MUSCLE RECOVERY AFTER EXERCISE, TRAINING AND COMPETITION: PHYSIOLOGICAL INDICATORS AND NON-INVASIVE MONITORING TECHNIQUES

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MUSCLE RECOVERY AFTER EXERCISE, TRAINING AND COMPETITION: PHYSIOLOGICAL INDICATORS AND NON-INVASIVE MONITORING TECHNIQUES

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The desire to improve muscle function and prevent overuse injuries from exercise and throughout training has led to the development of various methods to aid recovery and track readiness to perform. Ergogenic aids such as cold-water immersion, massage, and dynamic recovery procedures may have positive effects but the results of the related research remain equivocal. Furthermore, novel interventions in this scenario, like compression garments, ice vests, and photobiomodulation therapy are promising but need more evidence-based data to support their effectiveness.

Similarly, to properly monitor individual physical conditioning, there is a growing interest toward unobtrusive measures to accurately represent physiological status during and/or after exercise. There are several techniques being used, such as subjective ratings of well-being, heart rate monitoring, hormonal and hematological profile assessments. However, more sensitive indexes like heart rate variability and muscle activation (voluntary and/or involuntary) are arising as attractive alternatives that may delineate physical conditioning status and readiness to perform more precisely than the aforementioned measures.

The purpose of this Research Topic is to critically evaluate and summarize recent data from observational and intervention studies related to non-invasive methods designed to promote recovery and objectively monitor training status. Their association to physical performance and physiological recovery in athletes during training and competition is a major focus of this Topic.

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ORIGINAL RESEARCH
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Green Tea Extract Preserves Neuromuscular Activation and Muscle Damage Markers in Athletes Under Cumulative Fatigue

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A main implication of cumulative fatigue is the muscle damage that impairs neuromuscular function and training adaptations. These negative effects may limit performance when athletes exercise in consecutive days. In this regard, antioxidant supplementation has gain popularity among athletes. Green tea supplementation has been advocated as a strategy to improve exercise recovery due to the activity of its catechins with high antioxidant and anti-inflammatory potential. Here we performed a triple blinded placebo control experiment to determine the effect of green tea extract (GTE) from *Camellia sinensis* on muscle damage, oxidative stress, and neuromuscular activity in athletes submitted to consecutive sessions of exercise and fatigue. Sixteen trained amateur male athletes were randomly assigned to a GTE supplemented (500 mg/day) or placebo group during 15 days. Effects of supplementation were tested during repeated trials of submaximal cycling at 60% of peak power output performed after a protocol for cumulative fatigue of knee extensors. Muscle damage and oxidative stress showed lower magnitudes in response to fatigue after GTE supplementation. Placebo group showed impaired neuromuscular activity and higher muscle damage and oxidative stress compared to the GTE group during the cycling trials under fatigue. In summary, GTE supplementation showed positive effects on neuromuscular function in response to a condition of cumulative fatigue. It suggests GTE supplementation may have potential to serve as a strategy to improve performance and recovery in conditions of cumulative exercise.

Keywords: endurance, fatigue, exercise recovery, polyphenols, *Camellia sinensis*

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INTRODUCTION

Muscle fatigue is considered a limitant of athletic performance (Abbiss and Laursen, 2005). Its origin is multifactorial (Enoka and Duchateau, 2008), but it has been accepted that fatigue involves ATP depletion, muscle damage, and increased production of reactive oxygen species (ROS) resulting in a condition of oxidative stress (Hultman et al., 1986; Noakes, 1987; Steinbacher and Eckl, 2015). In general, fatigue negatively affects force production, and in the case of cycling,

pedal forces, power output, and cadence are impaired (Diefenthaeler et al., 2012). Repeated bouts of strenuous exercise lead to a condition of cumulative fatigue in which the capacity of force production reduces, and for quadriceps muscles the recovery of force production after fatigue may need up to 3 days (Stewart et al., 2008).

In addition to the acute effects of fatigue on performance, consecutive sessions of exercise under a fatigue state may result in poor performance during training sessions and competitions (Stewart et al., 2008; Rodríguez-Marroyo et al., 2017). Conditions of cumulative fatigue may also increase the risk of injuries (Shing et al., 2016) and promote negative psychobiological adaptations (Rodríguez-Marroyo et al., 2017). However, there are many situations in which athletes have no choice other than sustain the performance under fatigue. This is the case of ultra-marathons, trail running, cycling distance challenges, and professional or amateur cycling tours (Lucia et al., 2001; Rodríguez-Marroyo et al., 2017). Therefore, strategies to minimize the fatigue effects on performance of repeated bouts of exercise are of interest for both coaches and athletes. A plausible strategy to achieve this purpose is to promote a faster exercise recovery.

In this regard, supplementation with natural products has attracted interest of athletes from different competitive levels. Considering that fatigue and its effects on performance during repeated sessions of exercise have important participation of oxidative stress and muscle damage (Kyparos et al., 2007), there is a crescent interest in supplementation with antioxidants like the green tea extract (GTE) from *Camellia sinensis*. GTE is rich in polyphenols including epigallocatechin gallate, epicatechin, epigallocatechin, and epicatechin gallate, which result in a powerful antioxidant activity (Jowko, 2015; Schimidt et al., 2017). Previous studies showed that GTE supplementation might reduce oxidative stress (Sugita et al., 2016) and promote improvement in the maximal oxygen uptake during cycling to exhaustion (Richards et al., 2010). Furthermore, GTE can reduce muscle soreness resultant of eccentric exercise (Herrlinger et al., 2015) and decrease markers of muscle damage after eccentric exercise (da Silva et al., 2018), intense aerobic exercise (Kuo et al., 2015), and strength exercises (Herrlinger et al., 2015). Similar effects were not found when a single-dose of GTE was intake before intense muscle-endurance tests (Jowko et al., 2012). The effects described for GTE supplementation on muscle damage and oxidative stress suggest that GTE could be a valid strategy to preserve performance during repeated bouts of exercise leading to a cumulative fatigue. To the best of our knowledge, our study is the first to address this question.

The potential effect of GTE supplementation on performance under a fatigue state has important practical applications. For instance, amateur competitions can involve consecutive days racing without a proper time for recovery (Shing et al., 2007; Magrini et al., 2017), and among professional athletes the recurrent performance along several consecutive days is a common condition. Therefore, the main goal of our study was to determine whether GTE supplementation minimizes muscle damage and oxidative stress contributing to the preservation of neuromuscular function in trained athletes exposed to consecutive sessions of exercise leading to cumulative fatigue.

MATERIALS AND METHODS

Participants

We performed a randomized triple blinded placebo control experiment. Upon start of the study, 22 healthy trained men were recruited, but 16 completed all the phases of the study and had the data included in the analysis. They were randomly assigned to an intervention (green tea, $n = 8$) or placebo group ($n = 8$).

Participants were enrolled in systematic competitive cycling and/or running training including at least three sessions/week and had exercised more than 5 h/week in the past 12 months. The competitive level included participation in state and national competitions. During the study participants were requested to avoid ingestion of any medicine or stimulants, and to keep their regular routine of training and diet. Participants were followed regarding any injury, abrupt changes in their training volume and/or intensity. They should inform the need to start any medical treatment during the entire experimental phase. Six participants were excluded due to these criteria and therefore we had eight participants in each group. Intervention group was supplemented with green tea extract and the control received capsules with placebo. **Table 1** describes the study participants.

Experimental Design

Experiments started with the participants completing an incremental maximal cycling test to determine the individual peak power output (PPO). In the following days they performed submaximal cycling trials combined or not with sessions of knee extension exercise to fatigue.

The whole experiment lasted 23 days for each participant. In the different visits to the laboratory, neuromuscular parameters were determined based on electrical neuromuscular activity; muscle damage and oxidative stress were determined from blood samples; and a cardiac monitor recorded heart rate. Data were compared between the GTE and placebo groups and between the conditions with or without the fatigue. All participants were evaluated with or without fatigue. In the case of non-fatigue condition, we ensured at least 5 days without supplementation and without performance of vigorous exercise (Chow et al., 2003, 2005), being at least 2 of the 5 days without performance of any exercise (Ahtiainen et al., 2003) before the laboratory tests. We call fatigue the condition of being tested after completing knee extension trials until exhaustion in two consecutive days before a submaximal cycling trial. **Figure 1** illustrates our

TABLE 1 | Characteristics of study participants.

Characteristic	Green tea extract ($n = 8$)	Placebo ($n = 8$)
Age, years	37 (10)	37 (7)
Body mass, kg	77 (8)	76 (10)
Height, cm	170 (1)	170 (1)
Training experience, months	88 (64)	59 (48)
Training frequency, hours per week	7.2 (1.1)	7.2 (1.4)

Data are presented as mean (standard deviation).

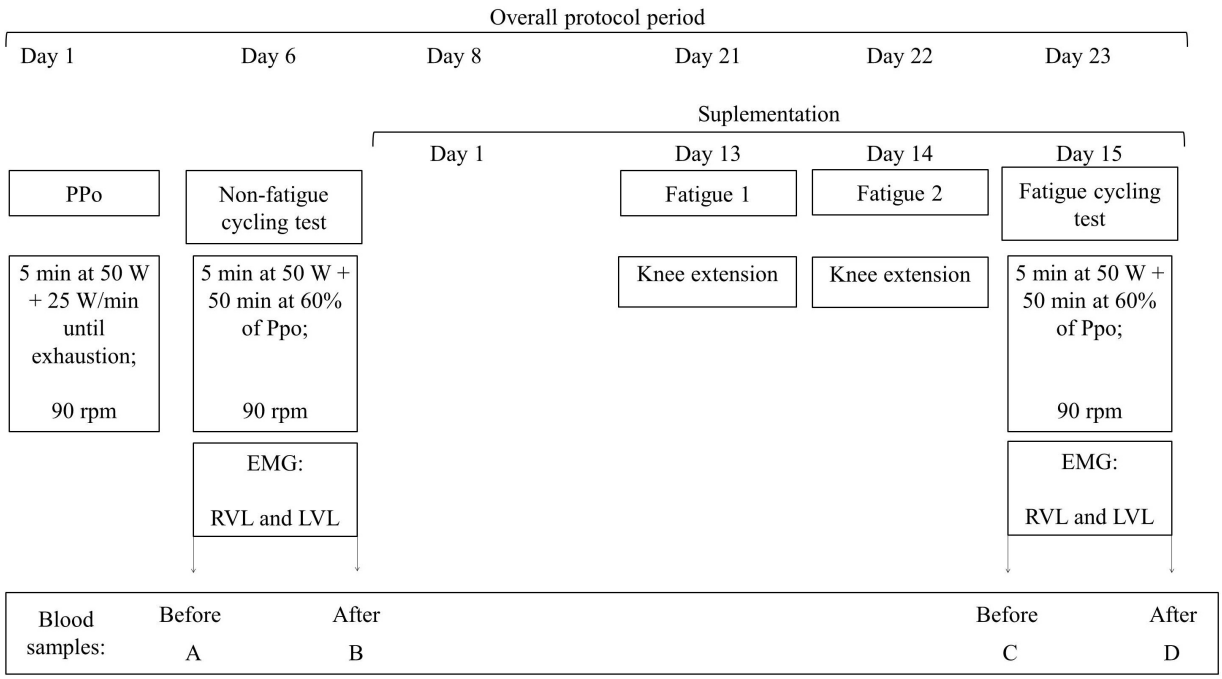

FIGURE 1 | Experimental design. All participants completed the same protocols. Experiment started with the incremental maximal test to determine peak power output (PPO). Five days later, participants completed a submaximal cycling trial at constant load of 60% PPO, and the randomization of GTE and placebo supplementation was performed. In the last 3 days of supplementation participants repeated the submaximal cycling trial after two sessions of knee extensors exercise to fatigue. To avoid learning effects from the first test on the results after fatigue condition, half of the participants from each group performed the non-fatigue submaximal cycling test after the fatigue period, and the other half before. rpm: cadence, in revolution per minute, PPO: PPO, EMG: record of electrical muscle activation by surface electromyography, RVL: vastus lateralis from right leg, left vastus lateralis (LVL): vastus lateralis from left leg.

experimental design. All participants signed a consent term before starting participation in the study; procedures were conducted in agreement with declaration of Helsinki, and this research was approved by the institutional committee of ethics in research (IRB no. 60376216.4.0000.5323).

Cycling Protocols

Cycling trials were performed always between 3 and 6 pm on a cycle ergometer (Lode Excalibur Sport, Lode, Netherlands) properly adjusted to the individual body posture of the participants. The incremental maximal test for determination of PPO started with a 5 min warm up at 50 W and cadence of 90 rpm followed by increments of 25 W/min until the participant was no longer able to keep pedaling cadence higher than 70 rpm. The last workload completed was therefore named the PPO (Priego Quesada et al., 2016). The submaximal tests started with workload of 50 W during 5 min and then the workload was increased to 60% of the individual PPO (Priego Quesada et al., 2016), which was sustained for 50 min.

