periods (mean values)				
ΣVT (L)	281.1 ± 70.8	239.0 ± 67.0‡‡	308.4 ± 71.3‡‡**	291.9 ± 61.9**$$
ΣVO_2 (L)	5.6 ± 0.9	5.5 ± 0.8	5.9 ± 0.8‡‡**	5.9 ± 0.8‡‡**
ΣVCO_2 (L)	6.2 ± 1.5	6.3 ± 1.3	6.3 ± 1.3	6.6 ± 1.3
HR (bpm)	137.1 ± 16.1	123.6 ± 18.6‡	139.5 ± 18.1**	139.2 ± 16.4**
HR_{peak} (bpm)	167 ± 12	164 ± 12	169 ± 12‡**	171 ± 10‡‡**
HR_{min} (bpm)	116 ± 20	104 ± 20‡	118 ± 22**	119 ± 19**
ΔHR (bpm)	51 ± 19	59 ± 16‡	50 ± 16**	52 ± 15**
Recovery periods				
First 2 min				
ΣVT (L)	166.6 ± 43.9	151.2 ± 39.0‡‡	196.4 ± 41.2‡‡**	182.4 ± 35.0‡‡**$$
ΣVO_2 (L)	3.512 ± 0.603	3.890 ± 0.520‡‡	3.972 ± 0.493‡‡	4.029 ± 0.471‡‡*
ΣVCO_2 (L)	3.693 ± 1.017	4.231 ± 0.925‡‡	4.144 ± 0.870‡‡	4.207 ± 1.041‡‡
Last 2 min				
ΣVT (L)	114.5 ± 28.6	87.8 ± 28.8‡‡	112.1 ± 33.7**	109.5 ± 28.4**
ΣVO_2 (L)	2.073 ± 0.337	1.633 ± 0.296‡‡	1.910 ± 0.347‡‡**	1.888 ± 0.336‡‡**
ΣVCO_2 (L)	2.537 ± 0.528	2.065 ± 0.446‡‡	2.086 ± 0.434‡‡	2.263 ± 0.464‡‡*$

*BFR_{60}, blood flow restriction during the two first minutes of recovery; HYP, a hypoxic condition in a chamber (FiO_2 13.0%); G-BFR, gravity-induced BFR; NOR, control; ΣVT, cumulated ventilation; $\dot{V}E_{peak}$, peak minute ventilation; $\dot{V}E_{min}$, minimum minute ventilation; $\Delta\dot{V}E$, $|\dot{V}E_{peak}-\dot{V}E_{min}|$; ΣVO_2, cumulated oxygen consumption; $\dot{V}O_{2peak}$, peak oxygen consumption rate; $\dot{V}O_{2min}$, mean oxygen consumption rate; $\Delta\dot{V}O_2$, $|\dot{V}O_{2peak}-\dot{V}O_{2min}|$; ΣVCO_2, cumulated carbon dioxyde release; $\dot{V}CO_2$peak, peak carbon dioxyde release rate; $\dot{V}CO_2$min, minimum carbon dioxyde release rate; $\Delta\dot{V}CO_2$, $|\dot{V}CO_{2peak}-\dot{V}CO_{2min}|$; HR_{mean}, mean heart rate; HR_{peak}, peak heart rate; HR_{min}, minimum heart rate; ΔHR, $|HR_{peak}-HR_{min}|$. Data are expressed as mean ± SD. *Significantly different from G-BFR; ‡significantly different from BFR_{60}; $significantly different from HYP. The statistical significance threshold is set at $p < 0.05$ (one symbol) and $p < 0.01$ (two symbols).*

lower in the first sprint compared to the other sprints, and in the second sprint compared to the third and the fifth ones ($p < 0.05$): 128.4 ± 5.8, 136.8 ± 5.2, 141.3 ± 5.5, and 143.1 ± 4.4 bpm, respectively. Only a condition main effect was found for HR_{peak} ($p < 0.01$). Values were lower in G-BFR compared to the other conditions ($p < 0.05$, $d = -0.8$, -0.5, and -0.4 for NOR, HYP, and BFR_{60}, respectively) and in BFR_{60} compared to NOR ($p < 0.05$, $d = -0.4$).

Muscular O_2 Extraction

Results are presented in **Table 2**, **Figure 3**.

We found a significant condition main effect on TSI ($p < 0.01$). TSI was higher in BFR_{60} compared to HYP and G-BFR ($p < 0.01$, $d = 0.8$, and 1.3, respectively), and lower in G-BFR compared to the other conditions ($p < 0.01$, $d = -0.9$, -0.5, and -1.3 for NOR, HYP, and BFR_{60}, respectively). Mean session (both sprint and recovery periods included) TSI was lower in G-BFR compared to the other conditions ($p < 0.05$). A main effect of condition was detected for [tHb], $[tHb]_{max}$, and $[tHb]_{min}$ ($p < 0.01$). Values were higher in BFR_{60} compared to the other conditions ($p < 0.01$, [tHb]: $d = 0.4$, 0.7, and 0.7; $[tHb]_{max}$: $d = 0.5$, 0.6, and 0.7; $[tHb]_{min}$: $d = 0.4$, 0.6, and 0.7 for NOR, HYP, and G-BFR, respectively).

$[O_2Hb]_{max}$, $[O_2Hb]_{min}$, and $[O_2Hb]$ also presented a main effect of condition ($p < 0.01$). Values were higher in BFR_{60} compared to the other conditions ($p < 0.05$, $[O_2Hb]_{max}$: $d =$

TABLE 2 | Near-infrared spectroscopy (NIRS) parameters during the interventions.

	BFR_{60}	G-BFR	HYP	NOR
Exercise				
TSI (%)	63.7 ± 6.3	54.0 ± 8.2‡‡	58.2 ± 8.0‡‡**	61.0 ± 6.9**
TSI_{max} (%)	80.7 ± 6.4	72.3 ± 10.9	78.8 ± 3.8**	80.7 ± 4.6**
TSI_{min} (%)	56.0 ± 8.1	46.4 ± 10.0‡	49.8 ± 11.1‡*	52.0 ± 11.2‡*
ΔTSI (%)	24.7 ± 9.4	25.9 ± 10.5	29.0 ± 11.5	28.7 ± 12.5‡‡*
[tHb] (μM)	126.6 ± 35.1	105.7 ± 18.4‡‡	107.1 ± 22.3‡‡	113.2 ± 25.6‡‡
$[tHb]_{max}$ (μM)	135.9 ± 38.1	113.5 ± 19.9‡‡	115.6 ± 25.8‡‡	120.0 ± 27.3‡‡
$[tHb]_{min}$ (μM)	120.1 ± 33.8	101.0 ± 17.1‡‡	102.3 ± 20.4‡‡	107.9 ± 23.8‡‡
Δ[tHb] (μM)	15.7 ± 11.5	12.5 ± 4.6	13.2 ± 7.4	12.1 ± 7.2
$[O_2Hb]$ (μM)	81.0 ± 25.7	56.7 ± 11.3‡	61.7 ± 11.7‡	68.1 ± 11.7‡*\$
$[O_2Hb]_{max}$ (μM)	108.9 ± 36.5	89.7 ± 21.3‡	81.0 ± 19.8‡	94.8 ± 21.3‡*
$[O_2Hb]_{min}$ (μM)	68.9 ± 23.3	47.0 ± 10.5‡	50.9 ± 11.8‡	56.2 ± 11.5‡*
$Δ[O_2Hb]$ (μM)	40.0 ± 20.6	33.9 ± 13.7	38.8 ± 21.4	38.6 ± 22.5
[HHb] (μM)	45.6 ± 14.4	49.0 ± 14.5	45.4 ± 16.2	45.4 ± 16.2
$[HHb]_{max}$ (μM)	55.6 ± 18.9	57.6 ± 18.9	54.8 ± 20.9	56.5 ± 24.7
$[HHb]_{min}$ (μM)	24.4 ± 8.4	30.2 ± 12.5	23.7 ± 6.6**	22.6 ± 8.3**
Δ[HHb] (μM)	31.2 ± 18.0	27.4 ± 15.3	31.1 ± 17.0	34.0 ± 21.8**
Recovery periods				
First 2 min				
TSI (%)	72.5 ± 6.6	65.9 ± 10.9	73.7 ± 4.5	77.3 ± 3.0**
TSI_{max} (%)	83.4 ± 5.6	78.7 ± 9.2	82.3 ± 3.2	84.4 ± 3.2
TSI_{min} (%)	54.6 ± 8.0	45.7 ± 14.4	50.8 ± 9.8	53.0 ± 7.9
ΔTSI (%)	28.8 ± 9.1	33.0 ± 13.5	31.5 ± 10.3	31.5 ± 8.5
[tHb] (μM)	146.6 ± 52.6	113.2 ± 20.8	113.8 ± 24.4	123.5 ± 30.2
$[tHb]_{max}$ (μM)	178.5 ± 72.7	121.7 ± 24.2‡	123.2 ± 29.0‡	140.4 ± 46.8
$[tHb]_{min}$ (μM)	111.0 ± 41.0	99.8 ± 20.5	100.7 ± 21.8	105.8 ± 23.0
Δ[tHb] (μM)	67.5 ± 39.6	21.9 ± 11.8‡	22.5 ± 9.7‡	34.6 ± 30.0‡
$[O_2Hb]$ (μM)	108.5 ± 47.6	74.7 ± 18.2‡	84.3 ± 19.7	95.5 ± 23.3
$[O_2Hb]_{max}$ (μM)	141.0 ± 71.3	92.7 ± 24.2	99.7 ± 26.1	116.4 ± 39.5
$[O_2Hb]_{min}$ (μM)	67.1 ± 27.1	47.9 ± 14.8	52.4 ± 11.2	58.7 ± 9.3
$Δ[O_2Hb]$ (μM)	73.9 ± 49.5	44.7 ± 23.7	47.2 ± 21.6	57.7 ± 33.4
[HHb] (μM)	39.1 ± 12.7	39.3 ± 14.2	29.6 ± 7.6	28.0 ± 8.4‡
$[HHb]_{max}$ (μM)	61.3 ± 22.1	61.5 ± 24.9	54.9 ± 20.3	56.5 ± 24.7
$[HHb]_{min}$ (μM)	21.8 ± 7.3	24.9 ± 9.2	20.4 ± 4.5	19.8 ± 6.9
Δ[HHb] (μM)	39.5 ± 18.8	36.6 ± 21.2	34.5 ± 17.7	36.6 ± 21.6
Last 2 min				
TSI (%)	77.7 ± 6.7	73.0 ± 10.6	79.5 ± 4.1	81.6 ± 3.6*
TSI_{max} (%)	84.5 ± 5.1	79.1 ± 8.8	82.3 ± 3.2	84.3 ± 3.5
TSI_{min} (%)	62.7 ± 13.5	63.0 ± 14.5	75.0 ± 6.0‡*	77.7 ± 6.1‡*
ΔTSI (%)	21.8 ± 12.0	16.1 ± 10.2	7.3 ± 4.5‡‡	6.6 ± 3.9‡‡*
[tHb] (μM)	136.7 ± 49.7	113.7 ± 20.3	114.5 ± 24.9	123.9 ± 28.0
$[tHb]_{max}$ (μM)	167.2 ± 65.4	120.6 ± 23.6‡	121.1 ± 27.4	136.6 ± 41.0
$[tHb]_{min}$ (μM)	112.7 ± 43.9	104.6 ± 21.8	105.8 ± 21.9	111.4 ± 23.2
Δ[tHb] (μM)	54.5 ± 33.9	16.0 ± 12.8‡‡	15.4 ± 7.3‡‡	25.2 ± 23.9‡‡
$[O_2Hb]$ (μM)	108.4 ± 45.8	83.3 ± 20.2	91.5 ± 22.6	100.8 ± 21.3
$[O_2Hb]_{max}$ (μM)	136.4 ± 62.3	93.1 ± 23.6	99.0 ± 25.4	113.7 ± 34.8
$[O_2Hb]_{min}$ (μM)	79.6 ± 42.4	70.9 ± 22.1	82.3 ± 19.2	88.9 ± 16.0
$Δ[O_2Hb]$ (μM)	56.9 ± 41.1	22.2 ± 16.9‡	16.7 ± 9.1‡‡	24.8 ± 22.9‡
[HHb] (μM)	28.3 ± 11.6	30.4 ± 12.9	23.0 ± 5.7	23.0 ± 8.8

(Continued)

TABLE 2 | Continued

	BFR_{60}	G-BFR	HYP	NOR
$[HHb]_{max}$ (μM)	45.8 ± 21.6	38.9 ± 19.6	26.5 ± 7.7‡	26.3 ± 9.5‡
$[HHb]_{min}$ (μM)	19.2 ± 6.6	23.8 ± 9.7	20.2 ± 4.6	20.1 ± 7.8
Δ[HHb] (μM)	26.6 ± 16.5	15.1 ± 12.3‡	6.2 ± 4.3‡‡	6.2 ± 3.0‡‡

*BFR_{60}, blood flow restriction during the two first minutes of recovery; HYP, hypoxic condition in a chamber (FiO_2 13.0%); G-BFR, gravity-induced BFR; NOR, control; TSI, tissue saturation index; TSI_{max}, maximum TSI; TSI_{min}, minimum TSI; ΔTSI, $|TSI_{max}-TSI_{min}|$; [tHb], total hemoglobin content; $[tHb]_{max}$, maximum [tHb]; $[tHb]_{min}$, minimum [tHb]; Δ[tHb], $|[tHb]_{max}-[tHb]_{min}|$; $[O_2Hb]$, oxyhaemoglobin content; $[O_2Hb]_{max}$, maximum $[O_2Hb]$; $[O_2Hb]_{min}$, minimum $[O_2Hb]$; $Δ[O_2Hb]$, $|[O_2Hb]_{max}-[O_2Hb]_{min}|$; [HHb], deoxyhaemoglobin content; $[HHb]_{max}$, maximum [HHb]; $[HHb]_{min}$, minimum [HHb]; Δ[HHb], $|[HHb]_{max}-[HHb]_{min}|$. Data are expressed as mean ± SD. *Significantly different from G-BFR; ‡significantly different from BFR_{60}; \$significantly different from HYP. Statistical significance threshold is set at $p < 0.05$ (one symbol) and $p < 0.01$ (two symbols).*

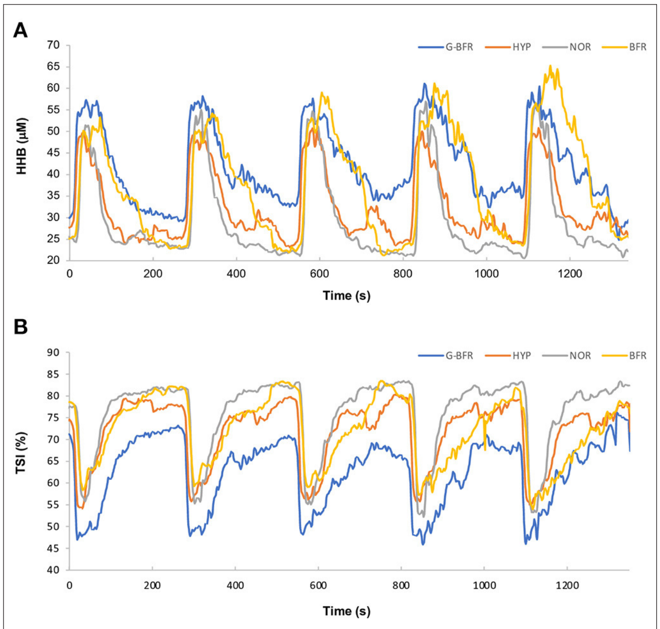

FIGURE 3 | Average deoxyhemoglobin **(A)** and tissue saturation index **(B)**. BFR_{60}, blood flow restriction during the two first minutes of recovery; HYP, hypoxia in a chamber at 13.0% FiO_2; G-BFR, gravity-induced BFR; NOR, control.

0.5, 0.7, and 1.0; $[O_2Hb]_{min}$: $d = 0.7$, 1.0, and 1.2, $[O_2Hb]$: $d = 0.7$, 1.0, and 1.2 for NOR, HYP, and G-BFR, respectively), and in NOR compared to G-BFR ($p < 0.05$, $[O_2Hb]_{max}$: $d = 0.7$; $[O_2Hb]_{min}$: $d = 0.8$; $[O_2Hb]$: $d = 1.0$). Moreover, $[O_2Hb]$ was lower in HYP compared to NOR ($p < 0.05$, $d = -0.5$). Finally, an effect of the sprint number was found on $Δ[O_2Hb]$ ($p < 0.01$). Values increased between the first sprint and the last two sprints ($p < 0.01$): 30.5 ± 2.9, 40.8 ± 6.0, and 42.3 ± 3.9 μM, respectively.

Recovery Periods

During the first 2 min of recovery, ΣVT, $ΣVO_2$, and $ΣVCO_2$ presented a main effect of condition ($p < 0.01$). ΣVT was lower in G-BFR ($p < 0.01$, $d = -0.8$, -1.1, and -0.4 for NOR, HYP, and

BFR_{60}, respectively) and higher in HYP ($p < 0.01$, $d = 0.4$, 0.7, and 1.1 for NOR, BFR_{60}, and G-BFR, respectively) compared to the other conditions, and higher in NOR compared to BFR_{60} ($p < 0.01$, $d = 0.4$). During this first part of recovery, ΣVO_2 was lower in BFR_{60} compared to the other conditions ($p < 0.01$, $d = -1.0$, −0.8, and −0.7 for NOR, HYP, and G-BFR, respectively) and in G-BFR compared to NOR ($p < 0.05$, $d = -0.3$). ΣVCO_2 was also lower in BFR_{60} compared to the other conditions ($p < 0.01$, $d = -0.5$, −0.5, and −0.6 for NOR, HYP, and G-BFR, respectively). Concerning NIRS data, a main effect of condition was found for TSI ($p < 0.01$). It was lower in G-BFR compared to NOR ($p < 0.01$, $d = -1.5$). Oxyhemoglobin concentration ($[O_2Hb]$), deoxyhemoglobin concentration ([HHb]), and the difference of hemoglobin concentration (Δ[tHb]) showed a main effect of condition ($p < 0.05$). $[O_2Hb]$ was higher for BFR_{60} than G-BFR ($p < 0.05$, $d = 0.9$). [HHb] was higher in BFR_{60} compared to NOR during this period ($p < 0.05$, $d = 1.1$). Δ[tHb] was higher in BFR_{60} compared to the other conditions ($p < 0.05$, $d = 0.9$, 1.6, and 1.5 for NOR, HYP, and G-BFR, respectively).

During the last 2 min of recovery, ΣVT, ΣVO_2, and ΣVCO_2 showed a main effect of condition ($p < 0.01$). ΣVT and ΣVO_2 were lower in G-BFR compared to the other conditions ($p < 0.01$, ΣVT: $d = -0.8$, −0.8, and −0.9, ΣVO_2: $d = -0.8$; −0.9 and −1.4 for NOR, HYP, and BFR_{60}, respectively). Of note, ΣVO_2 and ΣVCO_2 were higher in BFR_{60} compared to the other conditions ($p < 0.01$, ΣVO_2: d = 0.5, 0.5, and 1.4; ΣVCO_2: $d = 0.6$, 0.9, and 1.0 for NOR, HYP, and G-BFR, respectively). Furthermore, a main effect of condition was observed on TSI, TSI_{min}, $[tHb]_{max}$, and $[HHb]_{max}$ ($p < 0.05$). As previously, TSI was lower in G-BFR compared to NOR ($p < 0.05$, $d = -1.2$). Detailed results are presented in **Tables 1**, **2**, **Figures 2**, **3**.

DISCUSSION

The aim of the present study was to compare the acute effects of systemic hypoxia, BFR_{60}, and G-BFR on mechanical output, cardiorespiratory responses, and O_2 muscle extraction during SIT exercises in healthy moderately trained men. The main results were that SIT associated with G-BFR displayed lower mechanical and cardiorespiratory responses than the other modalities. G-BFR also showed lower skeletal muscle oxygenation. BFR_{60} induced greater blood accumulation within working muscles compared to the other conditions. Moreover, HYP at 13% FiO_2 and G-BFR increased local hypoxia within the working muscles, with a higher level of hypoxia observed for G-BFR.

The primary results showed that SpO_2 was lower for HYP throughout the exercise session and decreased at the end of the session (i.e., sprints four and five) for all the conditions. According to Fick's law of diffusion, systemic hypoxemia occurred in HYP because alveolo-capillary oxygen pressure difference decreases under hypoxic conditions. Consequently, $\dot{V}E_{peak}$ increased in HYP, as a compensatory response aiming to maintain oxygen consumption. In accordance, previous studies observed that the magnitude of the ventilatory response appears a critical factor of performance during a Wingate test in hypoxia (Fallon et al., 2015). Indeed, the authors showed that ventilation was elevated at the beginning and throughout the exercise when F_iO_2 was decreased from 20.9 to 10% (Fallon et al., 2015). However, the augmentation of ventilation observed in the present study was not sufficient, as total and peak oxygen uptake were still lower in HYP compared to NOR. In addition, mean ventilation was higher in HYP during the first period of recovery. Hypoxemia had an impact on muscle oxygenation parameters. Specifically, oxyhemoglobin concentration during sprints was lower in HYP compared to NOR. Therefore, as total work was the same between the conditions, except for G-BFR, this suggests that the anaerobic metabolism is more solicited in HYP. Furthermore, we analyzed SpO_2 during recovery when the cuffs were applied and did not observe any difference between the conditions. Similarly, no effect was detected in the study from Willis et al. (2019) using continuous BFR during the RSH protocol at 45% of arterial occlusive pressure.

In the current protocol, fatigue increased progressively throughout the session as performance variables (i.e., peak and mean power, time to reach mean power) were altered, and HR increased with the repetition of sprints. On the other hand, ΣVO_2 and $\dot{V}O_{2peak}$ were lower during the first sprint probably because metabolic demands are ensured by the anaerobic metabolism (i.e., phosphocreatine hydrolysis and glycolysis). Interestingly, $\dot{V}O_{2peak}$ was higher in NOR compared to the other conditions, which is in accordance with a study from Willis et al. comparing NOR and BFR (Willis et al., 2018). Furthermore, no effect of the condition or the number of sprints was found on FI. A study by Fallon and coworkers (Fallon et al., 2015) observed higher FI in HYP but their study included a single sprint of 30 s with a higher level of hypoxia ($FiO_2 = 10\%$). In the current study, the BFR_{60} application induced a decrease in gas exchange rates, which increased after its release. This phenomenon has already been observed during partial occlusion in dogs where oxygen consumption was decreased during BFR application and increased when pressure was removed. These responses relied on blood flow, which means that BFR causes vascular resistance (Fales et al., 1962). Importantly, in the current study, BFR was applied for the first 2 min of recovery. This probably delayed and reduced overall recovery, which may influence in turn performance, energy system usage, and metabolite accumulation in the following sprints. The BFR protocol used in the present study was applied during recovery periods between exercise bouts and as such has a different effect to the continuous application that may be seen during other studies. For HYP, performance variables were unaffected but average and maximal oxygen consumption and muscular oxyhemoglobin availability decreased. For all conditions, peak and average power decreased after each sprint excepting between sprints four and five where values were similar. Time to reach peak power increased between the three first sprints and sprint five. RPE increased throughout the exercises. Altogether, these data suggest that during SIT, both BFR and HYP enhance cellular stress (i.e., metabolite accumulation and/or hypoxia) without affecting total work during the training sessions.