Neuromuscular Assessment

Neuromuscular electrical activity was determined during the submaximal cycling tests using surface electromyography (EMG). EMG signals were recorded bilaterally from the vastus lateralis, which was selected due to its main role for power production in cycling (Bini et al., 2008). Data were sampled at 1.5 kHz using an EMG acquisition system (miniDTS and MyoMuscle, Noraxon, United States) following SENIAM guidelines for electrode placement and subject preparation (Hermens et al., 2000). EMG signals were filtered using a band-pass digital Butterworth filter with cut off frequency of 0.5–250 Hz. Onset and offset of neuromuscular electrical activity for each contraction burst were determined using the criteria of variation of two standard deviation for increase and decrease considering the average activation recorded during rest (Hodges and Bui, 1996). From each contraction burst during the cycling trials, the root mean square (RMS) value was determined as an indicator of magnitude of activation (Moritani et al., 1986) and the fast Fourier transform was computed to determine the median frequency, which was used as an indicator of fatigue (Cifrek et al., 2009). EMG signals were recorded alternating between 5 min of recording EMG (named "moment" 1–5, in the Section "Results") and 5 min without recording. At the end, for each participant we had five moments of 5-min EMG record. EMG data from moment 1 (5–10 min of exercise) was considered the reference to the normalization of RMS values obtained during the exercise.

Fatigue Condition

We aimed to elicit a condition of cumulative fatigue by combining 2 days of strenuous knee extension exercises until exhaustion and the performance of a submaximal cycling trial

on the subsequent day. The trials for knee extension were also performed between 3 and 6 pm using a seated knee extensor machine with the participant performing concentric-eccentric knee extensions until exhaustion. The first set had the workload equivalent to 50% of the individual body mass. A metronome set at 20 beats per minute controlled the movement velocity. In the first set of repetitions the maximal number of voluntary repetitions was determined. After 30 s of rest, participants performed the second set aiming at a number of repetitions corresponding to 75% of the maximal number of voluntary repetitions performed in the first trial. It was repeated until participants were no longer able to perform more than 50% of the maximal number of voluntary repetition in the first trial. Cycling trial was performed 24 h after the second fatigue protocol.

GTE Supplementation and Placebo

Participants received 15 capsules not identified and were advise to intake one capsule per day, before breakfast, with a glass of water. The capsules from GTE and placebo groups were identical. Supplementation was administrated in capsules because this strategy results in larger bioavailability (Henning et al., 2004). Capsules content were GTE and celulomax E, an inactive excipient that served as a placebo. GTE dose was defined considering the results from a previous study in which the same supplementation dose reduced fatigue-induced muscle damage (da Silva et al., 2018). GTE was purchased from a local commercial supplier, manipulated by a pharmaceutics registered professional, and tested using high performance liquid chromatography (HPLC) to ensure the presence of epigallocatechin gallate (1.60 mg/g), epicatechin (1.59 mg/g), epigallocatechin (16 mg/g), and epicatechin gallate (17.80 mg/g). HPLC was performed with a Shimadzu Prominence Auto Sampler (YL9100) system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator, and YL9160 diode array detector. To determine compounds profile the extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column (4.6 × 250 mm). The mobile phase was composed of water (pH = 3): acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100% acetonitrile. At 38 min water (pH = 3): acetonitrile (5:95, v/v) was used again, in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8 mL/min was used and 20 μL of sample were injected. Phenolic compounds were identified and quantified by comparing the retention time and UV–Visible spectral data to known previously injected standards. The chromatography peaks were confirmed by comparing the retention time with those of reference standards and by DAD spectra. Calibration curves were determined for EGC (y = 101,79x − 10,283); EC (y = 91,872x + 7657); EGCG (y = 103,5x − 93,211); ECG (y = 112,17x − 81,22). All chromatography operations were performed at ambient temperature and in triplicate. During the supplementation period participants were requested to report any consume of stimulants, other supplements, medications, and teas originated from *C. sinensis* or other plant. Furthermore, they were requested to avoid consume of fruits, milk, caffeine, and alcohol on the day before each cycling tests when blood samples were collected (Sugita et al., 2016). Participants received daily messages to recall them about the orientations and to avoid mistakes in capsules intake.

Blood Samples and Biochemistry Essays

Blood samples (10 mL) were collected from the ulnar vein before and after each cycling submaximal test. Samples were centrifuged (10 min, @3500 rpm) to separate the plasma that was stored at −80°C to further determination of total activity of creatine kinase (CK) (Noakes, 1987) using enzymatic commercial kits (Labtest). The blood samples for biochemical analyzes of oxidative damage were collected in tubes with heparin. The analysis of substances reactive to the thiobarbituric acid (TBARS) served to determine the lipid peroxidation (Ohkawa et al., 1979). To ensure that participants had no damage in soft tissues that could increase CK (for instance, a muscle strain, tendon, or ligament injury, etc.) we determined the serum levels of the C reactive protein (Pritchett, 1996) using immunological kits (Labtest). The blood analyses are named in the result section as: (A) pre cycling without fatigue, (B) post-cycling without fatigue, (C) pre cycling with fatigue, and (D) post-cycling with fatigue.

Statistical Analyses

Data are expressed as mean and standard deviation. Normality of data distribution was confirmed using the Shapiro–Wilk test. EMG signals within cycling trials were compared between the moments by one-way ANOVA with Bonferroni *post hoc*, and the comparison between the groups and fatigue conditions by two-way ANOVA with Bonferroni *post hoc*. Biochemical and heart rate data were compared within cycling trials by one-way ANOVA with Bonferroni *post hoc*. For non-parametric data Friedman and Wilcoxon testes were used. Comparisons between the groups were performed using independent t-test. Significance level was set at 0.05 for all analysis.

RESULTS

Age ($t_{(1)}$ = 0.457; P = 0.37), height ($t_{(1)}$ = 0.514; P = 0.49), body mass ($t_{(1)}$ = 1.016; P = 0.47), training experience ($t_{(1)}$ = 1.576; P = 0.159), and training frequency ($t_{(1)}$ = 0.242; P = 0.815) did not differ between GTE and placebo groups.

The PPO did not differ ($t_{(1)}$ = 0.333; P = 0.123) between the GTE 303 (52) W and placebo 309 (49) W, which resulted in a submaximal cycling workload that did not differ ($t_{(1)}$ = 0.333; P = 0.123) between the GTE 180 (27) W and placebo 181 (31) W.

Biochemical Essays

Results of C-reactive protein suggest that participants from GTE and placebo groups did not suffer macro injuries related to the experiments (data not shown).

Muscle damage was lower in the GTE supplemented participants. CK activity did not differ between GTE and placebo in pre cycling without fatigue (moment A, $F_{(1)}$ = 1.600; P = 0.300)

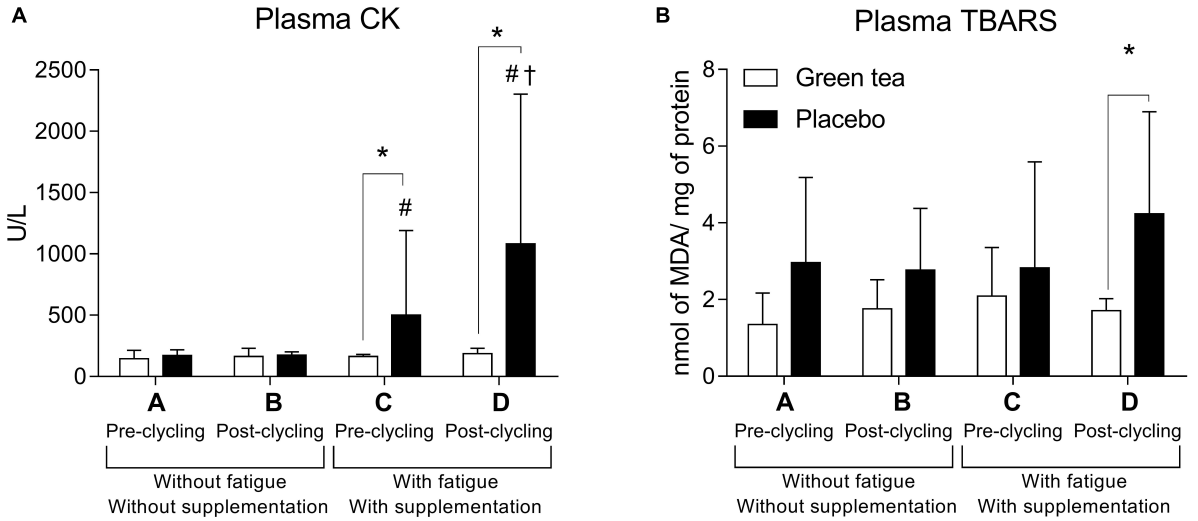

FIGURE 2 | Plasma **(A)** creatine kinase (CK) and **(B)** plasma lipid peroxidation measured by thiobarbituric acid reactive substances (TBARS). $^{*}P < 0.05$ between groups comparisons; $^{\#}P < 0.05$ higher than A and B for the same group; $^{\dagger}P < 0.05$ higher than C for the same group. MDA = Malondialdehyde.

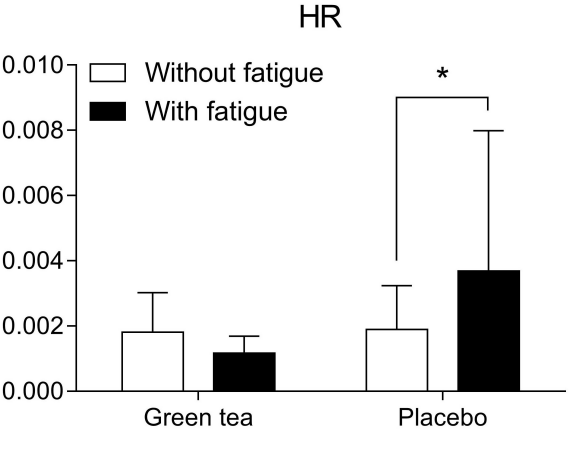

FIGURE 3 | Heart rate (HR) curve slope over time of exercise in the different groups and conditions. $^{*}P < 0.05$ between conditions.

and post-cycling without fatigue (moment B, $F_{(1)} = 1.890$; $P = 0.191$). However, CK was higher in placebo pre cycling with fatigue (moment C, $F_{(1)} = 19.496$; $P = 0.300$), and post-cycling with fatigue (moment D, $F_{(1)} = 18{,}917$; $P = 0{,}001$). GTE supplementation protected against muscle damage as estimated by CK activity ($F_{(3)} = 0.767$; $P = 0.522$, **Figure 2A**), which did not happen in placebo group ($Z_{(1)} = -2.100$; $P = 0.036$).

Placebo group showed higher oxidative stress in the fatigue condition, suggesting a protective role of GTE supplementation. Oxidative stress assessed by lipid peroxidation (TBARS, **Figure 2B**) did not differ between the groups in moments A ($F_{(1)} = 4.200$; $P = 0.600$), B ($F_{(1)} = 3.703$; $P = 0.075$), and C ($F_{(1)} = 0.522$; $P = 0.482$). In moment D, TBARS was higher in the placebo than GTE group ($F_{(1)} = 4.838$; $P = 0.045$).

Heart Rate

Cardiovascular responses estimated by heart rate showed that GTE supplemented group experienced lower cardiac workload than placebo group. Heart rate responses to the cycling trials (**Figure 3**) were analyzed by the angular coefficient of the regression curve considering second-to-second data recorded during the exercise. Higher heart rate increase during exercise in the fatigue condition was observed in the placebo ($F_{(1)} = 5.869$; $P = 0.030$) compared to GTE group ($F_{(1)} = 0.075$; $P = 0.788$).

Neuromuscular Activation

Neuromuscular activation from the left vastus lateralis (LVL) of the participants of placebo group showed significant impairment in the fatigue condition. Fatigue condition showed an effect for magnitude of neuromuscular activity estimated by the RMS values (**Figure 4A**) in the LVL that showed neuromuscular activation decreased by the end of the exercise in the fatigue condition in placebo ($F_{(4)} = 5.510$; $P = 0.020$) but not in GTE group ($F_{(4)} = 2.151$; $P = 0.140$). In the right vastus lateralis (RVL) fatigue condition did not showed an effect for magnitude of neuromuscular activity (**Figure 4A**) in both GTE ($F_{(4)} = 0.009$; $P = 0.920$) and placebo groups ($F_{(4)} = 3.570$; $P = 0.060$).

In GTE group, neuromuscular fatigue estimated by the median frequency during the submaximal cycling trial (**Figure 4B**) was not detected in the RVL ($F_{(4)} = 0.009$; $P = 0.920$) and LVL ($F_{(4)} = 1.914$; $P = 0.170$). Placebo group did not change median frequency of RVL ($F_{(4)} = 0.680$; $P = 0.170$) but showed a decrease in the LVL ($F_{(4)} = 5.510$; $P = 0.020$).