Importantly, several cardiorespiratory parameters (i.e., ΣVT, $\dot{V}E_{peak}$, $\dot{V}E_{min}$, $\Delta\dot{V}E$, ΣVO_2, ΣVCO_2, HR, HR_{peak}, HR_{min},

ΔHR), mean TSI (exercise and recoveries), TSI_{max} and TSI_{min} during exercise and/or recovery were lower for G-BFR compared to the other conditions. Of note, during sprints, the supine position induced lower mean, maximal, and minimal tissue saturation. Concerning training data, RPE appeared lower for G-BFR compared to BFR_{60} and HYP, peak power was lower compared to NOR, and time to achieve peak power was higher compared to BFR_{60} and HYP. Average power also appeared lower for G-BFR compared to the other conditions. Overall, these data are consistent with the literature. Indeed, an important decrease in strength of the knee flexor and extensor muscles is inherent to the supine vs. seated position (Houtz et al., 1957). Moreover, maximal exercise performance (such as maximal work rate, $\dot{V}O_{2max}$, $\dot{V}E_{max}$, and HR_{max}) during an incremental test has been shown to be impaired in supine position compared to upright cycle position, probably due to a lower perfusion (Hughson et al., 1991). Our results are consistent with the recent study of Preobrazenski and colleagues who showed a decrease in muscle oxygenation in their G-BFR model compared to the control group. However, they reported a higher RPE with G-BFR whereas we obtained the opposite result. This could be explained by the difference in exercise modality (aerobic vs. repeated all-out exercises). Altogether, the G-BFR condition may alter biomechanical and cardiorespiratory responses. However, G-BFR induced lower mean tissue oxygenation. Thus, G-BFR seems to induce specific stress compared to the other modalities.

Concerning BFR_{60} and HYP, present data indicate no difference with NOR for mechanical outcomes. These results are in line with the data from Willis et al. (2019) who compared NOR, BFR, and HYP during an RST protocol. Indeed, the authors found no difference in mean power, mean ventilation, or HR_{peak}. They also found a significant increase in total hemoglobin concentration with BFR (constant pressure of 45%). The present results also suggest that BFR_{60} promotes higher blood accumulation within working muscles than the other conditions, meaning that BFR_{60} may additionally affect training adaptations by confining metabolites. Importantly, BFR was suggested to induce greater neuromuscular fatigue while HYP produces central fatigue by impairing corticospinal excitability because of cerebral deoxygenation (Willis et al., 2018; Peyrard et al., 2019). During BFR application, deoxyhemoglobin concentration was higher and oxyhemoglobin content was not different compared to NOR. This result means that a partial occlusion (60%) has a greater impact on venous return than on arterial blood supply. Of note, power output strongly decreases for G-BFR, which may explain in turn the decrease of HR values while it should be increased with higher venous return. Furthermore, enhanced deoxyhemoglobin concentration matches the blood accumulation (increased Δ[tHb]) observed during BFR application. Its release induces bigger changes in oxyhemoglobin, deoxyhemoglobin, and total hemoglobin concentrations compared to the other conditions at the same period. On the contrary, our results suggest that G-BFR does not have a significant impact on venous return, as recently demonstrated by another group during aerobic exercises (Preobrazenski et al., 2021). During sprints, TSI was higher for BFR_{60} compared to the other conditions, due to an increased oxyhemoglobin concentration ([O_2Hb]). On the other hand, peak and mean HR were lower in BFR_{60} during the exercises. Interestingly, HYP and G-BFR induced greater local hypoxia within skeletal muscles, which was more prominent in G-BFR when considering both exercise bouts and recovery periods. Thus, G-BFR would represent an alternative to HYP to promote additional hypoxic stress within skeletal muscles cells. Additional studies are needed to compare the chronic effects of these conditions on both cellular adaptations to training and gains in muscle performance, especially because G-BFR lowers power output during sessions.

Furthermore, some limits must be acknowledged. First, the modalities of HYP, BFR_{60}, and G-BFR conditions were established according to preliminary work that allowed to identify the maximal stress that could be tolerated by the participants in combination with the SIT protocol of this study. Thus, further studies are needed to evaluate more precisely the nature and the degree of the stress generated by these conditions. On the other hand, BFR_{60} was the only stress which was not continuously applied. In such a situation, the BFR application still had a delayed impact on muscle oxygenation parameters during sprints. However, based on preliminary work, this mode of BFR has been chosen to set maximal stress that could be tolerated in this population in combination with the SIT protocol. Another limit can be that participants were moderately trained individuals, and it was a demanding protocol. This also makes it difficult to generalize the results, especially to untrained populations for which these training methods would be hard to complete. Moreover, TSI measurements cover a limited zone of the vastus lateralis muscle thus interpretations of the results at the whole muscle are to be considered with these limitations. Finally, the present study did not include women athletes. Indeed, some adaptations such as cardiovascular responses differ between men and women (Patel et al., 2021), thus introducing variability. Additional works are needed to compare outcomes according to gender because women remain underrepresented in sport science literature.

CONCLUSIONS

In conclusion, this study conducted in healthy moderately trained men showed that a session of SIT in combination with G-BFR showed lower mechanical, cardiorespiratory responses, and skeletal muscle oxygenation than the other conditions. Another important insight is that both SIT protocols conducted under HYP at 13% FiO_2 and G-BFR amplified local hypoxia within the working muscles. Importantly, a higher level of hypoxia was found with G-BFR when considering the measurement of the entire exercise session (i.e., exercise bouts and recovery periods). Furthermore, a single session of SIT associated with BFR_{60} promotes higher blood accumulation within working muscles compared to the other exercise modes. This suggests that BFR_{60} may additionally or differentially affect cellular homeostasis. Thus, each condition generates specific stress and further studies are needed to better understand subsequent consequences on long-term adaptation.

PERSPECTIVES

Further studies are needed (i) to compare the effects of these protocols on-field performance, (ii) to evaluate more precisely the degree of stress generated by each condition, even if high values for both BFR_{60} and hypoxia have been used based on literature for this kind of exercise, and (iii) to examine mechanistic insight since the mechanisms of action may be different. Investigations of the involvement of different cellular pathways, for example, those involved in mitochondrial adaptations (e.g., the axis of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha) are needed to improve our knowledge about the molecular benefits of these training methods. These research directions are important because they may help to improve the ability to develop more efficient hypoxic or BFR training modalities and to improve skeletal muscle function and whole-body metabolism. Finally, adding stress to training would promote adaptations in the long term, at least when recovery processes are sufficient.

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 experimental protocol was approved by the Local Ethics Committee (VD-2021-00597). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RS, FB, and AS: conceived and designed the experiments, analyzed the data, designed the tables and figures, wrote the manuscript, contributed reagents, materials, and analysis tools. RS, HB, and FB: performed the experiments. All authors read the manuscript and approved the final version.

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ORIGINAL RESEARCH
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The Effect of 16 Weeks of Lower-Limb Strength Training in Jumping Performance of Ballet Dancers

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Jumping ability is considered a determinant of performance success. It is identified as one of the predictors and talent identification in many sports and dance. This study aimed to investigate the effect of 16 weeks of lower-limb strength training on the jumping performance of ballet dancers. A total of 24 participants from the same dance school were randomly selected in the control group [CG; $n = 10$; aged 13.00 (1.49) years; 43.09 (9.48) kg and 1.53 (0.11) m] and the intervention group [IG; $n = 14$; aged 12.43 (1.45) years; 38.21 (4.38) kg and 1.51 (0.07) m], evaluated before and after the applied strength training program mainly using the body weight of each participant. Jump performance was assessed using MyJump2, a scientifically validated mobile phone app. Intergroup and intragroup comparisons were assessed, and the magnitude of change was calculated using the effect size (ES). While CG significantly decreased the relative power over time ($p < 0.001$, ES = −0.29: small), results from the intragroup comparisons suggest that IG significantly increased the countermovement jump (CMJ) height ($p < 0.001$, ES = 1.21: large), the relative force ($p < 0.001$, ES = 0.86: moderate), maximal velocity ($p < 0.001$, ES = 1.15: moderate), and relative power ($p < 0.001$, ES = 1.37: large). We concluded that a 16-week strength training program of lower limbs is an effective way to improve CMJ height in young dancers. Supplementary strength training appears to be the determinant for the improvement of the jumping performance of ballet dancers.

Keywords: jump, explosive, strength, power, dance

INTRODUCTION

Both sports and dance culminate in public performances. The intersections of dance and sports are reported since some athletes and coaches use strength training to improve their performance (Markula, 2018), particularly in aesthetic sports such as figure skating and gymnastics. In addition to the dramatic and artistic performance of dancers, dance demands high levels of motor performance, which include a high jumping ability (Koutedakis et al., 2005). Several authors recognize that dancers with a more developed jumping ability also improve the perception of aesthetic and artistic components of the choreography (Wyon et al., 2006; Angioi et al., 2009; Rafferty, 2010). Others underline the importance of jumping ability in talent identification in dance (Walker et al., 2010).

Studies involving dance students report that the increase in strength is crucial for the dancers to perform well, especially concerning the use of the lower limbs (essential in jumping) (Fração et al., 1999). In fact, given the abundance of ballistic actions in ballet (e.g., jumps and changes of direction), improvements in jump height may be beneficial for the dancers as a guide to specific training plans that can improve either maximal force or velocity capabilities (Alvarez et al., 2020). The countermovement jump (CMJ) is usually used to evaluate the power output of the lower limbs as ballistic movements that include both concentric and eccentric phases closer to the sports and ballet movements. In fact, the CMJ and drop jump performances are related to *grand jeté* leap performance in dancers with different skill levels, being considered useful tools for monitoring the power-generating capacity of the lower body of the dancer, thus giving insight into the overall jumping capacity of the dancer (Blanco et al., 2019). Consequently, both strength and speed development are fundamental to increasing jumping ability (Jimenez-Reyes et al., 2014).

Some studies observed the effect of training programs to improve jumping performance, either in dancers or in rhythmic gymnastics, which is the gymnastics discipline more equivalent to dance (Wang et al., 2010; Piazza et al., 2014; Komeroski et al., 2016; Mlsnová and Luptáková, 2017; Tsanaka et al., 2017; Dobrijević et al., 2018; Skopal et al., 2020; Stošić et al., 2020). Focusing on dance, previous studies have applied for a specific strength training program, for 9 weeks, based on and adjusted according to the force-velocity profile of each dancer (Escobar-Alvarez et al., 2019); evaluated whether a 9-week resistance training program could have a significant effect on the strength and power of the lower limbs in adolescent dancers (Dowse et al., 2020); applied, for 10 weeks, a modern and recreational dance exercise program and trunk and leg muscle strengthening exercises in university dance students (Stošić et al., 2020) and used their ballet classes, modified with a focus on lower-limb strength (reduction in bar duration (from 45′ to 20′) and the *petit* and *grand allegro* exercises at the beginning of center work, for 8 weeks, as an intervention to analyze jumping ability (Tsanaka et al., 2017). Regarding the results about the jump height, four studies (Tsanaka et al., 2017; Escobar-Alvarez et al., 2019; Dowse et al., 2020; Stošić et al., 2020) obtained positive results with significant differences, i.e., the applied training promoted improvements in the vertical jump height of the dancer. Concerning the instrument, two studies have used MyJump2 with ballet dancers, but only one conducted an intervention (Escobar-Alvarez et al., 2019; Alvarez et al., 2020). These findings of the authors suggest that the experimental group presented significant differences with large effect sizes (ESs) in CMJ height and other jumping performance variables, namely, the theoretical maximal force (Escobar-Alvarez et al., 2019). Additionally, measurements of the distance covered by the center of mass during push-off are highlighted (Samozino et al., 2008, 2012, 2014) to control the growth process of the participants over time.

Despite the known technical and physical demands of elite dance, traditionally, strength training has not been considered important to the ongoing development of adolescent dancers (Dowse et al., 2020). We found only one study that reported an intervention program aiming to improve the jumping performance of younger dancers using traditional external loads (Dowse et al., 2020). Our study aimed to promote strength training mainly using the body weight of an individual. We hope that this will raise the awareness of the dance teachers toward the strength training benefits to enhance the jumping performance of the dancers. Therefore, this study provides an example of a training program without any equipment that could be applied in dance classes of young ballet dancers.

This study aimed to investigate the effect of 16 weeks of lower-limb strength training in the jumping performance of classic ballet dancers. For this, we (1) evaluated the mechanical variables during the CMJ of dancers, (2) proposed a specific training program, for 16 weeks, to improve the jumping performance, and (3) compared the mechanical variables during the CMJ of dancers before and after a specific jump training program application. We hypothesized that the proposed training program will positively affect the CMJ height of the dancers.

MATERIALS AND METHODS

The following sample inclusion criteria were established: (1) enrollment at the educational institution participating in the study; (2) absence of injury that prevented them from training in the last 3 months, during the intervention and at the time of evaluation; (3) no involvement in any complementary physical training or sports activity during the 16 weeks of intervention; and (4) attendance at a minimum of 80% of training sessions.

Table 1 shows the sample characterization of the control group (CG) and the intervention group (IG).

Our sample was composed of 24 ballet dancers from the same school dance and was divided into the CG ($n = 10$) and the

TABLE 1 | Sample characterization [Mean (*SD*)].

Variables	CG ($n = 10$) mean (*SD*)	IG ($n = 14$) mean (*SD*)	*p*
Age (years)	13.00 (1.49)	12.43 (1.45)	0.357
Weight (kg)	43.09 (9.48)	38.21 (4.38)	0.156
Height (m)	1.53 (0.11)	1.51 (0.07)	0.487
BMI (kg/m^2)	18.05 (2.46)	16.77 (1.02)	0.148
HPO (cm)	0.36 (0.07)	0.39 (0.07)	0.348
Years of practice	5.00 (1.83)	6.50 (3.52)	0.189
Hours/training/week	28.50 (3.37)	27.93 (5.14)	0.762

$p \leq 0.05$, independent measures t-test; pretest between groups.
CG, control group; IG, intervention group; BMI, body mass index; HPO, height of push-off; SD, standard deviation.

IG ($n = 14$). Although the participants were selected randomly, the groups did not have the same number of subjects because four dancers from the CG were involved in a complementary sports activity, which led to their exclusion from the CG sample. The sample size was restricted to the number of higher-level dancers from the dance school that accepted and approved to carry out this study.

It is essential to highlight that the CG was composed of 9 female and 1 male dancers, with an average age of 13.00 (1.49) years, 5 years of regular dance practice, and 28.50 h of training per week (**Table 1**). The IG was composed of 10 female and 4 male dancers, with an average age of 12.43 (1.45) years, 6.50 years of practice, and 27.93 h of weekly training. No significant differences were found between the CG and the IG for any of the variables in **Table 1**.

All ethical procedures were carried out following the Declaration of Helsinki, and the study was approved by the local Ethical Committee (CE/FCDEF-UC/00742021). All participants and their guardians (dancers aged under 18 years) were informed of the benefits and risks of the research. Before the beginning of the study, they signed an informed consent document for testing, implementation of the training protocol, and publication of collected data.

The variables assessed in this investigation were the weight (kg), height (m), body mass index (BMI) (kg/m^2), CMJ (cm), relative force (N/kg), maximal velocity (m/s), relative power (W/kg), and the height of push-off (HPO) (cm) of each dancer. The relative values to body mass were calculated by dividing the output of each subject by their mass as previous findings, whereas relative force was calculated by dividing the force output of each subject by their mass (Dowse et al., 2020).

These mechanical variables were used in our study to better understand the CMJ high performance as stated by Cormie et al. (2011) that force and velocity are considered as the fundamental features of mechanical power output in sport movements.

Before the beginning of the study, the body weight (kg) and the stature (m) were collected using a Tanita SC-330 (TANITA Corp, Tokyo, Japan) and an aluminum stadiometer (Seca 713 model; Seca, Postfach, Germany), respectively. HPO assessment (Samozino et al., 2008, 2012, 2014) implicates two measurements of lower-limb length (in centimeters) by an experienced researcher using a tape measure (SECA, 201). First, with the participant lying down and the ankle fully extended, the distance from the iliac crest to toes and, second, squatting at 90° (knee flexion) from the iliac crest to the ground were measured. These measures were collected before each of the 3 evaluation moments to control the growth process of dancers along the 16 weeks of training. The time-lapse between the last training session and the evaluation moments was always proximally 72 h. Both groups were evaluated in pretest and after the 16 weeks of training. Since the IG experienced an unusual training strategy, an intermediated control moment of jump performance progression was programmed, which occurs at the 8th week only in IG.

Before all control time points, dancers performed their habitual warm-up routine consisting of 15 min of jogging, dynamic stretching (plantar flexors, hip extensors, hamstrings, hip flexors, and quadriceps femoris), and preparatory CMJs, also following previous orientations (Escobar-Alvarez et al., 2019). Before each jump, participants were instructed to remain in a standing position with their hands on their hips. From this position, participants performed a CMJ as described earlier (Jimenez-Reyes et al., 2014). A maximum effort CMJ was used to assess lower-body explosive power and the effect of the stretch-shorten cycle of each subject (Dowse et al., 2020). The instrument used was MyJump2, an app of iPhone 5 specially developed to monitor the vertical jump ability of the athlete in a valid, reliable, and economical way in adults (Balsalobre-Fernández et al., 2015; Jiménez-Reyes et al., 2017), and Samozino's method was used to monitor children (Morin and Samozino, 2016; Bogataj et al., 2020). The analysis of jumping performance using MyJump2 evoked recently in scientific research (Samozino et al., 2012, 2014; Balsalobre-Fernández et al., 2015; Jimenez-Reyes et al., 2016; Morin and Samozino, 2016). This method is based on the fundamental laws of mechanics, which proposes an accurate and reproducible field method to evaluate the power output of lower limbs and allows a precision similar to that obtained with specific laboratory ergometers (force platform method) (Samozino et al., 2008). This instrument can be used to monitor the performance of the athletes and dancers without expensive laboratory equipment or moving the athletes and dancers from their usual practice zone. It allows assessing the external force developed and the maximum speed capacity related to body mass (Jimenez-Reyes et al., 2014; Samozino et al., 2014; Jiménez-Reyes et al., 2017), thus personalizing the results to the characteristics of individual athletes or dancers.

Both groups (IG and CG) maintained the standardized training regimen (as presented in **Supplementary Table 1**). In addition, the IG followed a program of the lower-limb strength training session two times a week during the 16 weeks of intervention. Dancers performed a training program (20 min) mainly based on exercises using their body weight, following previous recommendations for youth training (Faigenbaum et al., 2009). The training program had four phases: phase 1 (weeks 1–4) was composed of full squat, single-leg squat, and step-up exercises; phase 2 (weeks 5–10) was composed of introducing box jumps, single-leg jumps, burpees, and lunges step-ups; phase 3 (weeks 11–13) was composed of Russian squats in pairs, bouncing, CMJ, and lateral step-ups; and phase 4 (week 14–16) was composed of isometric squats, single-leg squat jumps, leg press in pairs, and CMJ. Details about repetitions, sets, recovery, and duration of each phase are presented in **Supplementary Table 2**.

All data are presented as means (SDs) using IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY, United States. The normal distribution of the study variables was assessed using the Shapiro–Wilk test. Intergroup and intragroup comparisons were evaluated by an independent measure and a repeated measures t-test, respectively. We also conducted a repeated-measures ANOVA with Bonferroni adjustment (3 evaluation moments) to include a data collection performed at the 8th week only at the IG as a control measure of jump performance evolution. The level of significance was set at $p \leq 0.05$.

Intragroup magnitudes of change were calculated with the following S (Hopkins, 2004). The criterion for interpreting these magnitudes was as follows: < 0.2, trivial change; 0.2–0.6, small; 0.6–1.2, moderate; 1.2–2, large; > 2.0, very large (Hopkins et al., 2009). The probability that these differences exist was assessed *via* magnitude-based qualitative inferences (Batterham and Hopkins, 2006). Probabilities that differences were higher than, lower than, or similar to the smallest worthwhile difference were defined by the following scale: < 0.5%, almost certainly not; < 5%, very unlikely; < 25%, unlikely, probably not; 25%–75%, possibly, possibly not; > 75%, likely, probably; > 95%, very likely; > 99.5%, almost certainly. Finally, for the intergroup comparison, we used the ES from Cohen's *D*, using the following scale for interpretation: 0.2–0.5, small; 0.5–0.8, moderate; > 0.8, large (Cohen, 1988).

RESULTS

Although our sample is composed of female and male dancers, similar to a previous study (Dallas et al., 2014), no significant differences ($p \geq 0.05$) were found between the sexes in the jumping performance. Accordingly, we considered them as one group for the study analysis. **Table 2** shows the intergroup comparison (CG and IG) of the jumping performance variables during the pre-post intervention.

In pretest, the CG presented significantly higher CMJ ($p = 0.039$, ES = 0.911: large), relative force ($p = 0.004$, ES = 1.326: large), maximal velocity ($p = 0.035$, ES = 0.931: large), and relative power values ($p = 0.004$, ES = 1.328: large) in comparison to the IG. In posttest, no significant differences were found between groups in any of the study variables.

Table 3 shows the intragroup comparisons for anthropometric and jumping performance variables.

The CG significantly decreased relative power values over time ($p < 0.001$). In contrast, the IG significantly increased all variables, namely, the anthropometric measurements, weight ($p < 0.001$, ES = 0.43: small), height ($p < 0.001$, ES = 0.19: trivial) and BMI ($p \leq 0.003$, ES = 0.51: small), CMJ ($p < 0.001$, ES = 1.21: large), relative force ($p < 0.001$, ES = 0.86: moderate), maximal velocity ($p < 0.001$, ES = 1.15: moderate), and relative power ($p < 0.001$, ES = 1.37: large).

Table 4 presents the intragroup comparison in the IG dancers during the 3 evaluation moments (initial, after 8 weeks, and after 16 weeks of training protocol).

Finally, **Table 4** presents an intermediate evaluation moment, including the IG dancers as a control measure of jump performance evolution. It indicates that the anthropometric variables significantly increased over time, but the height of the dancer only differs significantly between moments 1–3 and 2–3 ($p = 0.002$). Regarding the jumping performance, CMJ ($p < 0.001$), maximal velocity ($p < 0.001$), and relative power ($p < 0.001$) increased significantly and progressively across all evaluation moments. Finally, relative force increased significantly between moments 1–2 and 1–3 ($p < 0.001$).