DISCUSSION

Here we set out to determine whether GTE supplementation could benefit performance under a condition of cumulative fatigue. GTE has been shown as a potential antioxidant,

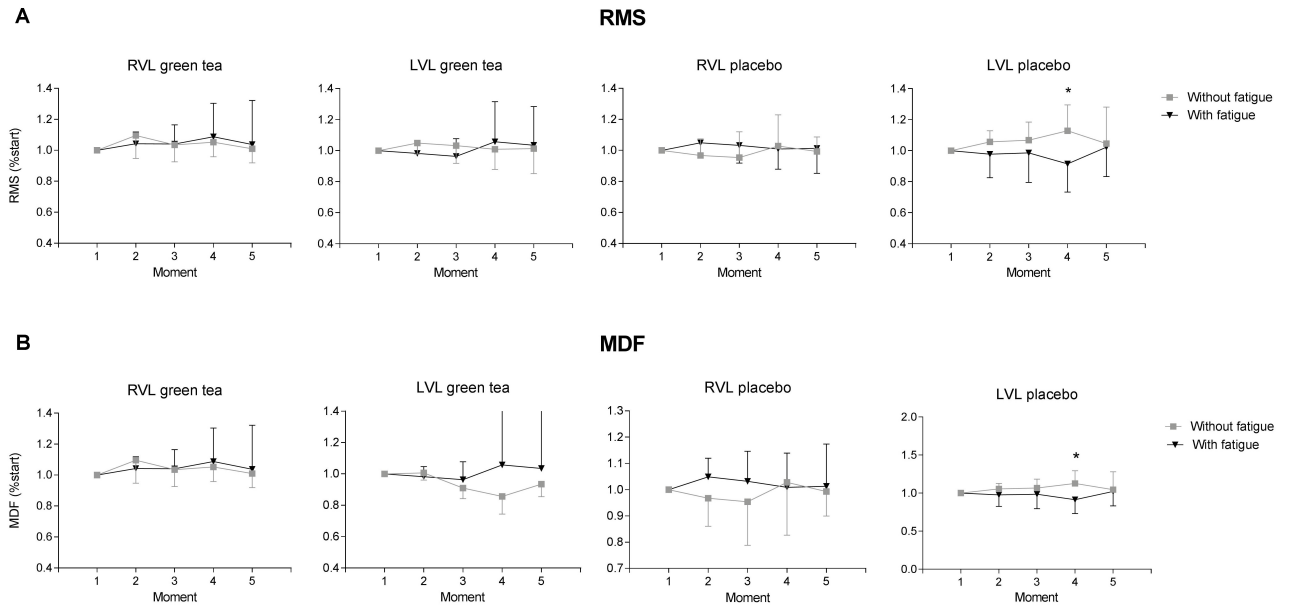

FIGURE 4 | Results of neuromuscular electrical activity obtained from GTE and placebo groups during the cycling trials with and without fatigue condition. Data are shown as mean (bars) and standard deviation (vertical lines) for **(A)** root mean square (RMS) and **(B)** median frequency (MDF) normalized to the moment 1 for right (RVL) and left vastus lateralis (LVL). $^*P < 0.05$ difference between conditions for the same group at a moment.

with positive effects on different tissues, and could be a good option for competitive sports. Despite of its popularity among athletes, few evidences of the benefits are available concerning amateur competitive sport. To the best of our knowledge, this is the first study demonstrating that GTE supplementation before cumulative fatigue minimizes muscle damage and oxidative stress in trained athletes, therefore playing a significant role in exercise recovery, and with important effects on neuromuscular and cardiovascular performance during exercise.

Previous studies on GTE supplementation in athletes were limited to the determination of performance improvement resultant of higher lipid oxidation due to GTE activity (Ichinose et al., 2011). Rather than an effect on energy supply, here we focused on performance during endurance trials of cycling under cumulative fatigue, which is close to the experienced by athletes in competitions lasting more than 1 day, and found GTE results supporting benefits of this supplementation on both muscle damage and recovery markers, as well neuromuscular function (Fuglevand et al., 1993). These are important implication for training and competition.

Placebo group showed higher muscle damage after fatigue. Increase in CK activity is commonly associated with damage resultant of mechanical stress and structural acute changes in the muscle, which happens in coexistence with increase in oxidative stress (Morillas-Ruiz et al., 2006). Such result supports the role of GTE in minimizing muscle damage resultant of exercise.

Oxidative stress is the most accepted explanation to the presence of muscle damage, and the results from GTE group support the lower oxidative stress as an explanation to the lower CK activity observed in the GTE supplemented group (Panza et al., 2008). CK activity determined from the circulating blood can be variable, and it is important to ensure the absence of other lesions that could influence CK activity. We found no changes in C-reactive protein and therefore attribute the changes in CK activity to the stress imposed by the exercise protocols (Pritchett, 1996).

Green tea extract supplementation resulted in stable lipid peroxidation, which was used as a marker of oxidative stress. It is known that oxidative stress is not cumulative along different days of exercise (Shing et al., 2007), but when we look at the oxidative stress data from the last session of cycling exercise we found lower oxidative stress in the GTE group. This result is in agreement with a previous study addressing sprints tasks that resulted in an oxidative stress condition in placebo but not in the GTE supplemented group (Jowko et al., 2015).

The exercise configuration used here leaded to an imbalance in the oxidative status resulting in oxidative stress (Vollaard et al., 2005). Oxidative stress has important implications on the contraction mechanisms and force output capacity (Prochniewicz et al., 2008). GTE catechins work as scavengers of reactive species of oxygen better than observed in response to other supplementation strategies commonly used in sports, such as vitamin C and E (Zaveri, 2006). It supports our idea that the antioxidant properties of GTE are the main explanation to our results in the fatigue condition.

The metabolic damage in the muscle tissue impaired muscle activation in a way visible considering simple markers of surface electromyography. Placebo group showed lower magnitudes of neuromuscular activity and higher indicators of muscle fatigue. The decrease in the magnitude of neuromuscular activation and its correlation with increase in markers of muscle fatigue is

expected in muscles exposed to repeated bouts of exercise under cumulative fatigue (Mendez-Villanueva et al., 2012). Our cycling trials involved constant workload relative to the individual PPO. It doesn't allow us to discuss decrement in force output as expected during a test for exhaustion (Diefenthaeler et al., 2012) because we fixed the mechanical load, but these results denote that our athletes were able to further recruit additional muscle fibers (Ericson et al., 1986) in order to sustain the exercise, and it had implications on the cardiovascular responses (Thomson et al., 2016), as we estimated by analyzing the heart rate data recorded during the cycling trials. While this rationale seems evident to the placebo group, GTE group showed better indicators of neuromuscular performance and cardiovascular demand.

Our study has limitations. We were unable to fully control the diet of the participants and it may have influenced the higher variability observed in the results, which is also a common issue in previous studies. To minimize this effect we delivered detailed recommendations to the participants, like avoiding intermittent fasting that affect oxidative stress (Dannecker et al., 2013). Also, we used the term "fatigue" despite of the difficulties to quantify this phenomena and the number of co-variables that can influence fatigue manifestations. We controlled the highest number of factors possible we could to minimize other variables of influence on our results. Neuromuscular results showed consistent impairments in the left leg, and it may have some relation with variables of motor control like leg preference, which deserves attention in future researches. Measurements of force would help to determine the extend of damage due to the exercise (McHugh et al., 1999). We were unable to evaluate knee extensors force. The pharmacokinetics of the catechins in the blood may have influenced our results. In rats dosed with green tea catechins, concentrations in the blood exhibited peak up to 3 h after intake (Janle et al., 2008). In humans, peak plasma concentrations are reached between 1.5 and 5 h depending on the catechin considered, but the variability and the level of metabolites are not clearly identified (Janle et al., 2008). We tried to minimize these effects by controlling the period of the day in which tests were performed according to the time when the supplementation was intake. Finally, although the dosage is different among the studies, a higher dosage is not related to better results on muscle soreness, for example (Arent et al., 2010; Rynders et al., 2014; Romain et al., 2017), and we selected a dosage that was shown to be effective in conditions of fatigue (da Silva et al., 2018).

CONCLUSION

Green tea extract supplementation before an event of cumulative fatigue minimizes muscle damage and oxidative stress in trained athletes. It also shows positive effects on neuromuscular parameters related to muscle activation and muscle fatigue. Therefore, GTE supplementation can be considered a valid strategy in the context of competitive endurance sport aiming at exercise recovery and performance of athletes.

ETHICS STATEMENT

This study was approved by the ethics committee from Universidade Federal do Pampa and all participants signed a consent term prior to start the participation in this research.

AUTHOR CONTRIBUTIONS

ÁM, WdS, MS, and FC designed the study, interpretated the data, and prepared the manuscript. ÁM, WdS, and MS collected and processed the data. All authors approved the final manuscript.

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frontiers in Physiology

BRIEF RESEARCH REPORT
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Basal Mild Dehydration Increase Salivary Cortisol After a Friendly Match in Young Elite Soccer Players

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A soccer match induce changes in physiological stress biomarkers as testosterone (T), cortisol (C), and testosterone:cortisol (T:C) ration. Hydration state may also modulate these hormones, and therefore may alter the anabolic/catabolic balance in response to soccer match. The role of hydration status before the match in this biomarkers has not yet been reported. The aim of this study was to compare the salivary T, C, and the T:C ratio responses after two friendly matches in well-hydrated and mild-dehydrated (MD) elite young male soccer player. Seventeen players (age, 16.8 ± 0.4 years; VO_2max 57.2 ± 3.6 ml/kg^{-1}/min^{-1}) were divided into two teams. Before the matches the athletes were assessed for hydration level by the urine specific gravity method and divided for the analysis into well-hydrated (WH; n = 9; USG < 1.010 g/mL^{-1}) and mild-dehydrated (MD; n = 8; USG 1.010 to 1.020 g/mL^{-1}) groups. Hormones were collected before and after each match by saliva samples. The mean (HRmean) and maximal (HRmax) heart rate were measured throughout the matches. A two-way ANOVA was used to compare T, C, and T:C between and within groups. Similar HRmean (WH, 83.1 ± 4.7%; MD, 87.0 ± 4.1; p = 0.12) and HRmax (WH, 93.2 ± 4.4%; MD, 94.7 ± 3.7%; p = 0.52) were found for both groups during the matches. No differences were found before the matches in the T (p = 0.38), C (p = 66), nor T:C (p = 0.38) between groups. No changes within groups were found after matches in neither group for T (WH, p = 0.20; MD, p = 0.36), and T:C (WH, p = 0.94; MD, p = 0.63). Regarding the C, only the MD group showed increases (28%) after the matches (MD, p = 0.03; WH, p = 0.13). In conclusion MD group exacerbate the C response to friendly matches in elite young male soccer players, suggesting that dehydration before match may be an added stress to be considered.

Keywords: hydration, hormone, endocrine, saliva, catabolic, football, recovery, sport health

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INTRODUCTION

Hormonal response to a soccer match is a hot topic, with anabolic (i.e., testosterone [T]) and catabolic (i.e., cortisol [C]) hormones potentially influencing the performance and health status of the athlete (Slimani et al., 2017). At the same time, their ratio testosterone:cortisol (T:C) is considered a physiological stress indicator associated to overtraining (Hayes et al., 2015).

Some studies such as Penailillo et al. (2015) showed a decrease on T, without changes on C, whereas others such as Thorpe and Sunderland (2012) describe increases on both hormones. The differences between studies can be explained due to differences in match intensity, biological, psychological, social factors and/or due to the degree or years of training, the latter being highly dependent to the age of the athletes (Casto and Edwards, 2016). In addition, it has been shown that the hormonal response during exercise might depend on hydration state (Roy et al., 2001). For instance, it has been observed greater C concentration on hypohydrated subjects before and after running at 70% of maximal oxygen consumption (VO_2max) in comparison to euhydrated runners (Maresh et al., 2006). Although in male soccer players, salivary cortisol, T, and T:C have been assessed as physiological stress biomarkers after the match, the role of hydration status before the match in this biomarkers has not yet been reported. This information could increase our understanding of the physical stress induced by a football match, which could improve the preparation and strategy to protect and/or enhance elite performance in subsequent matches. Therefore, the aim of this investigation was to assess the effects of hydration level before a soccer match on the T, C, and T:C response after the match in young elite soccer players.

MATERIALS AND METHODS

Subjects

Seventeen male soccer players (age: 16.8 ± 0.4 years; body mass: 67.5 ± 7.5 kg; height: 173 ± 6.8 cm; VO_2max: 57.2 ± 3.6 ml/kg^{-1}/min^{-1}), from a South American under-17 (U17) soccer national team, participated in this study. According to the hydration level assessed by the urine specific gravity (USG) before the matches, the subjects were divided for the analysis into two groups: well-hydration (WH) (USG < 1.010 g/mL^{-1}, n = 9) and mild dehydration (MD) (USG from 1.010 to 1.020 g/mL^{-1}, n = 8) (Casa et al., 2000). Injured players and goalkeepers were excluded. The legal guardians of the players signed an informed consent, while the players give their verbal assent, after the potential benefits, and risks where explained to them. The study was approved by the ethics committee of the Universidad Finis Terrae and conformed to the principles outlined in the Declaration of Helsinki.