DISCUSSION

This study aimed to investigate the effect of 16 weeks of lower-limb strength training on the jumping performance of ballet dancers. We have compared the jumping performance variables during the CMJ of dancers before and after a training program, for 16 weeks. Our findings confirm the hypothesis formulated initially since the training program positively affected the CMJ height of the dancers. While the CG significantly decreased relative power values over time ($p < 0.001$), intragroup comparisons indicate that the IG significantly increased the CMJ height ($p < 0.001$, ES = 1.21: large), relative force ($p < 0.001$, ES = 0.86: moderate), maximal velocity ($p < 0.001$, ES = 1.15: moderate), and relative power ($p < 0.001$, ES = 1.37: large) after the 16-week training program. The ES interpretation and the individual response results suggest that these improvements in CMJ, relative force, velocity, and relative power represent almost certainly (100%) a positive effect from the training program (**Table 3**), which supports its efficiency.

In fact, our findings suggest that in pretest, the CG presented significantly higher CMJ ($p = 0.039$, ES = 0.911: large), relative force ($p = 0.004$, ES = 1.326: large), maximal velocity ($p = 0.035$, ES = 0.931: large), and relative power values ($p = 0.004$, ES = 1.328: large) in comparison to the IG. Regarding the CMJ, this advantage of the CG over the IG in pretest [29.33 (5.73) cm vs. 25.10 (3.70) cm, respectively] with a large ES is in opposition with the previous findings where the IG

TABLE 2 | Intergroup comparison of jumping performance variables pre-post intervention.

Variables	CG pretest (*N* = 10) mean (*SD*)	IG pretest (*N* = 14) mean (*SD*)	*p*	ES	CG posttest (*N* = 10) mean (*SD*)	IG posttest (*N* = 14) mean (*SD*)	*p*	ES
Weight (kg)	43.09 (9.48)	38.21 (4.38)	0.156	0.704	43.46 (9.69)	40.21 (4.56)	0.345	0.456
Height (m)	1.53 (0.11)	1.51 (0.07)	0.487	0.293	1.54 (0.11)	1.52 (0.07)	0.657	0.187
BMI (kg/m^2)	18.05 (2.46)	16.77 (1.02)	0.148	0.728	18.07 (2.42)	17.32 (1.08)	0.377	0.428
CMJ (cm)	29.33 (5.73)	25.10 (3.70)	0.039*	0.911	28.60 (6.24)	29.85 (3.43)	0.534	–0.261
Relative force (N/kg)	17.74 (0.96)	16.38 (1.07)	0.004*	1.326	17.19 (1.76)	17.36 (1.09)	0.773	–0.121
Maximal velocity (m/s)	1.20 (0.11)	1.11 (0.08)	0.035*	0.931	1.18 (0.12)	1.21 (0.07)	0.460	–0.311
Relative power (W/kg)	21.25 (2.80)	18.14 (1.96)	0.004*	1.328	20.89 (2.96)	20.99 (2.01)	0.923	–0.041

*CMJ, countermovement jump; ES, effect size (Cohen, 1988). *p ≤ 0.05.*

TABLE 3 | Intragroup comparison of anthropometric and jumping performance variables pre-post intervention.

		Variables	Weight (kg)	Height (m)	BMI (kg/m^2)	CMJ (cm)	Relative force (N/kg)	Maximal velocity (m/s)	Relative power (W/kg)
CG (n = 10)		Pretest mean (*SD*)	43.09 (9.48)	1.53 (0.11)	18.05 (2.46)	29.33 (5.73)	17.74 (0.96)	1.20 (0.11)	21.25 (2.80)
		Posttest mean (*SD*)	43.46 (9.69)	1.54 (0.11)	18.07 (2.42)	28.60 (6.24)	17.19 (1.76)	1.18 (0.12)	20.89 (2.96)
		p	0.069	0.096	0.574	0.273	0.256	0.263	<0.001*
		ES	0.04 (0.03)	0.04 (0.04)	0.01 (0.03)	−0.12 (0.18)	−0.48 (0.72)	−0.14 (0.21)	−0.29 (0.42)
		95% CL	0, 0.07	0, 0.08	−0.02, 0.04	−0.3, 0.07	−1.2, 0.24	−0.35, 0.07	−0.71, 0.13
		Inference	Trivial	Trivial	Trivial	Trivial	Small	Trivial	Small
	Individual response	Probability	Almost certainly	Almost certainly	Almost certainly	Likely	Possibly	Possibly	Possibly
		Positive-trivial-negative	0-100-0	0-100-0	0-100-0	1-78-21	6-19-75	1-69-30	3-32-65
IG (n = 14)		Pretest mean (*SD*)	38.21 (4.38)	1.51 (0.07)	16.77 (1.02)	25.10 (3.70)	16.38 (1.07)	1.11 (0.08)	18.14 (1.96)
		Posttest mean (*SD*)	40.21 (4.56)	1.52 (0.07)	17.32 (1.08)	29.85 (3.43)	17.36 (1.09)	1.21 (0.07)	20.99 (2.01)
		p	<0.001*	<0.001*	0.003*	<0.001*	<0.001*	<0.001*	<0.001*
		ES	0.43 (0.13)	0.19 (0.07)	0.51 (0.24)	1.21 (0.26)	0.86 (0.22)	1.15 (0.25)	1.37 (0.3)
		95% CL	0.3, 0.56	0.12, 0.26	0.27, 0.75	0.95, 1.47	0.64, 1.08	0.9, 1.4	1.07, 1.67
		Inference	Small	Trivial	Small	Large	Moderate	Moderate	Large
	Individual response	Probability	Almost certainly	Likely	Almost certainly	Almost certainly	Almost certainly	Almost certainly	Almost certainly
		Positive-trivial-negative	100-0-0	39-61-0	98-2-0	100-0-0	100-0-0	100-0-0	100-0-0

*95% CL, 95% confidence limits. *p ≤ 0.05 (significant differences); ES – effect size (Hopkins, 2004).*

presented an initial advantage in this variable (Escobar-Alvarez et al., 2019). Besides this initial advantage (of 4.23 cm in comparison to the IG), the intragroup comparisons indicate that the training program allowed the IG to increase this variable by 4.75 cm [achieving 29.85 (3.43) cm]. In contrast, the CG jump height declined to 28.60 (6.24) cm after the 16 weeks, despite previous findings indicating minimal changes in CG jump height over time [e.g., 27.3 (2) cm vs. 27.5 (2) cm] (Escobar-Alvarez et al., 2019).

Previous studies that observed the effect of training programs in the jump height of dancers have also obtained improvements in this variable with values pre-post diverging between 16.9 (2.9) cm and 18.9 (2.7) cm ($p < 0.001$, $d = 0.36$: small ES) pre-post 30 weeks of a plyometric training program and in CMJ with arm swing dancers jumped 21.5 (3) cm vs. 25 (2.8) cm ($p < 0.001$, d = 1.21: large), an ES equal to our findings in CMJ (Mlsnová and Luptáková, 2017); 22.50 (4.21) cm and 25.47 (4.95) cm pre-post a 10-week modern and recreational dance exercise program and trunk and leg muscle strengthening exercises (Stošić et al., 2020); 26.93 (2.78) cm and 27.35 (3.06) cm pre-post an 8-week protocol (Tsanaka et al., 2017); and 29.3 (3.2) cm and 33.5 (3.7) cm with a significant improvement (Escobar-Alvarez et al., 2019) aligned with our findings. Still, in studies without interventions, CMJ height values ranged between 23.34 (1.72) cm in dancers aged 15 (1.07) years (Rojano-Ortega, 2020) and 28.29 (3.42) cm in dancers aged 18.94 (1.32) years (Alvarez et al., 2020). In comparison, in this study, the average age of IG dancers was 12.43 (1.45) years and the pre-post CMJ height ranged between 25.10 (3.70) cm and 29.85 (3.43) cm, underlining the proficiency of our training program in the jump height of IG dancers.

Regarding the significant improvements in the IG relative force (16.38 (1.07) N/kg vs. 17.36 (1.09) N/kg, $p < 0.001$, ES = 0.86: moderate) and relative power (18.14 (1.96) W/kg vs. 20.99 (2.01) W/kg, $p < 0.001$, ES = 1.37: large), and since our sample was composed of younger dancers, it was important to calculate these variables relative to body mass of each dancer from simple computation measures based on body mass, jump height (from flight time), and push-off distance (Jiménez-Reyes et al., 2017). A previous study obtained a significant improvement in lower-body peak force after a resistance training program in adolescent dancers (Dowse et al., 2020). We observed that our relative power values are lower than those observed in other studies with older dancers, such as 27.14 (1.80) W/kg (Rojano-Ortega, 2020) and values pre-post an intervention in rhythmic gymnastics ranging from 21.66 (4.09) W/kg to 23.98 (4.48) W/kg with a 9.67% improvement according to the authors (Grande Rodríguez et al., 2010). Although we also obtained significant improvements in the IG maximal velocity [1.11 (0.08) m/s vs. 1.21 (0.07) m/s, $p < 0.001$, ES = 1.15: moderate], previous studies have also obtained higher values, such as 2.30 (0.12) m/s vs. 2.31 (0.13) m/s in dancers (Tsanaka et al., 2017) and 2.33 (0.18) m/s vs. 2.45 (0.23) m/s in rhythmic gymnastics (Grande Rodríguez et al., 2010). Previous findings suggest that dance training mainly develops velocity capabilities, and supplemental force training may be beneficial regarding the high number of dramatic elevations that dance performance requires (Alvarez et al., 2020).

TABLE 4 | Intervention group, intragroup comparison (3 evaluation moments).

Variables	N	Pretest mean (*SD*)	Posttest mean (*SD*)	Posttest2 mean (*SD*)	*p* (Wilks Lambda)	*Post hoc* (Bonferroni)
Weight (kg)	14	38.21 (4.38)	38.78 (4.34)	40.21 (4.56)	<0.001*	All moments
Height (m)	14	1.51 (0.07)	1.51 (0.07)	1.52 (0.07)	0.002*	Moments 1–3, 2–3
BMI (kg/m^2)	14	16.77 (1.02)	16.95 (1.05)	17.32 (1.08)	0.010*	All moments
CMJ (cm)	14	25.10 (3.70)	28.03 (3.82)	29.85 (3.43)	<0.001*	All moments
Relative force (N/kg)	14	16.38 (1.07)	17.01 (0.98)	17.36 (1.09)	<0.001*	Moments 1–2, 1–3
Maximal velocity (m/s)	14	1.11 (0.08)	1.17 (0.08)	1.21 (0.07)	<0.001*	All moments
Relative power (W/kg)	14	18.14 (1.96)	19.92 (2.06)	20.99 (2.01)	<0.001*	All moments

*$p \leq 0.05$.

As stated previously, we controlled the growth of the dancers over the 16 weeks of training using HPO measurements. Still, younger dancers are inevitably in maturation and growing processes, similar to previous studies (Dowse et al., 2020), which may be a possible justification for these dissimilarities. Additionally, female and male dancers did not differ significantly in the jumping performance variables ($p \geq 0.05$). The apparent sample homogeneity could be explained by the maturation process, whereas girls could be at a higher maturity stage and balanced the performance of the boys. Accordingly, we considered them as one group for the study analysis.

Since we could not measure the jumping performance every 3 weeks during the program, and 1 week after the end of the program as recommended (Jiménez-Reyes et al., 2019), the additional evaluation moment at week 8, precisely in the middle of the protocol, represented a control measure of the IG jump performance evolution. After 8 weeks of avoiding external load and promoting working with the bodyweight of an individual as much as possible following the recommendations for youth training Faigenbaum et al. (2009), the training program included exercises where dancers had to overcome the strength of a partner to perform specific exercises (e.g., leg press with the partner sitting on their feet, see **Supplementary Material**). This adjustment to the training program reveals that an external resistance may be an essential aid in improving the jumping performance of dancers (Escobar-Alvarez et al., 2019). Additionally, it discloses the importance of controlling the training and adapting it to the individual needs of the athlete (Alvarez et al., 2020), that is, of including intermediate evaluation moments within the training program applications in scientific research; considering both training content and training duration together may enable more individualized, specific, and effective training monitoring and periodization (Jiménez-Reyes et al., 2019).

The training program of this study included exercises that develop the strength component, exercises that stimulated the explosive strength, and ballistic exercises that correspond to the stretch-shortening cycle action (Escobar-Alvarez et al., 2019). Although not significant, the CG CMJ height, relative force, and maximal velocity values decreased and the relative power decreased significantly ($p < 0.001$, ES = − 0.29: small) during the 16 weeks with standard ballet classes. These findings clearly correspond to previous research suggesting that dance training alone may not provide sufficient overload to evoke a physiological change in adolescent dancers and identified that the inclusion of a strength training program facilitated improvements in maximum lower-body strength (Koutedakis et al., 2007) and vertical CMJ height (Brown et al., 2007). While the literature provides contradictory results regarding the determination of the optimal plyometric volume to enhance jumping performance, two previous studies suggest that a low volume in plyometric jumps can lead to a higher increase in CMJ (Chen et al., 2013; Baena-Raya et al., 2019). In contrast, a previous meta-analysis stated that training protocols based on jumps (plyometric) or resisted training weightlifting exercises provide similar results in jumping performance (Berton et al., 2018).

In fact, the significant and progressive increment over all of the evaluation moments of the CMJ ($p < 0.001$) is aligned with previous outcomes that also conducted additional evaluation moments (Escobar-Alvarez et al., 2019), suggesting that these protocols are an effective way to improve CMJ height in female ballet dancers. This is a significant step forward for the dance conditioning literature and provides a platform for research and practice in dance-specific additional training (Véliz et al., 2016).

Other studies also showed significant improvements in the jumping performance of dancers (Wang et al., 2010; Komeroski et al., 2016; Tsanaka et al., 2017; Escobar-Alvarez et al., 2019; Stošić et al., 2020) and rhythmic gymnasts (Piazza et al., 2014; Dobrijević et al., 2018; Dallas et al., 2020) as a result of training interventions, and the improvements in jumping performance are consistent with previous findings in ballet dancers (Escobar-Alvarez et al., 2019; Alvarez et al., 2020) and in other sports disciplines (Jimenez-Reyes et al., 2016, Jiménez-Reyes et al., 2019). We suggest an exercise prescription based on the individual needs and the physical demands of ballet, jazz, and contemporary dancers as referred to in previous studies (Alvarez et al., 2020; Dowse et al., 2020).

Our results are aligned with previous findings, suggesting that incorporating resistance training may enhance strength and power adaptations in adolescent dancers, which can be achieved with minimal equipment and can be performed in the training space of the dancers, as our previous design of the training programs indicates (Dowse et al., 2020; Skopal et al., 2020). Our findings support earlier recommendations regarding integrating resistance training methods (Véliz et al., 2016; Tsanaka et al., 2017) or strength and conditioning coaches (Tsanaka et al., 2017) to deliver systematic resistance training to adolescent dancers. Other authors also refer that this may facilitate skill acquisition during growth and reduce the potential for injury (Brown et al., 2007) and that the inclusion of strength training may be able to manage growth and maturational-related changes that commonly lead to decrements in strength, balance, and the ability to master dance-specific technical skills (Daniels et al., 2001). Furthermore, by demonstrating the potential for adaptation within an adolescent cohort, it is hoped that this will increase the awareness of the strength training benefits and encourage dancers and support staff to consider a more integrated approach to training (Dowse et al., 2020).

We recognize that our study presents some limitations, such as a reduced sample size in IG and CG, including only ballet dancers. It is not known whether the results are generalizable to other dance styles. We also acknowledge that our sample was formed by younger dancers who were in the maturation process. Although we did not control the maturity of the participants, the growth process was perceived by the HPO measure, since it would have a direct influence on the validity and reliability of the instrument used. This marker did not show a significant modification along the intervention period, which led the authors to assume maturity stability during the study. We also included female and male participants, which can influence the results. Lastly, the evaluation of the transference of improvement in jump height in a specific dance skill was not conducted.

CONCLUSION AND PRACTICAL IMPLICATIONS

A 16-week training program of lower-limb strength training using mainly own body mass effectively improve CMJ height in young dancers and can present practical implications for dance training. Supplementary strength training seems to be effective for improving jumping performance in ballet dancers.

We suggest that the incorporation of 20 min of strength and plyometric additional training could improve the jump height of the ballet dancers.

The design of the training program suggests that this is possible with no equipment and may be easily incorporated in the dance training schedule and the typical dancer's training space.

FUTURE RESEARCH

We suggest more investigation in this area, seeking a better understanding of the dance physical needs, making more information available for dance professors to better complement their training programs. Future studies should aim for a more individualized, specific, and effective training monitoring and periodization (e.g., variables measured every 3 weeks during the program and every week after the end of the individualized program) (Jiménez-Reyes et al., 2019). It would be important to assess if the study results could be transferred to perform ballet-specific skills.

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 Ethical Committee, Faculty of Sports Science and Physical Education, University of Coimbra (CE/FCDEF-UC/00742021). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LÁ-C, FC, JE-Á, and LR participated in study design, data collection, and the writing of the first draft manuscript. LÁ-C, FC, JE-Á, BG, IL, and LR participated in the article collection and analysis. LÁ-C, FC, JE-Á, IL, and LR participated in the writing of the methodology and results, and the final revisions of the manuscript. All authors have read and approved the final version of the manuscript and agreed with the order of presentation of the authors.

SUPPLEMENTARY MATERIAL

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Issues on Trainability

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Trainability is an adaptive response to given exercise loads and must be localized to the targeted physiological function since exercise-induced acute and chronic adaptations are systemic. Lack of adaptation or moderate level of adaptation in one organ or one physiological function would not mean that other organs or functions would not benefit from exercise training. The most beneficial training load could easily be different for skeletal muscle, brain, the gastro-intestinal track, or the immune systems. Hence, the term of non-responders should be used with caution and just referred to a given organ, cell type, molecular signaling, or function. The present paper aims to highlight some, certainly not all, issues on trainability especially related to muscle and cardiovascular system. The specificity of trainability and the systemic nature of exercise-induced adaptation are discussed, and the paper aims to provide suggestions on how to improve performance when faced with non-responders.

Keywords: responders, non-responders, VO2max, resistance training, systemic adaptation

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INTRODUCTION

Trainability is an important issue not only in elite sport but also for recreational athletes. It is often observed that a repeated exercise load does not cause increased performance for some subjects and those are referred to as non-responders (Bouchard and Rankinen, 2001; McLaren et al., 2018; Hortobagyi et al., 2021; Hrubeniuk et al., 2021; Mattioni Maturana et al., 2021). It is known that exercise-induced adaptation is different from the general adaptation theory developed by Selye (1975) since exercise-induced adaptation is specific. This specificity guarantees the necessity of different types and loads of training in various sports and explains the different phenotypes of sprinters, long-distance runners, or body builders.

One can suggest that non-responders are probably non-responders to a given training load and they can be responders when a personalized training load is given. Let's take an example of those who are referred to as non-responders to improve maximal oxygen uptake (VO2max) by endurance exercise with moderate intensity (about 70% of VO2max). VO2max is referred to as a cardiovascular fitness measure, but VO2max is dependent on heart and arteriovenous oxygen difference (a-v difference) and can be measured by incremental exercise protocols in the laboratory or can be apprised by a huge number of field activities (Radák, 2018b). VO2max in very well trained athletes can reach 80 ml/kg/min. A-V difference is very much dependent on the capillarization and mitochondrial number of the skeletal muscle. The appropriate training to enhance cardiac output and to a-v difference could be easily different.

Most published work uses either moderate-intensity exercise, around 70% of the VO2max or high-intensity interval training (HIIT) to test the trainability of endurance. Moderate intensity training mostly results in general adaptation including enhanced cardiovascular function, while HIIT causes metabolism-induced adaptation to skeletal muscle (Radák, 2018a). It has been reported in a recent study that the adaptation to moderate-intensity training varied to a greater degree than after HIIT and non-responders were not found in the HIIT group (Maturana et al., 2021). Moreover, it would be interesting to test how non-responders adapt to mixed training, which contains both moderate and high-intensity training. In the present review, we aim to point out some important issues on trainability, in order to help understand exercise-induced systemic adaptation and we also offer some suggestions on how to improve performance, when faced with non-responders. In addition, it is important to note that exercise-induced adaptation is systemic, and non-responders in one function or organ could be responders in other functions and organs.

ADAPTATION TO A SINGLE BOUT OF EXERCISE

Due to the nature of a single bout of exercise, the adaptive response is limited but it is systemic. One of the driving mechanisms that modulate adaptive response is the exercise-induced changes in metabolism and the generated metabolites. Since the degree of the elevation, the type of metabolism and the generated metabolites, and the main energy source of adenosine triphosphate (ATP) production are dependent upon the intensity of exercise, adaptation is dependent on intensity. In general, in the skeletal muscle, low-intensity exercise of long duration increases the resistance to fatigue, and high-intensity exercise leads to muscle strength and growth. At the cellular level, it can be measured by increased mRNA and short-lived protein levels. Exercise with a short duration and high intensity could elevate mRNA levels of enzymes involved in glycolytic metabolism because of the break-down of carbohydrate under anaerobic conditions, and the shortage of O2 delivery can activate hypoxia-inducible factor 1 (HIF1), and thus the inadequate availability of ATP can lead to activation of adenylate kinase, which can lead to phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and dependent cellular signaling and so forth. The decreased capacity to maintain the Na-K pump at the sarcolemma, the accumulation of metabolites such as lactate, ammonia, and inorganic phosphate, and the drop in creatine phosphate (CrP) levels could cause not only fatigue in the skeletal muscle, but they are also important initiators of adaptive response. This is very much true for reactive oxygen and nitrogen species (RONS) as well, which are produced at significant levels during high-intensity exercise (Radak et al., 2008a, 2011, 2013). Single bouts of exercise with low and moderate intensity and long duration would cause increases in the mRNA expression of proteins involved in aerobic metabolism of sugars and fatty acids. Single bouts of exercise with low intensity can readily cause dehydration and increased body temperature. Blood glucose and muscle glycogen content could be significantly decreased. There are overlapping signaling pathways of high and low-intensity exercise bouts on various physiological processes, such as improving insulin sensitivity, regulation of mitochondrial network, etc. The release of microRNA-s from skeletal muscle to the circulation is also dependent on the intensity of exercise. Hence, resulting in different adaptive responses (Ramos et al., 2018; Torma et al., 2020).

It is very important to understand that exercise-induced adaptation is systemic. Lack of adaptation or moderate level of adaptation in one organ or one physiological function would not mean that other organs or functions would not benefit from exercise training. The most beneficial training load could be easily different for skeletal muscle, brain, the gastro-intestinal track, or the immune system. Hence, the term of non-responders should be used with caution and just referred to a given organ, cell type, molecular signaling, or function.