Study Design

One week before the matches VO_2max was assessed to all participants with an incremental test. In the matches day the evaluated soccer team was divided into two teams (A and B). The first match (team A) was played at 11:00 am, and the second match (team B) at 11:30 pm (the same day), in preparation for the FIFA U17 World Championship 2015, carried out in Chile. Climatic conditions were similar between matches. The both friendly matches were played against a professional soccer team of the Chilean professional league. The assessed teams won both matches (first match, 2-0; second match, 3-1). During the two matches, the players were asked to play as it was an official match. The matches follow international rules (FIFA). The USG was assessed 30 min before the match with a portable Refractometer (Robinair, model SPX, United States) in triplicate according to previous suggestions (Castro-Sepulveda et al., 2015). Nutritional recommendations were not made prior to matches and during the friendly matches players consumed water *ad libitum*. According to Penailillo et al. (2015), for the assessment of the T and C, saliva was collected 30 min before each match (Pre-), and 5–10 min after each match (Post-). Briefly, the players were sat, with their eyes open, their head slightly tilted forward and making minimal orofacial movement. All saliva (± 3 ml) was collected for about 2 min. The saliva samples were centrifuged at 1,500 *g* for 15 min and stored at −20°C until analysis. The T and C were determined by enzyme immunoassay using a commercial kit (Salimetrics, State College, PA, United States). The optical density was determined with a microplate reader (Multiskan, Thermo®) at 450 nm. All analyses were performed in duplicate according to the manufacturer's procedures. The intra-assay coefficient of variation was 2.5 and 2.8% for the T and C, respectively. Only players that played >80 min were considered. The mean (HRmean) and maximal (HRmax) heart rate was measured throughout the match using the Polar Team system (MARCA, PAIS). The hearth rate values were reported as relative values, according to age-expected maximum values (220-age).

Maximal Oxygen Uptake

The VO_2max was determined by a breath-by-breath pulmonary gas exchange system (Ergocard, Medisoft, Belgium) during an incremental treadmill test. The starting speed was 3 km^{-1}/h^{-1}, with speed increments of 1 km^{-1}/h^{-1} every 60 s. Prior to the tests, the gas analyser was calibrated using gases of known concentrations (VO_2 = 16.0% and VCO_2 = 4.0%), and the airflow was calibrated using a 3-liter syringe (Hans Rudolph, Kansas, MO, United States).

Body Mass Loss

Body mass loss (kg) was calculated by measuring body mass before and after matches (body mass after match – body mass before match) using the same scale (SECA model M20812, Germany), with a precision of 0.1 kg.

Statistical Analysis

Data is shown as mean ± standard deviation (SD). The normality of the data was analyzed by the Shapiro–Wilk test, showing that data was normally distributed. An unpaired t-test was used to compare hydration level, basal characteristics, body mass loss, and HR during matches between groups. A two-way ANOVA was used for the comparison of the T, C, and T:C between and within of WH and MD groups, with a Tukey *post hoc* test when significate main effect was found. The alpha value was set at $p < 0.05$. Statistical analyses were performed in GraphPad Prism® 6.0 (GraphPad Software, San Diego, CA, United States).

RESULTS

In A team were found five players in WH condition and four players in MD condition and in B team were found four players in WH condition and four players in MD condition. As expected, the USG was lower ($p < 0.0001$) in the WH group (1.006 ± 0.002 g/mL^{-1}) compared to the MD group (1.014 ± 0.002 g/mL^{-1}). No differences were found between groups in basal characteristics (age: WH 16.3 ± 0.7 and MD 16.9 ± 0.5 years, $p = 0.61$; body mass: WH 68.5 ± 8.6 and MD 67.3 ± 8.4 kg, $p = 0.68$; height: WH 171 ± 9.1 and MD 175 ± 8.0 cm, $p = 0.21$; VO_2max: WH 56.3 ± 3.8 and MD 57.8 ± 7.2 ml/kg^{-1}/min^{-1}, $p = 0.74$). Neither were found differences between groups in HRmean (WH 83.1 ± 4.7% and MD 87.0 ± 4.1%; $p = 0.12$) or HRmax (WH 93.2 ± 4.4% and MD 94.7 ± 3.7%; $p = 0.52$) during the matches. After the matches no differences between groups were found in body mass loss (WH 1.6 ± 0.3 kg and MD, 1.4 ± 0.7 kg; $p = 0.33$).

Before the match no differences were found between groups in salivary T (WH 33.41 ± 22.56 pg/mL^{-1} and MD 43.70 ± 18.52 pg/mL^{-1}; $p = 0.38$), C (WH 3.17 ± 0.19 pg/mL^{-1} and MD 3.22 ± 0.18 pg/mL^{-1}; $p = 0.66$), nor T:C (WH 10.41 ± 6.96 and MD 13.52 ± 5.65; $p = 0.38$). The within-group analysis show that salivary T did not change after matches in WH (49.4 ± 18.6 pg/mL^{-1}, $p = 0.20$) nor MD (57.1 ± 22.5 pg/mL^{-1}, $p = 0.36$) (**Figure 1A**). With respect to the C level after the match (MD 4.1 ± 0.9 pg/mL^{-1}; WH 3.8 ± 0.7 pg/mL^{-1}), the MD group exhibit an increase (28%; $p = 0.03$), while no significant change was observed in the WH group ($p = 0.13$) (**Figure 1B**). Regarding the T:C ratio, no changes were found within group in neither group after matches (WH 13.3 ± 5.7, $p = 0.94$; MD 14.4 ± 5.7, $p = 0.63$) (**Figure 1C**). No relationship was found between HR during matches and changes in C (HRmean vs. changes in C, $r = 0.13$, $p = 0.37$; HRmax vs. changes in C, $r = 0.25$, $p = 0.17$).

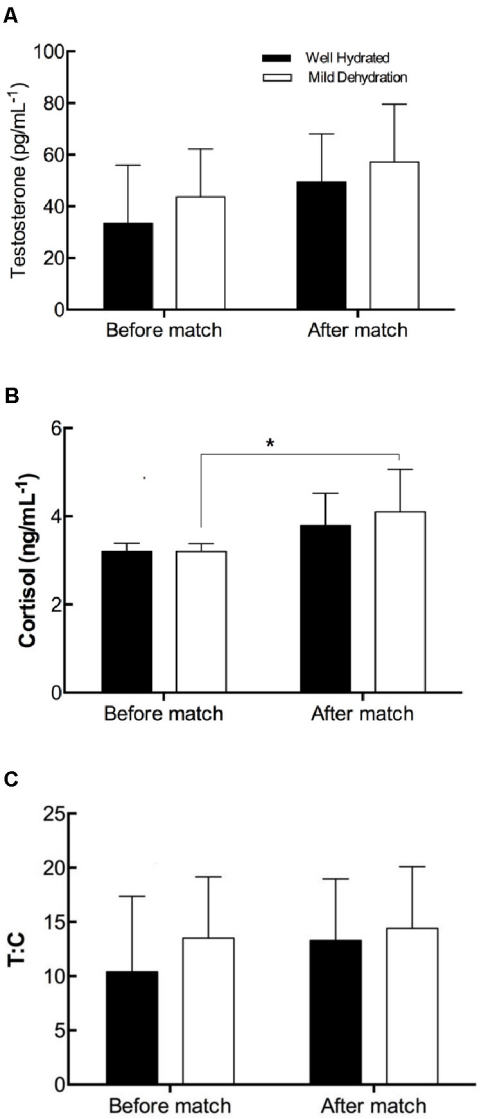

FIGURE 1 | Comparison within well and mild dehydration groups in testosterone **(A)**, cortisol **(B)**, and their quotient **(C)** before and after a friendly match in elite young soccer players. *$p < 0.05$.

DISCUSSION

The aim of this study was to assess the effects of hydration level before a soccer match on the T, C, and T:C after two friendly matches in young elite soccer players. The main results indicate that MD group showed an exacerbated increase in C response after the matches. These results suggest that C response to soccer match is sensitive to hydration state.

With respect to T, different to our results (no changes after match) Penailillo et al. (2015) showed a decrease in T after a friendly match in elite soccer players. It is possible that the different results with the former study, is the difference in years of training experience of athletes. Penailillo et al. (2015) the players had an average of 26 years of age, while in our study, subjects averaged 17 years of age. Therefore, the greater chronological age, hence greater training experience of soccer players in the study of Penailillo et al. (2015), could have induced a reduced hormone response during a competitive match compared to the less experienced U17 soccer players recruited in our study. In addition, several other factors have been identified to play a role in the hormone response to a soccer match (Kobayashi and Miyazaki, 2015; Slimani et al., 2017).

Similar to Penailillo et al. (2015) our results showed no changes in C after match in WH group. However, our results indicate that the MD group showed a significant increase after the match. Considering that no relationship was found between HR during matches and changes in C ($r = 0.13$–0.25, $p = 0.17$–37), and that VO_2max (i.e., fitness level) was similar in the WH and the MD groups (56.3 and 57.8 ml/kg^{-1}/min^{-1}, $p = 0.74$), is unlikely that potential differences in physiological stress during match or differences in fitness level explain the increase in C observed in the MD group and the lack of increase in the WH group. Therefore, the hydration level probably played a key

significant role. Regarding the T:C, this marker did not change at the end of the match. This may be due to the fact that T:C only decreases during periods of high intensity training, but remains stable during periods of competition (Filaire et al., 2001). In this sense, the role of the hydration level probably played a minor role on the T:C ratio as compared to its role on C levels after the match.

Our study is the first to assess the effects of MD before the match on the response of T, C, and T:C in young elite male soccer players. Our results showed that MD group increase the C response after two friendly matches without alters in effort intensity during matches evaluated by heart rate. Sensibility of C to hydration state was observed previously in seven adults with different levels of hydration completed a strength test. They showed that the most dehydrated subjects (−5% of body weight) showed greater increases in C after exercise, with no changes in T (Judelson et al., 2008). Moreover, another study showed that C increased in hypohydrated (USG = 1,034 ± 0.001 g/mL^{-1}) young cross-country athletes (Maresh et al., 2006). Finally, a recent study reported that body weight loss during the match (dehydration) was associated with the increases in C levels in male professional tennis players (López-Samanes et al., 2018). Therefore, our results, in a collective sport, confirm previous findings, expanding the knowledge for young elite male soccer players, regarding the effects of dehydration on stress response (i.e., C increase) during a soccer match.

The C concentrations is a well-recognized physiological stress marker. This steroid hormone plays an important role in response to stress and skeletal muscle recovery after exercise because of the activation of the hypothalamic-pituitary-adrenocortical axis. This finding may be of upmost importance for coaches and medical staff of football teams to consider, since MD before training or competition is very common in football soccer players (Castro-Sepulveda et al., 2015). A previous study in Cushing's syndrome shows a relationship between HR and C (Chandran et al., 2013). Our results do not show this relationship, this inconsistency could be explained by the different mechanisms that modify the HR in the Cushing's syndrome and during the exercise.

One of the limitations of this study is not having evaluated other variables that influence C levels and responses as (1) sleep quality before the matches (Bassett et al., 2015) and (2) natural daily response of C (Pritchard et al., 2017). Another potential limitation is that players were free to hydrate during the match. This, although ethically sound, may have altered the after-match hydration level. However, after the match no differences between groups were found in body mass loss (WH, 1.6 ± 0.3; MD, 1.4 ± 0.7; $p = 0.33$).

CONCLUSION

In conclusion, MD before soccer match increase C response after match. These results show that C response to soccer match is sensitive to hydration state which suggests that dehydration before match may be an added stress to be considered.

AUTHOR CONTRIBUTIONS

MC-S and HZ-F designed the study. MC-S, RR-C, and HZ-F collected and analyzed the data. MC-S, RR-C, FA-C, CM, LP, JC, and HZ-F interpreted the data and prepared the manuscript. All authors approved the final version of the paper.

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Syzygium cumini Nectar Supplementation Reduced Biomarkers of Oxidative Stress, Muscle Damage, and Improved Psychological Response in Highly Trained Young Handball Players

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The purpose of this study was to investigate the effects of *Syzygium cumini* (SC) nectar supplementation on performance, markers of oxidative stress, muscle damage, and psychological response in Handball players. Twenty-five young athletes (age = 18.6 ± 2.4 years) from an elite high school national level Brazilian Handball team were randomized into two study groups: SC/Jamelon nectar (SC, n = 12) and placebo (n = 13). The subjects ingested 10 mL/kg/day of Jamelon nectar or placebo 30 min before the training sessions and immediately after training cessation, for 28 days. Body mass index (BMI) and percentage of fat mass were assessed using bioelectrical impedance analysis. Biomarkers of oxidative stress were measured by lipid peroxidation, which was quantified by malondialdehyde (MDA). Total antioxidant capacity (TAC), creatine kinase (CK) activity, and lactate dehydrogenase (LDH) were determined. The 20 m shuttle run test, vertical jump, and running anaerobic sprint test were assessed to verify performance and the fatigue index was calculated. The Profile of Mood States (POMS) questionnaire was used for psychological evaluation. Both groups demonstrated improved vertical jump performance and a decreased fatigue index over time but without significant differences between them regarding performance. There was statistically significance only for SC in CK, LDH, and MDA, and TAC was greater in

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the SC compared to placebo. Furthermore, only the SC group demonstrated improved mood disturbance and confusion after the intervention. In conclusion, the present study suggests that SC nectar supplementation reduced biomarkers of oxidative stress and muscle damage, and improved psychological response in young handball players.

Keywords: performance, nutrition, sport, athletes, recovery

INTRODUCTION

Handball is a sport modality characterized by performing many different movements with intensive effort, such as running, jumping, changing direction, shooting, and technical movements (Michalsik et al., 2015). In addition, handball players are involved in repeated bouts of training and games with high physical contact, increasing the mental and physical demand on the players (Michalsik et al., 2014). Excessive training can generate imbalance in the pro and antioxidant status due to the inefficient ability of the antioxidant system to reduce overproduction of reactive oxygen species (ROS), thus this condition may lead to oxidative stress (Hattori et al., 2009; Martarelli and Pompei, 2009; Mrakic-Sposta et al., 2015). Marin et al. (2013) investigated the response of oxidative stress and antioxidant status in handball players for 6 months with different training loads and competitions. The authors observed an increase in antioxidant capacity and biomarkers of oxidative stress (superoxide anion and hydrogen peroxide) according to training load.

In addition, Tanskanen et al. (2010) demonstrated an increase in the resting biomarker of oxidative stress (protein carbonyls) in over-trained athletes; however, there was a significant relationship between over-training and low antioxidant capacity, demonstrating that excessive training plus insufficient recovery periods can induce overproduction of ROS (Marin et al., 2013). Therefore, oxidative stress plays a role in the development of overtraining syndrome, which can be associated with muscle damage, inflammation and reductions in psychological aspects (e.g., motivation and mood) as well as performance in athletes (Purvis et al., 2010; Tanskanen et al., 2010; Carfagno and Hendrix, 2014). In this perspective, other studies show association between oxidative stress with muscular fatigue, indicating that oxidative stress during exercise decreases the skeletal muscle contractility by impairing the release of calcium by the sarcoplasmic reticulum (Moopanar and Allen, 2005; Cheng et al., 2016), which could result in a lower interaction between actin-myosin filaments and force output (Powers et al., 2011).

For this reason, studies have investigated the influence of different type of dietary antioxidants on performance and oxidative stress in athletes, demonstrating an efficient strategy to counter high ROS production in this population (Peternelj and Coombes, 2011; Pingitore et al., 2015). *Syzygium cumini* (SC), commonly known as Jambolan, is used for the treatment of several diseases including diabetes, inflammation, sore throat, bronchitis, asthma, coughs, and dysentery (Ayyanar and Subash-Babu, 2012), as the health benefits of SC consumption have been associated with high phenolic compounds, such as gallic acid and flavonoids (catechin, epicatechin, epigallocatechingallate, and epicatechingallate; Ayyanar and Subash-Babu, 2012).

Investigation of the health effects of SC supplementation in animals is widely explored in the literature, demonstrating antioxidant and anti-inflammatory activity, as well as hypoglycemic effects (Ravi et al., 2004; Sharma et al., 2008; Siani et al., 2013). Ulla et al. (2017) showed that SC seed powder prevents oxidative stress in rats with high fat diet induced obesity through a decrease in malondialdehyde (MDA) levels and increased activity of antioxidant enzymes, superoxide dismutase, glutathione peroxidase, and catalase. Whether SC supplementation influences exercise performance, oxidative stress, muscle damage, and psychological response in humans is currently unknown in the literature. We hypothesized that SC supplementation could reduce oxidative stress, muscle damage, and improves performance as well as psychological response in handball players.

Thus, the purpose of this study was to investigate the effects of SC nectar supplementation on performance, markers of oxidative stress and muscle damage, and psychological response in handball players.

MATERIALS AND METHODS

Subjects

The study was carried out at the Federal University of Piauí (UFPI), Teresina, Brazil. Twenty-five young male athletes, age between 16 and 23 years (n = 25; age = 18.6 ± 2.4 years) from the elite high school national level handball team (CaicBalduíno school/GHC team and positions of the players: 13 center, 9 wing, and 3 pivot) were included, who had been training for at least 1 year with a weekly frequency of five training sessions, 90–120 min per day, and had regularly participated in international and national competitions during the previous 2 years. The team is currently twice world champion and national champion in the category.

The athletes were randomized into two study groups: SC (n = 12) and placebo (n = 13), using simple randomization techniques for allocation, which ensure that each athlete has an equal chance of being allocated to a treatment group. None of the participants reported any physical limitations that could prevent completion of the assessments and exercise interventions; being smokers or continuously using any medication, in addition to which, none were taking any dietary or performance enhancing supplements that could have affected the outcome of the study. During the study, the participants were required not to consume nectar or derivatives other than the intervention. The project

was approved by the Ethics Research Group of the UFPI, Teresina, Brazil (Protocol Number: 1755888) and the research was conducted according to the 2008 Revision of the Declaration of Helsinki. All participants and their parents signed a written consent form and were informed about the purpose of the study and the possible risks.

Experimental Design

This study used a randomized, double-blind design. Initially, the participants were submitted to body composition, dietary intake, and psychometric assessments, and collection of blood samples; 24 h later, they performed aerobic, anaerobic, and strength tests. Subsequently, the participants were randomized, using simple randomization[1], into two groups (SC or Placebo) and after 28 days of supplementation the same initial evaluation was performed. After intervention (48 h), blood samples were collected and performance tests were conducted after 72 h.

Procedures

Training Program and Supplementation Protocol

The training program following a model proposed by Verjoshanski (1990) and adapted by Oliveira (1998), which we used Phase A1 (4 weeks) of periodization, according to Souza et al. (2006). During 28 days of intervention, the coach maintained the same training session, which the athletes performed general strength and medium-intensity endurance training about 60 min in the morning, three times a week. Twice a week, the subjects trained maximal power and maximal speed and 5 days a week in the evening (6 to 7:30 p.m.) trained sport-specific strength and power and techno-tactical skills. Participants were instructed to maintaining heart rate (HR) between 75–90% of maximum HR and the rating of perceived exertion between 6 and 9 (Foster et al., 2001).

The individuals consumed 10 mL kg/day of Jamelon nectar, divided into the moments before the training session (30–45 min pre-training) and immediately after the training session, for 28 days, according to the protocol adopted by Toscano et al. (2015). On days when there was no training, supplementation was consumed with snacks, at the time of choice of the athletes.

For supplementation, a Jamelon nectar drink was produced with 100 g of filtered drinking water, 4 g of crystal sugar (Cristal®), and 33.3 g pulp (edible fraction with skin). In the processing of the nectar, the Jamelon pulp was thawed at 25°C and homogenized with sugar and water in an industrial blender (Siemsen, D560484, Jaraguá do Sul, Brazil). Each nectar drink was poured by hand into a plastic bottle with a capacity of 1 L, previously sanitized in hypochlorite solution, and capped with a plastic lid. The bottles containing the nectar drink were stored in a refrigerator at 5°C.

The phenolic profile of the Jamelon nectar used in the present study was evaluated by the Embrapa Semi-Arid Oenology Laboratory (Petrolina, Pernambuco, Brazil) using a validated and internationally published procedure (Kschonsek et al., 2018). Twelve phenolic compounds were positively identified and quantified by HPLC using an Aliance Waters 2695 system (Milford, MA, United States) equipped with a diode array detector (DAD) and fluorescence detector (FLD). Data acquisition and analysis were performed with Waters Empower^{TM} 2 software (Milford, MA, United States).

The control group received a carbohydrate beverage that was administered in isocaloric, isoglucose, and isovolumetric form, by means of a conversion chart according to the weight of each athlete, as proposed in the study by Toscano et al. (2015) who used carbohydrate drink as a control for fruit juices. The nutritional composition of the two beverages is shown in **Table 1**.

Anthropometric, Body Composition, and Dietary Intake Assessment

Body weight was measured using an electronic scale (Filizzola PL 50, Filizzola Ltda., São Paulo, Brazil), with a precision of 0.1 kg. Height was measured on a fixed stadiometer of the brand Sanny (Sanny brand, São Paulo, Brazil) with an accuracy of 0.1 cm and length of 2.20 m. The body mass index (BMI) was calculated as body mass divided by the square of the body height (kg/m^2). The percentage of fat mass was assessed using bioelectrical impedance analysis and accompanying software (OMRON® BIA, model 214, OMRON HEALTHCARE Co., Ltda., Kyoto, Japan) and the athletes were positioned in a supine position and remained still throughout the examination and they were wearing light clothing. Twenty-four daily records were conducted via 3-day food diaries that consisted of one weekend day, Sunday, and two weekdays, Monday and Wednesday or Tuesday and Thursday. All food intake records were analyzed for total kilocalorie and macronutrient intakes to ensure that dietary

TABLE 1 | Nutritional composition and HPLC-evaluated phenolic compounds of the supplemented beverages.

Nutritional composition	Nectar of Jamelon (33 g of pulp + 4 g of sugar + 100 mL of water)	Drink control (8 g of maltodextrin + 100 mL of water) 100 mL of water)
Calories (kcal)	30	30
Carbohydrates (g)	7.5	7.5
Proteins (g)	0.17	0
Lipids (g)	0.03	0
Fibers (g)	0.6	0
Gallic acid	2.61 ± 0.09	–
Flavonoids (mg/L)	0.61 ± 0.00	–
(+) – Catechin	0.53 ± 0.00	–
(–) – Epicatechin	1.39 ± 0.07	–
(–) – Epicatechin gallate	2.12 ± 0.05	–
(–) – Epigallocatechin gallate	0.74 ± 0.00	–
Procyanidin A2	0.71 ± 0.00	–
Procyanidin B1	0.46 ± 0.00	–
Procyanidin B2		
Anthocyanins (mg/L)	7.53 ± 0.16	–
Cyanidin-3.5-di-*O*-glycoside	1.44 ± 0.13	–
Cyanidin-3-*O*-glycoside	205.7 ± 2.51	–
Malvidine-3.5-di-*O*-glycoside	48.85 ± 1.08	–

Data are mean ± standard deviation.

[1]www.randomizer.org

intake was similar between baseline and post-intervention. Food questionnaires were analyzed by the same nutritionist using the software NutWin, version 1.5 (Nutrition Support Program, Federal University of São Paulo, São Paulo, Brazil, 2002).

Biomarkers of Oxidative Stress

After an overnight fast (12 h), venous blood samples were collected, which it was 24 h before the beginning of the intervention and 48 h after 28 days of supplementation. Blood samples (10 mL) were immediately allocated into two 5 mL vacutainer tubes (Becton Dickinson, Juiz de Fora, Brazil) containing EDTA for plasma separation. The tubes were centrifuged at 3000 rpm for 15 min at 4°C, and plasma and serum samples were stored at −20°C until analysis. Biomarkers of oxidative stress were measured by lipid peroxidation, which was quantified by MDA. The thiobarbituric acid reaction in plasma was adopted according to the method described by Ohkawa et al. (1979). In addition, total antioxidant capacity (TAC) was determined. TAC was quantified in plasma by measuring the activity of elimination of the 2,2-diphenyl-1-picrylhydrazyl free radical using the method described by Brand-Williams et al. (1995). The absorbance was obtained in the Labmax 240 premium automatic analyzer (Labtest, Minas Gerais, Brazil), at 520 nm wavelength.

Muscle Damage

The plasma creatine kinase (CK) activity and plasma lactate dehydrogenase (LDH) were determined 48 h after the supplementation period, after an overnight fast (12 h) using the catalytic activity method and pyruvate lactate method, respectively, both with a commercial Kit (Labtest, Minas Gerais, Brazil) and were quantified using Lab-Max 240 Premium (Labtest, Minas Gerais, Brazil) according to the manufacturer's instructions.

Performance

Lower Body Power

Vertical jump was assessed using the Jump test on the Mat (Multi-Sprint® software, Huntsville, AL, United States). Subjects were instructed to stand on the mat with feet hip width apart and perform a rapid lower body eccentric movement followed immediately by a maximal intensity concentric movement. Subjects were instructed to jump straight up and minimize any in-air hip flexion. The best of three trials separated by a 1 min rest interval was recorded as vertical jump height (cm), according to Hermassi et al. (2011) and Silva et al. (2016). The lower body power test was performed immediately post body composition assessment at baseline and after 28 days.

Cardiorespiratory Fitness

The 20 m shuttle run test (20MST) was used to determine maximum oxygen consumption (VO_2max). The test was performed incrementally at a distance of 20 m back and forth. The VO_2max was determined by the following equation: age < 18 years: VO_2max = –24,4 + (6*Vmax) and age > 18 years: VO_2max = 31.025 + (3.238*Vmax) – (3.248*age) + (0.1536*Vmax*age); where Vmax is the speed (km/h^{-1}) obtained in the final completed stage (Leger et al., 1988).