Physical exercise impacts the immune system via complex regulations, which involve proper adjustment of pro- and anti-inflammatory cytokine, and neopterin production (Scheffer and Latini, 2020). It is suggested that exercise-induced elevation of proinflammatory interleukin 6 (IL-6) even can block tumor necrosis factor alpha and attenuate IL-1β signaling, hence exhibiting anti-inflammatory effects (Pedersen, 2017). It also has been shown that exercise-induced modulation of metabolism alters proinflammatory responses in macrophages and the modulation can involve the energy sensor, AMPK (Nieman and Pence, 2020). Sirtuin 1 (SIRT1), the activity of which and its contents readily respond to exercise training (Radak et al., 2020) also promotes anti-inflammatory and tolerance programs in multiple immune cell types (Yoshizaki et al., 2010). Metabolites, which are the product of catabolic processes of proteins, carbohydrates, and fats are involved in immune defense and acute phase responses, complement activation, and humoral responses mediated by circulating immunoglobulins (Nieman and Pence, 2020). It is suggested that following a single bout of exercise, the immune system efficiency decreases, which provides an open window, which increases the chance of upper respiratory diseases (Kakanis et al., 2010). High-intensity exercise appears to increase the risk of upper respiratory track-related infections (Wang et al., 2012) and extreme exercise loads can also lead to the temporary weekend immune system (Simpson et al., 2020).

Indeed, the immune response is intensity dependent, since a single bout of high-intensity exercise is associated with a greater acute phase leukocyte count and redox response than aerobic exercise (Jamurtas et al., 2018). However, T lymphocyte and monocyte are important parts of the immune system, due to their angiogenic potential they are also important contributors of adaptive response by initiating to blood vessel growth and repair. It has been shown that a single bout of high-intensity exercise results in greater elevation of T lymphocyte and angiogenic Tie2-expressing monocytes than low-intensity acute exercise (O'Carroll et al., 2019).

Hormonal secretions can be changed by a single bout of exercise (Kraemer et al., 2020; Luse et al., 2020), which, due to the short time period, might not cause a long term adaptive response, but could be important to sharpen the sensitivity of receptors and initiate signaling processes. It has been shown that high-intensity exercise can increase the level of circulating anabolic hormones to a greater degree than high volume training (Wahl et al., 2013). Moreover, the effects of exercise on insulin sensitivity are also well described (DiMenna and Arad, 2021). A single bout of exercise, due to the altered regulation of blood supply, could readily cause ischemia in the liver, kidney, and gastro-intestinal track and affect the bacterial flora of the microbiome (Radak et al., 2019). Moreover, a single bout of exercise increases the levels of circulating microRNA (miR) (Denham and Prestes, 2016). These skeletal muscle-originated myo-miRs play a significant role in acute exercise-associated miR elevation. MiR(s) also provide a further control of translation, since they can readily lead to degradation of targeted mRNA (s) and thus prevent protein synthesis. Due to the systemic effects of exercise, oxygen and energy supply of the brain can be altered, which could cause modulation of neurotrophins, lactate uptake, etc. (Radak et al., 2019). Acute exercise can lead to increases in the level of circulating brain-derived neurotrophic factor (BDNF), and the results of animal studies have revealed that BDNF is increased following a single bout of exercise in the hippocampus of rats (Shahandeh et al., 2013).

When resistance exercise is done, acute resistance exercise with high muscle tension can cause damage to sarcomeres, which might be important in satellite cell proliferation, that can later on lead to myonuclear accretion (Damas et al., 2018). Moreover, high-intensity single exercise bouts activate phosphorylate transcription factors that, after repeated stimulation (which happens during chronic exercise), leads to increased muscle mass. When mouse skeletal muscle was treated with 50 high-intensity eccentric exercise, it turned out that serum response factor (SRF) activity is linked to a histone modification cascade starting with the phosphorylation of serine 10 on histone 3 (H3S10ph) (Solagna et al., 2020). The phosphorylation of histone can lead to increased protein synthesis which requires mitogen- and stress-activated kinase 1 (Solagna et al., 2020). The acute response of high-intensity exercise also causes phosphorylation of myocardin—related transcription factors on Ser66 (Solagna et al., 2020). The reversible modification of histone by acute exercise indicates that repeated exercise causes epigenetic modifications. Indeed, the effects of acute straight line running and running with 180-degree change of direction (mimicking ball game running) were studied on the DNA methylation of skeletal muscle and results revealed that overlapping methylation of many genes, and exercise specific methylation were also observed (Maasar et al., 2021). Acute exercise results decreased methylation of the whole genome, which could be an important step to initiate exercise-induced gene activation (Barres et al., 2012). It is important to note that the degree of the methylation of peroxisome proliferator-activated receptor gamma, coactivator 1 α (PGC-1 α), pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4), and peroxisome proliferator-activated receptor δ (PPAR-δ), promoter regions were dependent on the intensity of a single bout of exercise (Barres et al., 2012), which further supports the idea that exercise-induced adaptation is dependent on the intensity of the exercise. The methylation of other organs is also modified by a single bout of exercise. It has been shown that acute restraint stress decreases global DNA methylation in the hippocampus, cortex, and periaqueductal gray in brain of rats, and this alteration was attenuated by a single bout of exercise, suggesting that exercise has the potential to modulate changes in DNA methylation and gene expression (Rodrigues et al., 2015).

The cellular and systemic responses to exercise with different intensities and durations are different. One of the well-known concepts of exercise-induced adaptation is shown in **Figure 1**. There is no reason to believe that a single bout of exercise, with over a certain intensity and duration, does not cause some of the biochemical, and physiological changes, which are the initiative of long-term adaptive responses. However, there is no guarantee that chronic training is always associated with enhanced performance.

ADAPTATION TO LONG TERM EXERCISE

The adaptation to long-term exercise is much more complex than the biochemical, and physiological responses to a single bout of exercise. Depending on the level of physical fitness, 4–12 weeks of training, 3–20 training sessions a week, with 30 to 180 min duration, could be necessary to improve sport performance. In order to increase the performance from a high level of physical fitness, one has to work very hard. Even daily 4–8 h of training is not exceptional for certain sports. This period of repeated training sessions with rest periods can lead to the synthesis of training intensity/duration-dependent targeted proteins and related improvement of targeted biochemical and physiological processes, including metabolism, receptor sensitivity, regulation of autonomic nervous system, and so forth.

Overall, the targeted reversible changes in histone and DNA modifications, the activation of signaling pathways, microRNA, and mRNA expressions that are observed as a result of a single bout of exercise, turn into production of proteins, alteration of neuro-endocrine regulation, and the immune system leads to improved physiological function and altered phenotype following repeated cyclic training loads (Denham et al., 2014; Howlett and McGee, 2016; da Silva et al., 2017; Quan et al., 2020). Regular exercise is carried out to bring about functional changes although in a different manner in most of the organs.

On the other hand, it is also clear that long-term adaptation is not a linear process. Even with the best personalized training loads, there are periods with the absence of increased performance, which can last for weeks, months, or even for years (**Figure 2**). Plateaus are a normal part of the adaptive response, and the presence of a plateau does not mean that athletes are non-responders.

Among other influencing factors, adaptation is mainly dependent on the appropriate loading and resting cycles. It is generally accepted, that the improvement of those performance influencing factors is most meaningful, and the most significantly limiting factor for the performance. If we take an example of the athlete with an 8 L/min cardiac output, and 70% type I fibers in the significantly working skeletal muscle groups of his/her event, and whose training plan contains only moderate-intensity exercise loads, he/she might easily plateau during

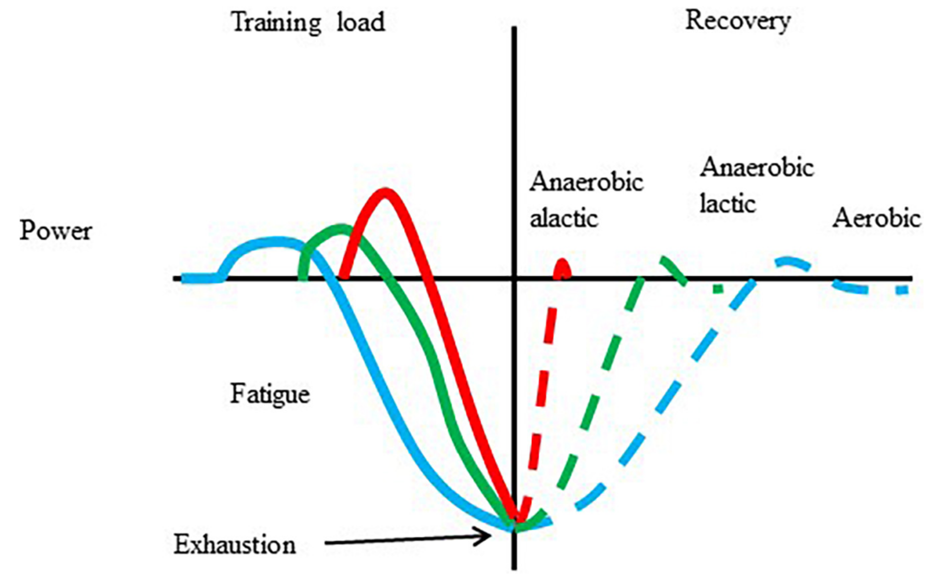

FIGURE 1 | Adaptation to a single bout of exercise. Exercise results in increased metabolism, and power, which, after repeats can lead to fatigue and exhaustion. The length of recovery is dependent on the type of exercise that caused the fatigue and the level of fatigue. During the recovery periods overcompensation is possible.

preparation, while this kind of training could benefit the same size of athletes with 6 L/min cardiac output. Sprint interval training (SIT) sessions or HIIT, on the other hand, could be quite useful to the 8 L mentioned athlete above, since these training sessions have very significant effects on muscle metabolism. It is also known that, due to the different recruiting thresholds of type I and type IIB and IIx fibers, the mitochondrial mass is increased by intensity and duration-dependent manner (Bishop et al., 2014). Therefore, if the given training intensity/duration is not tailored to the person it is easy to accept that the training does not cause measurable increase in the performance, and we can call those individuals non-responders. Given the enormous resources available to sport science it is not very difficult to design individual tailored training programs, to minimize the duration of plateaus and avoid non-responding training periods. It is important to note that exercise-induced adaptation is systemic, yet the question arises whether non-responders are non-responders in a systemic sense or simply in some physiological function.

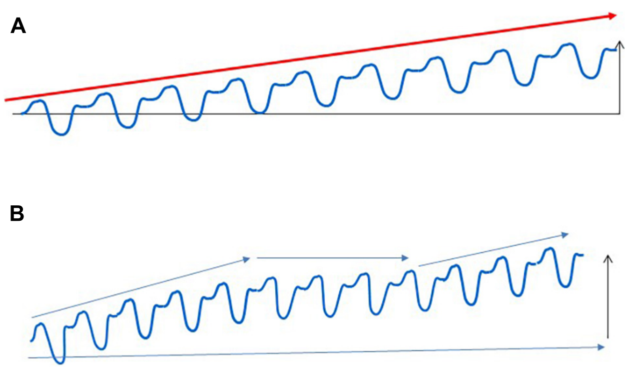

FIGURE 2 | Adaptation to regular exercise bouts. Regular exercise does not cause a linear improvement in sport performance, which is shown in **(A)**. Long-term exercise training generally leads to increased performance for the untrained, but the adaptive response of trained subjects contains many periods with unchanged, sometimes even decreased performance **(B)**. These adaptive plateaus are a normal part of the adaptive response, and athletes whose preparation contains these plateaus cannot be regarded as non-responders.