After 24 h of 20MST, anaerobic capacity was evaluated using the running anaerobic sprint test (RAST; Silva et al., 2016). After a pre-warm-up by stretching and light jogging for 10 min and an active recovery of 5 min, athletes performed six full-length races over a distance of 35 m at the maximum possible speed, with a 10 s rest interval between each repetition. The times in seconds and hundredths of a second and the speed in m/s were recorded using Hidrofit® brand photocells and Multi-Sprint® software.

The anaerobic power of each sprint in W/kg was obtained by multiplying the current weight of the athlete by the distance squared and then dividing by the time obtained in the race cubed. This power output (W) was then divided by the current weight (kg) of the individual, obtaining the maximum power in $W.kg^{-1}$ (Bangsbo, 1998). All performance tests were conducted 72 h after 28 days of supplementation and the participants were instructed to avoid strenuous physical exercise and had not used any caffeinated beverages for at least 24 h prior tests.

Psychological Evaluation

In order to evaluate the initial state of mood of the athletes, associated with psychological stress, the subjects responded to the version of the Profile of Mood States (POMS) questionnaire adapted to the sport by Raglin and Morgan (1994) at the beginning and after the 28-day intervention. This questionnaire is composed of 36 items, distributed in six dimensions – tension, depression, hostility, fatigue, confusion, and stamina.

Statistical Analysis

We performed a power analysis of this study based on the observation from a previous study that verified a difference of 0.2 for VO_{2max} ($mL.kg^{-1}{\cdot}min^{-1}$) with a standard deviation of 0.2 in the experimental and control means after 28 days of integral purple grape juice on the performance of recreational runners (Toscano et al., 2015). Using a power (1-type II error) of 0.80 and a type I error of 0.05 by PS software (ver 3.1.2, Dupont and Plummer[2]), it was estimated that we would need 17 participants per group. Therefore, we were able to reject the null hypothesis that this response difference is zero with a power (1-type II error) of approximately 70%.

A mixed model was used to compare placebo and SC nectar across time using a two-way ANOVA for repeated measures. When a significant interaction (group x time) was observed, a Bonferroni *post hoc* test was conducted. For all measured variables, the estimated sphericity was verified according to the Mauchly's W test and the Greenhouse–Geisser correction was used when necessary. The partial eta-squared (η^2) was calculated for time. Statistical significance was set at $p < 0.05$. The data were analyzed using StatSoft Statistica (version 10.0).

RESULTS

Table 2 presents the dietary and macronutrient intake, anthropometry, body composition, and performance variables

[2]http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize

TABLE 2 | Comparison between placebo and supplementation group on dietary intake, anthropometry and performance.

Variables	Placebo (n = 13)		SC (n = 12)	
	Baseline	Post	Baseline	Post
CHO (g)	900.7 ± 413.1	1153 ± 545.8	1041 ± 341.7	1430 ± 665.1
PRO (g)	461.9 ± 172.5	404.5 ± 204.2	404.2 ± 129	486.1 ± 188.2
FAT (g)	590.3 ± 388.5	613.3 ± 295.7	702 ± 247.9	869.8 ± 389.8
Total Intake (kcal)	1963 ± 775	2265 ± 865	2116 ± 732	2562 ± 913
Vitamin A (μg ER)	346.5 ± 266.9	311.6 ± 184.9	280.9 ± 127.9	337.4 ± 256.6
Vitamin C (mg)	21.8 ± 16.6	23.4 ± 18.6	30.9 ± 7.7	36.4 ± 16.3
Vitamin E (mg)	13.1 ± 6.7	19.2 ± 9.2	12.2 ± 7.8	14.6 ± 3.3
Copper (mg)	0.9 ± 0.5	1.1 ± 0.4	0.9 ± 0.2	1.3 ± 0.5
Iron (mg)	13.2 ± 6.1	16.8 ± 5.7	14.9 ± 3.2	20.5 ± 9.0
Zinc (mg)	11.3 ± 7.2	9.8 ± 3.2	12.5 ± 3.4	13.9 ± 7.4
Selenium (μg)	102.9 ± 44.8	106.6 ± 39.5	108.3 ± 24.4	99.9 ± 36.9
Body mass (kg)	71.2 ± 7.1	71.9 ± 7.1	70.7 ± 7.6	70.8 ± 7.9
BMI (kg/m^2)	22.1 ± 1.8	22.1 ± 1.7	22.2 ± 2.6	22.3 ± 2.5
Body fat (%)	18.0 ± 3.5	17.9 ± 3.5	18.1 ± 4.5	18.1 ± 4.6
$VO2_{max}$ (mL·kg^{-1}·min^{-1})	45.7 ± 3.8	46.5 ± 5.4	48.3 ± 6.3	49.1 ± 6.9
RAST (W/kg^{-1})	8.6 ± 1.2	8.7 ± 1.2	7.8 ± 1.3	8.1 ± 1.1
Fatigue index (W·seg^{-1})	8.1 ± 2.4	6.8 ± 2.7¥	6.7 ± 2.3	5.7 ± 1.9¥
Vertical Jump (cm)	36.2 ± 5.6	37.2 ± 5.2¥	35.8 ± 3.3	36.3 ± 3.2¥

¥ *Main effect of time with significant difference from baseline. VO_{2max}, maximal oxygen consumption; RAST, running anaerobic sprint test.*

at baseline and after 28 days of intervention in the placebo and SC groups. There were no statistically significant differences between groups at baseline and across time for any variable investigated.

Performance

For vertical jump, both groups improved over time (F = 4.641, p = 0.042, η^2 = 0.17), with no significant differences in changes between groups (F = 0.117, p = 0.736) and interaction (F = 0.587, p = 0.451). For fatigue index, there was a main effect of time (F = 8.668, p = 0.008, η^2 = 0.30) but no difference between groups and interaction was observed ($p > 0.05$). For VO_2max and RAST, there were no main effects of time, statistically significant interactions, or differences between groups ($p > 0.05$).

Biomarkers of Oxidative Stress and Muscle Damage

Table 3 shows the comparison between placebo and supplementation groups for muscle damage and oxidative stress.

For CK, there was a statistically significant interaction (F = 5.981, p = 0.023) with lower CK concentration in the SC group after intervention; however, there was no significant difference between groups (F = 0.010, p = 0.891). For LDH, there was a statistically significant interaction (F = 4.365, p = 0.048). The Bonferroni *post hoc* showed a decrease in the SC group after 28 days of supplementation but no change was observed in the placebo.

For MDA, there was a main effect of time (F = 5.865, p = 0.024, η^2 = 0.20) and a statistically significant interaction was observed (F = 15.847, p = 0.001). The *post hoc* test demonstrated that only the SC group presented decreased MDA in relation to baseline but there was no significant difference between groups (F = 0.713, p = 0.407).

TABLE 3 | Comparison between placebo and supplementation groups on muscle damage and oxidative stress.

Variables	Placebo (n = 13)		SC (n = 12)	
	Baseline	Post	Baseline	Post
CK (U/L)	179.6 ± 52.2	186.1 ± 60.5	210.2 ± 65.34	150.2 ± 58.2*
LDH (U/L)	292.7 ± 45.3	296.8 ± 55.8	331.9 ± 52.4	288.9 ± 37.4*
MDA (μM)	4.0 ± 1.0	4.3 ± 0.7	4.6 ± 1.1	3.2 ± 0.9*
TAC (%)	17.2 ± 2.8	17.7 ± 2.3	18.3 ± 3.1	21.8 ± 2.7*$

*Data are mean ± standard deviation. *Bonferroni's post hoc with statistic significant difference from baseline; $statistic significant difference between groups. CK, Creatine kinase; LDH, lactate dehydrogenase; MDA, malondialdehyde; TAC, total antioxidant capacity.*

For TAC, there was a main effect of time (F = 19.378, $p < 0.001$, η^2 = 0.46), statistically significant interaction (F = 11.400, p = 0.003), and significant difference between groups (F = 6.820, p = 0.016). The Bonferroni *post hoc* showed a greater mean of CAT in the SC group than the placebo (p = 0.004).

Psychological Response

Table 4 presents the difference between placebo and supplementation groups for the psychological response.

For mood disturbance, there was a main effect of time (F = 10.509, p = 0.004, η^2 = 0.31) and statistically significant interaction (F = 5.336, p = 0.030). The *post hoc* only identified an improvement in the SC group across time (p = 0.004), however, without differences between groups. Regarding confusion, there

TABLE 4 | Comparison between placebo and supplementation groups on the psychological response.

Variables	Placebo (n = 13)		SC (n = 12)	
	Baseline	Post	Baseline	Post
Mood disturbance	99.9 ± 12.4	97.7 ± 12.7	100.7 ± 17.0	89.7 ± 11.6*
Confusion	−1.5 ± 2.1	−1.6 ± 1.8	−1.7 ± 2.6	−3.8 ± 2.1*
Tension	−0.31 ± 2.1	−0.46 ± 2.0	0.25 ± 2.9	−1.1 ± 1.7
Depression	5.1 ± 4.6	4.8 ± 5.0	3.9 ± 7.8	2.2 ± 1.6
Hostility	5.1 ± 2.1	5.0 ± 2.4	4.2 ± 4.3	3.2 ± 2.3
Vigor	13.8 ± 6.8	14.1 ± 5.6	12.0 ± 6.7	15.8 ± 4.7
Fatigue	5.1 ± 3.3	4.1 ± 2.6	6.2 ± 5.2	4.9 ± 3.7

*Data are mean ± standard deviation. *Bonferroni's post hoc with statistic significant difference from baseline.*

was a main effect of time (F = 11.923, p = 0.002, η^2 = 0.34) and statistically significant interaction (F = 10.344, p = 0.004) with a decrease in the SC group but not the placebo; however, there was no significant difference between groups (F = 2.120, p = 0.159).

Regarding correlations, there was a statistically significant relationship between absolute changes in the CK and mood disturbance only for the SC group (r = 0.74, p = 0.006) but no correlation was observed for the placebo (r = 0.06, p = 0.833) and a tendency to significant correlation was observed between CK-delta and confusion-delta in the SC group (r = 0.55; p = 0.06) but not in the placebo (r = 0.13; p = 0.67).

DISCUSSION

The main findings of this study were that SC nectar decreased muscle damage markers (CK and LDH) and MDA after 28 days of intervention in handball players and increased TAC. Furthermore, SC nectar improved psychological response with lower mood disturbance compared to baseline and a significant relationship was observed between changes in the CK concentration and mood disturbance only in the SC group.

It has been reported that oxidative stress caused by excessive training may compromise performance in athletes (Hattori et al., 2009; Tanskanen et al., 2010; Marin et al., 2013). Oxidative stress plays a critical role in overtraining syndrome in athletes (Tanskanen et al., 2010), which can be associated with fatigue, as well as lower muscle contractile function (Moopanar and Allen, 2005; Powers et al., 2011; Cheng et al., 2016), suggesting that oxidative stress is a target that needs to be controlled in athletes (Purvis et al., 2010; Pingitore et al., 2015). Previous studies have shown that supplementation with natural antioxidants is an efficient strategy to prevent oxidative stress in different kinds of athletes (Pingitore et al., 2015). For example, it has been demonstrated that supplementation with integral purple grape juice for 28 days improved running time-to-exhaustion in recreational runners as well as increasing antioxidant activity (Toscano et al., 2015).

In the present study, we demonstrated that 28 days of SC nectar was effective for reducing MDA and increasing TAC in young handball players, leading to protective effects against oxidative stress. Currently, there is a lack of studies investigating the effects of SC in humans. However, our findings are in agreement with a study conducted by Ulla et al. (2017), which investigated the effects of SC seed powder supplementation on oxidative stress in rats with high fat diet induced obesity. The findings showed that SC prevented oxidative stress by decreasing MDA levels and increasing activity of antioxidant enzymes (SOD, CAT, and GPX).

The improvements in redox homeostasis by SC supplementation may be explained by the high levels of phenolic compounds (Ayyanar and Subash-Babu, 2012), such as gallic acid and flavonoids (i.e., catechin, epicatechin, epigallocatechingallate, and epicatechingallate) as demonstrated in the present study, as these bioactive nutrients can generate antioxidant and anti-inflammatory activity as well as hypoglycemic effects (Meydani and Hasan, 2010; Kosuru et al., 2018). Studies have demonstrated that gallic acid, epicatechin, and epigallocatechingallate can activate a transcription factor denominated nuclear factor erythroid 2-related factor 2 (Nfr2), which in turn is translocated to the nucleus cell to synthesize antioxidant enzymes (Done and Traustadottir, 2016; Shin et al., 2016; Feng et al., 2017). We hypothesize that SC nectar supplementation increased TAC mediated by Nfr2 activation; however, future research is required to test this hypothesis.

Serum CK and LDH levels may be increased due to skeletal muscle damage as a consequence of intense training and are directly associated with training load (Horta et al., 2017). In addition, muscle damage can induce delayed-onset muscle soreness and performance loss (Peake and Neubauer, 2017). For this reason, persistently increased levels of serum CK are considered a biomarker of overtraining, often used for monitoring athletes in sport medicine (Brancaccio et al., 2007; Carfagno and Hendrix, 2014). We observed that SC supplementation influenced resting CK and LDH levels, with a significant reduction only in the SC group compared to baseline. These results may be explained by the influence of ROS production on the etiology of muscle damage through ion transport system oxidation (Kourie, 1998), as oxidative stress can lead to the release of muscle constituents to blood, such as CK and LDH (Armstrong, 1990; Taghiyar et al., 2013). Supporting this, previous studies have demonstrated that dietary antioxidant ingestion reduced biomarkers of muscle damage after fatiguing exercise (Taghiyar et al., 2013; Pereira Panza et al., 2015). Recently, Machado et al. (2018) demonstrated that green tea extract supplementation before an event of cumulative fatigue reduced muscle damage and oxidative stress in trained athletes. Thus, lower oxidative stress in the SC group may explain the reduction in serum CK and LDH levels, demonstrating that SC nectar supplementation could be an important strategy to maintain lower CK and LDH concentration during rest in young athletes.

Furthermore, the routine of athletes can induce changes in psychological response, such as mood disturbance, depression, difficulty in concentrating, and emotional instability, which are possible factors that explain performance loss during overtraining conditions (Purvis et al., 2010;

Carfagno and Hendrix, 2014). In addition, increases in mood disturbance are associated with training load (Morgan et al., 1987), demonstrating that monitoring mood state may be a potential method of preventing performance reduction in athletes. It has been demonstrated that brain oxidative stress is associated with neurodegenerative and psychological disorders, contributing to mood disturbance (Salim, 2014, 2017). Our results showed that SC nectar supplementation improved psychological response with lower mood disturbance after 28 days of intervention. We hypothesize that the improvements in redox homeostasis through decreased MDA levels and increased TAC may be one potential mechanism that SC nectar supplementation reduced mood disturbance, although further studies are necessary to confirm the influence of natural antioxidants on psychological response in athletes and also in patients with psychological disorders. In addition, we observed significant correlations between changes in CK concentration and mood disturbance only for the SC group but not the placebo. Corroborating with our data, Hollander et al. (2016) found an association between serum CK and psychological disorders, suggesting that CK may be used as a potential biomarker of affective state. Thus, further studies are necessary to understand the relationship between CK levels and psychological response.

Finally, our results showed that despite the improvement in psychological parameters and redox homeostasis with SC nectar supplementation, we did not observe a significant difference between the placebo and SC groups for performance gains, as both groups presented increased vertical jump and anaerobic performance. These findings may be explained as the subjects are well trained and only 1 month of supplementation may be insufficient to verify significant improvement in performance. Therefore, we suggest further studies analyzing the effects of SC supplementation for more than 28 days on performance in different kinds of athletes and practitioners of physical activity.

Despite the importance of our data, some limitations need to be considered; the short-term of the intervention (28 days); the fact that data collection was performed during the pre-season and cannot be broken down into player positions to investigate the differences between positions, since there were a small number of athletes, mainly pivot position and physical activities in the remained hour of the day was not assessment. Also, we suggest further studies investigating the pharmacokinetics of the compound, once the effects could be of the last dose rather than an adaptation to the continuous SC supplementation.

CONCLUSION

The present study suggests that SC nectar supplementation reduced biomarkers of oxidative stress and muscle damage, and improved psychological response in highly trained young handball players.

CLINICAL IMPLICATIONS

The present study suggests that SC nectar supplementation can be used as a natural compound to help in exercise recovery and damage prevention in athletes. The results of this study may be applied by coaches and nutritionists could be an important non-pharmacological and a natural compound for minimize oxidative stress, muscle damage, and improve psychological response and maybe an interesting strategy to prevent overtraining syndrome in highly trained young athletes.

AUTHOR CONTRIBUTIONS

MdS, FR, and LC devised the study design, participated in the interpretation of data, and drafted the manuscript. FR, AS, AKB, and ACB arried out the data collection, participated in the interpretation of data, and assisted in the writing the manuscript. MeM, MdF, MdS, SR, AS, and RdM participated in the interpretation of data and drafted the manuscript. FR performed all statistical analysis, participated in the interpretation of data, and assisted in the writing of the manuscript. All authors read and approved the final manuscript.

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Acute and Delayed Neuromuscular Alterations Induced by Downhill Running in Trained Trail Runners: Beneficial Effects of High-Pressure Compression Garments

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Introduction: The aim of this study was to examine, from a crossover experimental design, whether wearing high-pressure compression garments (CGs) during downhill treadmill running affects soft-tissue vibrations, acute and delayed responses in running economy (RE), neuromuscular function, countermovement jump, and perceived muscle soreness.

Methods: Thirteen male trail runners habituated to regular eccentric training performed two separate 40-min downhill running (DHR, –8.5°) sessions while wearing either CGs (15–20 mmHg for quadriceps and calves) or control garments (CON) at a velocity associated with ∼55% of VO_{2max}, with a set of measurements before (Pre-), after (Post-DHR), and 1 day after (Post-1D). No CGs was used within the recovery phase. Perceived muscle soreness, countermovement jump, and neuromuscular function (central and peripheral components) of knee extensors (KE) and plantar flexors (PF) were assessed. Cardiorespiratory responses (e.g., heart rate, ventilation) and RE, as well as soft-tissue vibrations (root mean square of the resultant acceleration, RMS A_r) for *vastus lateralis* and *gastrocnemius medialis* were evaluated during DHR and in Post-1D.

Results: During DHR, mean values in RMS A_r significantly increased over time for the *vastus lateralis* only for the CON condition (+11.6%). RE and cardiorespiratory responses significantly increased (i.e., alteration) over time in both conditions. Post-DHR small to very large central and peripheral alterations were found for KE and PF in both conditions. However, the deficit in voluntary activation (VA) was significantly lower for KE following CGs (–2.4%), compared to CON (–7.9%) conditions. No significant differences in perceived muscle soreness and countermovement jump were observed between conditions whatever the time period. Additionally, in Post-1D, the CGs condition showed reductions in neuromuscular peripheral alterations only for KE (from –4.4 to –7.7%) and perceived muscle soreness scores (–8.3%). No significant differences in

cardiorespiratory and RE responses as well as countermovement jump were identified between conditions in Post-1D.

Discussion: Wearing high-pressure CGs (notably on KE) during DHR was associated with beneficial effects on soft-tissue vibrations, acute and delayed neuromuscular function, and perceived muscle soreness. The use of CGs during DHR might contribute to the enhanced muscle recovery by exerting an exercise-induced "mechanical protective effect."

Keywords: compression garments, soft-tissue vibrations, muscle fatigue, running economy, muscle damage, downhill running

INTRODUCTION

Trail running is characterized by the succession of long uphill and downhill sections in a natural environment (*for review*, see Giandolini et al., 2016b). In trail running races where the distance may vary from short (<42 km) to ultra-long (≥100 km), severe alterations in neuromuscular function were reported with substantial failures in both central and peripheral neuromuscular mechanisms for knee extensors (KE) and plantar flexors (PF) (Millet et al., 2003, 2011b; Easthope et al., 2010; Saugy et al., 2013). In these studies, peripheral muscle fatigue may be greatly associated with exercise-induced muscle damage (EIMD) over repeated and prolonged eccentric muscle actions through downhill sections. Intense and/or prolonged downhill running (DHR) is well known to induce a substantial peripheral fatigue and/or low-frequency fatigue in lower limb muscles, assessed from reduced M-waves amplitudes and a decrease in the ratio between force evoked by low-frequency stimulation (e.g., 10–20 Hz) and force evoked by high-frequency stimulation (50–100 Hz) (Martin et al., 2004; Giandolini et al., 2016a). Cellular mechanisms underpinning peripheral fatigue may be attributed to longer muscle lengths (i.e., overstretched sarcomeres) during eccentric muscle actions over braking phases, leading to myofibrillar damage such as disrupted weaker sarcomeres and/or excitation–contraction coupling failure (Proske and Morgan, 2001; Douglas et al., 2017). Although the contribution of central component to neuromuscular fatigue is less important during DHR, central fatigue assessed by a decline in maximal voluntary activation (VA) (2.5–8.0%) was found for KE and PF following a 30-min treadmill DHR (−20%) and a 6.5 km downhill trail run (∼ −16%) (Giandolini et al., 2016a; Martin et al., 2004). This central fatigue could originate from supra-spinal level or from inhibitory reflexes mediated by free endings of group III and IV afferents, stimulated by metabolites and damage to muscle spindles (Martin et al., 2005).

From a biomechanical perspective, foot-ground impacts cause sudden decelerations of soft-tissue packages inducing muscle oscillations. According to the "muscle tuning" paradigm, muscle activity is tuned in response to impact forces to dampen soft-tissue vibrations (Wakeling et al., 2001). During DHR, substantial increases in vertical impact force peaks (>50%) and horizontal braking force peaks (>70%) at steep slopes (i.e., –9°) were observed, compared to level running (Gottschall and Kram, 2005). Regarding the knee and ankle joints, which are considered as net absorbers and generators of force during DHR, the negative work period as a percentage of total stance time is significantly greater for these two joints during DHR than level running sessions (Eston et al., 1995). This longer negative work period combined with a reduced upward displacement of the center of mass causes a gradual disappearance of the bouncing mechanism during DHR as speed and slope become greater (Dewolf et al., 2016). Consequently, the negative work done by both KE and PF muscles is about twofold greater during DHR with a −8.3% slope than during level running as the same speed (4.5 $m.s^{-1}$) (Buczek and Cavanagh, 1990). Therefore, KE or PF fatigue is greater after DHR than level running as a consequence of important absorption function and increased electromyographic activities (Giandolini et al., 2016a, 2017; Maeo et al., 2017). As a matter of fact, the increase in vertical downward velocity associated with higher ground reaction forces experienced during DHR might accentuate soft-tissue vibrations (Dewolf et al., 2016) and in turn, muscle activity. For instance, *triceps surae* soft tissue vibrations increased during prolonged and intense running sessions (Friesenbichler et al., 2011; Khassetarash et al., 2015). Since these findings were obtained during level treadmill running (∼40 min) at a relatively low velocity (from 3 to 4 $m.s^{-1}$), one could assume that prolonged exposures of KE and PF to higher loading rate induced by a strenuous DHR may cause greater soft-tissue vibrations and in turn, increased electromyographic activity, which might contribute to greater EIMD and muscle fatigue.

On the physiological side, muscle fatigue may be associated with acute and delayed alterations in running economy (RE), i.e., oxygen demand for a given running speed, following trail running events (Vernillo et al., 2017) or laboratory-based DHR sessions (Chen et al., 2007). In a recent review, Vernillo et al. (2017) have suggested that muscle fatigue needs to be compensated by a greater neural input to the active muscles to produce the same amount of force, particularly during the push-off phase of the running step, leading to an altered RE (Vernillo et al., 2017). Interestingly, following a 65-km mountain ultramarathon, RE was significantly altered during downhill treadmill running whereas no significant changes in either level or uphill RE were observed. These results suggest that different contraction regimens specifically affect RE during exercise (Vernillo et al., 2015). It was also described that repeated and prolonged muscle eccentric actions induced by a 30-min treadmill DHR durably affect level RE at high metabolic

intensities (>70%VO_{2max}) in the recovery phase (up to 5 days after DHR) in untrained and moderately trained subjects (Chen et al., 2007, 2009).

Several strategies including DHR training sessions and the use of lower limb compression garments (CGs) have been tested in an attempt to reduce RE alterations and detrimental effects of muscle damage and/or muscle fatigue induced by trail running or DHR events (Bieuzen et al., 2014; Hill et al., 2014; Peake et al., 2017; Toyomura et al., 2017; Vercruyssen et al., 2017). Although recent reviews and meta-analyses indicated that wearing CGs during recovery may be effective in the attenuation of EIMD (Beliard et al., 2014; Hill et al., 2014; Brown et al., 2017), the beneficial effects of CGs on acute physiological responses during running are still debated.

In this regard, MacRae et al. (2011) reported that discrepancies in the findings might be population- and exercise-dependent (e.g., training status, treadmill slopes) or related to CGs features (e.g., intensity of compression). Using magnetic resonance imaging, Miyamoto and Kawakami (2014) found that wearing short tights with a high-pressure intensity of 15–20 mmHg reduced muscle fatigue during treadmill running. Additionally, a reduction in KE force decline was identified following a 15.6 km short trail running only for subjects wearing high-pressure compression stockings (>15 mmHg) during exercise (Bieuzen et al., 2014). The use of high-pressure CGs (>15 mmHg) might thus induce a beneficial effect on muscle damage during exercise and potentially, on RE. Investigations with participants not accustomed to DHR showed that the use of CGs may be an effective method to reduce muscle damage induced by DHR (Valle et al., 2013), by attenuating soft-tissue vibrations during exercise (Bieuzen et al., 2014). However, this mechanistic hypothesis has never been validated during eccentric endurance exercises either in recreational subjects or trained runners. The interest of wearing CGs in trail runners habituated to DHR, and for whom adaptations due to the repeated bout effects have already been induced by eccentric training (Hyldahl et al., 2017), remains to be elucidated. Therefore, it seems important to assess the effectiveness of wearing high-pressure CGs during DHR within a homogeneous group of well-trained trail runners habituated to eccentric contractions, through several outcome measurements including soft-tissue vibrations, RE, neuromuscular function, countermovement jump performance, and perceived muscle soreness.