TRAINABILITY

It has been known for a long time that the same training program can result in different adaptive responses to different individuals and this observation was the basis for the development of personalized training loads. Interestingly, the training load which is used in most of the reported training studies is the same for all subjects. This can possibly explain the great variability of different training responses. Each subject participating in these studies could have a very different training experience, related to his/her inherited genetic setting, with different limits that influence training responses to the given load. As explained earlier, the ratio of fiber types could significantly influence the increase of mitochondrial mass to a given endurance training program, and the mitochondrial mass could influence VO2max, which often is used as a measure of trainability in endurance events. An animal model was recently introduced to study trainability (Koch et al., 2013). This model was set up using a genetically heterogeneous rat population (N/NIH stock) to develop lines named low response trainers (LRT) and high response trainers

(HRT). Selection was based on the change in maximal running distance evaluated by a treadmill-running test to exhaustion. In the untrained condition, LRT and HRT rats were similar for exercise capacity. However, after receiving 9 weeks of a standard amount of endurance training, HRT rats improved, on average, by 200 meters for distance run whereas those bred as LRT failed to improve and, on average, declined in running capacity by 65 meters (Koch et al., 2013). We tested the different adaptive response of LRT and HRT rats after 3 month of endurance training at 70% of VO2max (Marton et al., 2015). We found that the alterations in the levels of VO2max, RONS, SIRT1, NAD (+)/NADH ratio, proteasome (R2 subunit), and mitochondrial network-related proteins such as mitochondrial fission protein 1 (Fis1) and mitochondrial fusion protein (Mfn1) were not related to trainability for these rats. However, data suggested that PGC1-α, nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), and Lon protease might be linked to trainability to the given exercise protocol. These results further suggest that HIIT training could possibly be more effective to train the LRT group, since HIIT more readily induced PGC1-α, NRF1, TFAM than aerobic exercise (Williams et al., 2019). The lack of non-personalized training, therefore, may be one of the reasons for the lack of a training response.

Nutritional habits could directly impact trainability. One interesting example is the possible effect of antioxidant supplementation on performance. The general belief is that antioxidants cannot directly improve exercise performance but could play an important role in preventing or attenuating exercise-induced muscle damage (Sureda et al., 2014; Decroix et al., 2018; Nocella et al., 2019). However, research-based opinions suggest pro and contra roles on the effects of antioxidant supplementation on adaptive responses. RONS are important signaling molecules of exercise-induced adaptation (Davies et al., 1982; Radak et al., 2008b; Torma et al., 2020), but is it possible that Vitamin C and E cocktails eliminate the systemic effects of exercise? This is certainly not the case. Antioxidant supplementation could down-regulate some cellular signaling processes (Gomez-Cabrera et al., 2008) and it has been shown that although the VO2max of the subjects increased from 41.2 to 45.6 ml/kg/min after 9 weeks exercise with daily 1 g of Vitamin C supplementation the increases were not significant, while untreated groups showed significant increases (Gomez-Cabrera et al., 2008). Therefore, the Vitamin C supplementation attenuated the effects of exercise, in a given group, but this did not eliminate or curb the beneficial effects. Indeed, it seems unlikely that the complex effects of exercise can be blocked by antioxidant supplementation (Higashida et al., 2011). The beneficial effect or the possible attenuation of exercise-induced adaptation of antioxidant supplementation could be dependent on the timing of supplementation and the level of physical fitness (Radak et al., 2017). A great number of elite athletes use antioxidant supplementation in order to support exercise performance. However, it seems improbable that antioxidant supplementation would increase or decrease exercise performance to a measurable degree (Reid, 2016; Bowtell and Kelly, 2019; Higgins et al., 2020; Arazi and Eghbali, 2021). According to our present knowledge, it seems unlikely that the antioxidant supplementation could create a non-responding group to exercise training.

What about trainability and resistance training? It is clear from the literature that protein uptake can directly influence the rate of muscle metabolism and, up to a degree, the development of muscle hypertrophy (Morton et al., 2018a). However, the intensity and duration of resistance training are the main factors of exercise-induced muscle plasticity. Results suggest that sensitivity of tension-mediated signaling pathways and the number of androgen receptors could make the difference between responders and non-responders (Morton et al., 2018b). Moreover, it has also been suggested that the difference in biogenesis of ribosomes (Figueiredo et al., 2015) and satellite cell proliferation capacity (Petrella et al., 2008) could be factors. Knocking out the paired box 7 (Pax7) gene, which is often used to identify satellite cells, results in muscle weakness and early death (Kuang et al., 2006). The difference in the expression patterns of miR (s) (Davidsen et al., 2011; Ogasawara et al., 2016) could also account for the different training responses to resistance training. All of these training adaptation limiting factors could be the result of a different genetic setting, in other words, the adaptation to training is very individual including epigenetic modifications that are due to exercise training, nutritional habits, and other lifestyle and environmental factors (Voisin et al., 2015).

One of the exercise-dependent controlling factors of adaptation is the methylation of DNA and post-translational modification of histone residues. Hypo-and hyper-methylation of CpG island of the promoter regions of genes can activate and silence transcription and directly alter adaptive responses. A single bout of exercise results in hypomethylation of whole DNA, and the promoter region of some genes which are important in exercise-induced adaptation (Barres et al., 2012), suggesting a regulatory role of methylation in trainability. Due to modulating role of exercise on methylation, the plasticity of this system makes it possible to convert non-responders to responders and vice versa. The adaptability or trainability to a given exercise program is initiated by methylation-controlled transcription, followed by translation. This notion is supported by the observation that the short-chain fatty acid, butyrate, which is produced at an elevated rate by the gut microbiome is an adaptive response to exercise training (Abraham et al., 2019) can readily change DNA methylation in fibroblasts (Parker et al., 1986). Although the direct evidence that methylation readily impacts trainability is rare, the health-associated consequences of exercise modulated DNA methylation are well known. It turns out that regular exercise hypermethylates the TRIM59 gene, which is a powerful oncogene, and KLF14 genes, which regulates inflammation (Spolnicka et al., 2018). The downregulation of these genes is associated with the anti-tumor and anti-inflammatory activities of regular exercise (Spolnicka et al., 2018).

Trainability is a complex phenomenon because exercise-induced adaptation is systemic (**Figure 3**). Most trainability studies focus on the adaptive response to the targeted training. However, adaptation in organs distant from skeletal muscle could significantly affect sport performance and health. The exercise-induced adaptation of liver directly affects exercise performance

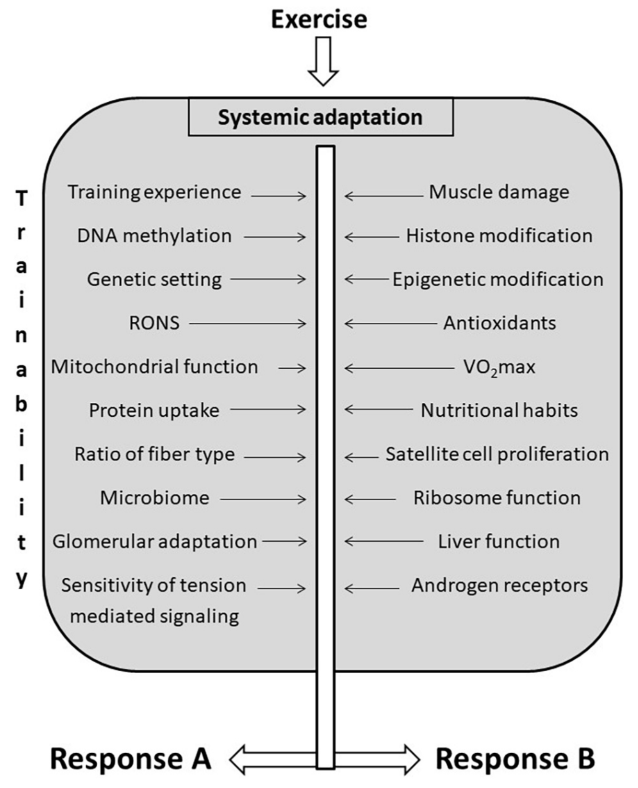

FIGURE 3 | Factors that influencing trainability. There are a great number of factors that influence trainability of different organs. Exercise-induced adaptation is systemic, and the same training load could cause different adaptive responses in different organs indicated as **(A,B)**, to different functions, and to different individuals.

because liver controls lactate (Proia et al., 2016), carbohydrate, fat, and protein metabolism, partly by the generation of hepatokines such as Fibroblast Growth Factor 21, Fetuin-A, Angiopoietin-like protein 4, and Follistatin (Ennequin et al., 2019). Glomerular adaptation to exercise training, which involves filtration of lactate, Na^{+} and K^{+} and the adjustment of pH and hydration also directly alters exercise performance (Mallie et al., 2002). The adaptive response of training could lead to increased contractile response to sympathetic agonists of renal arteries as a result of a significantly reduced blood flow during exercise bouts (Kocer et al., 2011). Moreover, the systemic effects of adaptation to exercise training cover the adaptive response of the microbiome as well. It has been shown that endurance exercise resulted in elevation of the relative abundance of *Veillonella atypica* in the gut microbiome (Scheiman et al., 2019). This bacterium converts fatigue-inducing lactate into energy-providing propionate, and hence, supports endurance performance (Scheiman et al., 2019). At the moment we don't have enough information on how the intensity of exercise training influences microbiome and the relative abundance of different bacterial strains. It is known that very long term exhaustive exercise can easily cause gut ischemia and gastrointestinal problems (de Oliveira and Burini, 2009), which most probably would differently alter the microbiome status of the gastrointestinal track. Using Zucker rats it was found that although HIIT was more effective to decrease epididymal fat mass than moderate-intensity exercise, the microbiome contents did not differ significantly (Maillard et al., 2019).

The repeated exercise bouts associated with energy demands result in adaptive responses to liver, increasing fat oxidation, which serves a protective role against fatty liver diseases (Shephard and Johnson, 2015). Regular exercise beneficially affects the function of kidneys by maintaining mitochondrial function and suppressing inflammation (Radak et al., 2020). Regular exercise can easily lead to increased brain-derived neurotrophic factor (BDNF) concentration in the brain, and elevated BDNF beneficially affects brain plasticity, memory, mood, and viability of neurons (Gomez-Pinilla and Hillman, 2013; Kujach et al., 2019; Quan et al., 2020).

The present paper intends to show the complexity of exercise-induced systemic adaptation and point out how difficult it could be to divide the subjects into responders and non-responders, when the whole body is responding. Despite the systemic adaptation, the organ-dependent adaptation varies a lot at different intensities and loads, and the so-called optimal training load is different for different organs. Overall, trainability is considered to be the adaptive response of the targeted condition. Nonetheless, it must be kept in mind that the exercise performance is dependent on the response of many organs and a large range of influencing factors. The lack of or attenuated improvement of, the targeted condition to exercise training, might have many causes including adaptive plateau, improper loading, genetic limitation, epigenetic alteration, and so forth.

LIMITATION OF THE STUDY

One of the great limitations is the complexity of trainability, which appears to be very individual, and as of it, uniform approaches can provide limited information. Indeed, vast range of trainability studies used only one exercise protocol to all subjects without pointing out the individual limiting factor (s) of the targeted training goal. Therefore, the review of the results of these studies probably cannot provide realistic data on trainability. There is a huge limitation to gain functional results of heart, brain, liver, kidney, immune system, microbiome, and other organs especially in human studies, however, these elements can directly or indirectly affect trainability. The other limiting factor could be the possible plasticity of trainability, as we change one limiting factor the following limiting factor could be changed by very different training and the possible interactions are not well known. The present paper could just show a small part of trainability, which is part of an extremely complex adaptive response to exercise.

FUTURE PERSPECTIVES

Appropriate testing of physiological functions of different systems and organs, like muscle and cardiovascular system and brain, before and after different training loads would be important to better understanding exercise-induced adaptive response. Moreover, using biomarkers to assess liver-, kidney-, and immune function along with the status of the microbiome would also add a lot to understanding the enigma of trainability.

AUTHOR CONTRIBUTIONS

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

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