Accordingly, the objective of the current work was to examine the effects of wearing high-pressure CGs (>15 mmHg) during a 40-min treadmill DHR on acute and delayed neuromuscular responses and RE in well-trained trail runners accustomed to eccentric work. We hypothesized that wearing CGs during exercise would reduce soft-tissue vibrations and thus, acute and delayed central and peripheral fatigue and improve RE.

MATERIALS AND METHODS

Subjects

Thirteen well-trained male trail runners [(mean ± SD) age: 38.6 ± 5.7 years; height: 175.8 ± 5.1 cm; body mass: 72.1 ± 4.7 kg] participated to this study. Participants had a mean of 8.8 ± 3.4 years of trail running practice and were regularly involved in short-distance races (20–45 km). The average weekly training mileage during the two weeks before the first laboratory visit was 51.0 ± 20.6 km including 1913 ± 1181 m of positive/negative elevation. The sample size was calculated according to a previous study by Bieuzen et al. (2014) investigating the effect of a short trail running exercise [model inducing similar decrements in maximal voluntary contractions (MVC) than DHR] with the use of different running apparels on acute and delayed muscle fatigue (i.e., decline in MVC over time considered as the primary outcome), with a statistical power of 80% and a significance at $P \leq 0.05$. Their mean VO_{2max} and maximal heart rate (HR_{max}) were 64.6 ± 5.0 $ml.kg^{-1}.min^{-1}$ and 183.1 ± 8.1 $beats.min^{-1}$, respectively. All subjects had previous experience with CGs for at least 2 years but none of them wore CGs on a regular basis during racing. This study was carried out in accordance with the recommendations of local institutional review committee (University of Toulon) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the local institutional review committee (University of Toulon).

Experimental Design

Participants visited the laboratory on five different occasions. During the first visit, subjects performed a maximal test on a motorized treadmill (Venus® 200/100r, HP cosmos®, Germany) with a 10% slope that aimed at determining VO_{2max} and HR_{max}. During this test, HR and breath-by-breath VO_2 values were averaged every 10 s by the Oxycon Alpha metabolic measurement cart (Jaeger®, Germany). VO_{2max} was determined from the three highest consecutive values (i.e., over a 30-s interval) reached during the last stage of the protocol. Following a 30-min recovery period which enabled to return to baseline VO_2 and HR values (i.e., before the VO_{2max} protocol), subjects were instructed to run on the treadmill for the determination of velocity associated with 55% VO_{2max} of DHR (i.e., V_{DHR}, −8.5°). In this session, subjects were familiarized with all experimental procedures. During the second and the fourth visits (separated by one week), athletes performed one 40-min treadmill DHR while wearing different running garments, with a set of measurements immediately before (Pre-) and after (Post-DHR). Each DHR session was followed by a similar set of measurements 1 day after (Post-1D, i.e., third and fifth visits) to evaluate the delayed effects of DHR (**Figure 1**). These blocks of 2 days (i.e., DHR + Post-1D) were performed in a counterbalanced and randomized order[1].

Before starting DHR or Post-1D, subjects first carried out a warm-up which consisted of 7 min of level running ($3.05\ m.s^{-1}$) and 3 min of DHR ($3.33\ m.s^{-1}$; −10% slope). Then, perceived muscle soreness, countermovement jump, and neuromuscular function were evaluated in this order. During this set of measurements, subjects were asked to wear control garments (CON condition, loose-fitting conventional running garments, compression intensity <5 mmHg), whatever the running apparel

[1]www.randomizer.org

assigned to DHR. After the neuromuscular protocol, subjects kept CON garments or wore lower limb CGs (CGs condition, SALOMON® S/LAB EXO garments, stocking with 20–25 mmHg at the middle of calf and 18–20 mmHg at the upper site of calf, short-thigh with 16–18 mmHg at the middle of thigh, and 18–20 mmHg at the lower site of thigh) to begin DHR sessions. No "*in vivo*" CGs measurements (i.e., using a pressure sensor) were performed in our subjects during the DHR sessions. Prior to laboratory running sessions, CGs were re-fitted to obtain the required range of compression level according to manufacturer's guidelines and based on subject's circumference (i.e., upper, middle, and lower sites of thigh; middle and upper sites of calf) and limb lengths (Vercruyssen et al., 2017).

Running economy, HR, ventilation (V_E), respiratory exchange ratio (RER), and step frequency (f) were determined at different time periods of DHR conditions but also, at V_{DHR} (−8.5°) during the Post-1D run bouts. Accelerations of soft-tissue packages were exclusively measured during DHR conditions, allowing to assess soft-tissue vibrations at different time periods. At the end of each DHR, subjects were asked to report perceived muscle soreness scores and took off their CGs (for subjects wearing them during DHR) to complete a pair of countermovement jumps. No CGs were used during the set of measurements after DHR and within the recovery phase. The order of measurements in Post-1D was standardized as follows: perceived muscle soreness, countermovement jumps, neuromuscular function, and RE during the 5-min running bout at V_{DHR} (**Figure 1**). Between the end of each DHR and the beginning of Post-1D testing bouts, participants were instructed not to perform any interventions including massage, icing and nutritional strategies (e.g., protein intake) possibly affecting the recovery process. Each subject received an isotonic carbohydrate (CHO)-sports drink (600 ml) after DHR and the quantity of CHO feedings was standardized (i.e., 8–10 g CHO per kg body mass) during the recovery phase. The training program was also standardized during the 7-day washout period separating the two exercise blocks (i.e., a 1-day passive rest between the end of DHR and Post-1D sessions for a given block but also, before the second DHR block, with intermediate sessions of 60-min swimming and 40-min low-intensity flat running at a mean HR <75% HR_{max}). All experimental sessions were performed at the same time of day for a given subject and conducted between 10:00 and 16:00 hours under similar laboratory conditions (18–20°C, 35–40% relative humidity).

Downhill Running

Following 3 min of high-intensity level running (i.e., corresponding to 3.88 $m.s^{-1}$), the treadmill slope was immediately set to a −8.5° and V_{DHR} was also set to induce the equivalent of a metabolic intensity of 55% VO_{2max}. Then, the treadmill velocity was not changed within and between DHR exercises. Based on various pilot testing, V_{DHR} was considered as a severe intensity which could be mechanically tolerated (in terms of repeated braking forces) by subjects during a 40-min period. According to Chen et al. (2009) and the reality of trail running, the gradient was set at −8.5° to induce substantial mechanical impairments. Mean V_{DHR} represented 4.20 ± 0.23 $m.s^{-1}$ during the DHR conditions and the Post-1D run bouts (−8.5°). During the DHR conditions, mean VO_2 were 35.7 ± 3.5 $ml.min^{-1}.kg^{-1}$ for CON (i.e., 54.3 ± 4.6% VO_{2max}) and 36.4 ± 2.0 $ml.min^{-1}.kg^{-1}$ for CGs (i.e., 55.5 ± 2.9% VO_{2max}).

Neuromuscular Function

The neuromuscular function was tested using the method of electrical stimulation as recommended by Millet et al. (2011a) similarly on the right KE and PF muscles in Pre, Post-DHR (7 min and 10-min after exercise termination for KF and/or PF), and Post-1D (same order of measurements than in Post for KF and PF). The evaluation of neuromuscular function was randomized for KE and PF in Post-DHR for a given condition. Before each test, the optimal stimulation intensity was identified by delivering successive single electrical stimuli at increasing intensities on relaxed muscles on the femoral (for KE) and tibial (for PF) nerves. The stimulation intensity used during all tests was 130% of optimal intensity to ascertain full spatial recruitment. The optimal stimulation intensities ranged from 110 to 160 mA for KE and from 110 to 130 mA for PF through the Pre-, Post-DHR, and Post-1D sessions. For the KE testing, subjects seated upright in a custom-built chair with hips at 100° of flexion and knees at 90°. The subjects' ankle was strapped by non-compliant straps to the calibrated force transducer (F 501 TC 200 daN, TME 78 Orgeval, France) located slightly above the malleoli. The subjects were firmly attached to the ergometer with a rally car harness to avoid lateral and frontal displacements and were instructed to grip the harness during the test to standardize arm placement. For PF testing, subjects seated in the same custom-built chair and placed their foot, in a 0° dorsiflexion position, on a customized ergometer equipped with an instrumented pedal (SMTR 500 Nm, Sensel Measurement, Vincennes, France) located in the chair alignment. The forefoot was strapped to the pedal to limit heel lift and subjects were asked to perform a plantar flexion while keeping arms on their chest. The strain gauge and the pedal force were used to record the mechanical responses during MVC and electrically evoked contractions.

During the neuromuscular tests, transcutaneous electrical stimulations were applied to the femoral and posterior tibial nerves *via* a self-adhesive electrode cathode (10 mm in diameter) pressed manually by a researcher into either the femoral triangle (for KE) or the popliteal fossa (for PF) (Jubeau et al., 2017). The self-adhesive rectangular anode (50 mm × 90 mm, Dura-Stick Premium, Compex) was located either in the gluteal fold (for KE), or on the patella (for PF). A constant current stimulator (model DS7A, Digitimer, Hertfordshire, United Kingdom) delivered a square wave stimulus of 1 ms duration and 400 V maximal voltage and the interval of stimuli in the doublet were 100 and 10 ms for doublets at 10 Hz (Db_{10}) and 100 Hz (Db_{100}), respectively. Surface EMG signals were continuously recorded from the *vastus lateralis* and the *gastrocnemius medialis* muscles with a pair of self-adhesive surface (10 mm diameter) electrodes (Controle Graphique Medical, Brie-Comte-Robert, France) in bipolar configuration with a 20-mm interelectrode distance. The reference electrode was attached on the patella. Signals were amplified with a bandwidth frequency ranging from 1 Hz to 5 kHz (common mode rejection ratio = 110 dB, impedance

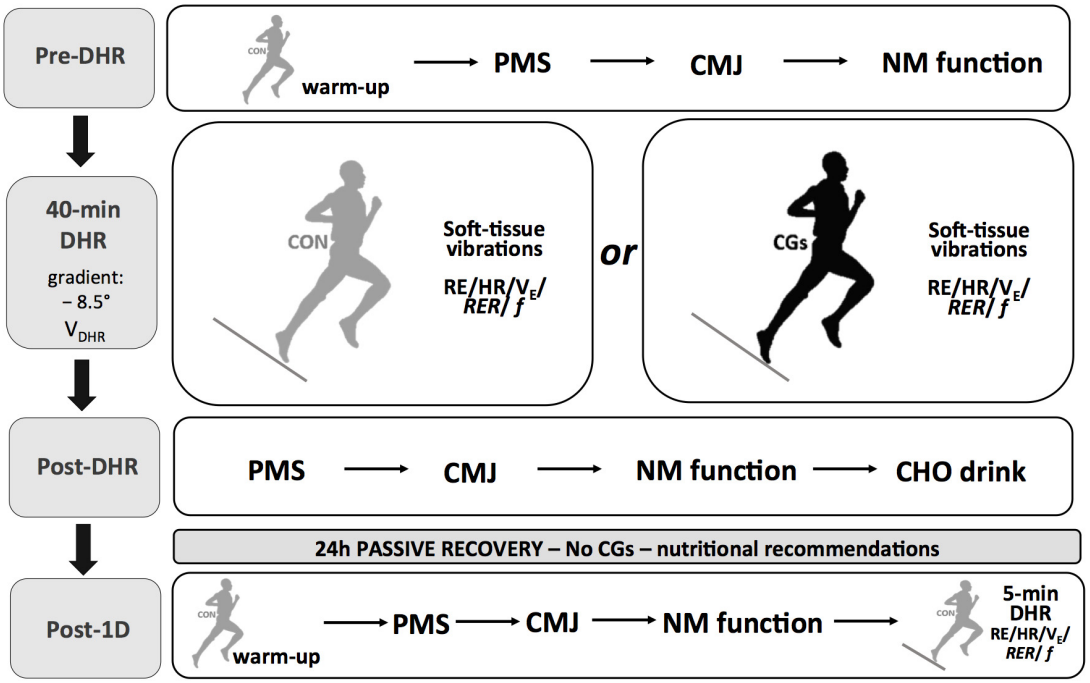

FIGURE 1 | Schematic representation of the experimental design. Abbreviations: CON, control garments; CGs, compression garments; DHR, downhill running; V_{DHR}, velocity associated with 55% VO_{2max}; PMS, perceived muscle soreness; CMJ, countermovement jump; NM function, neuromuscular function; RE, running economy; HR, heart rate; V_E, ventilation; RER, respiratory exchange ratio; *f*, step frequency.

input = 1000 MΩ, gain = 1000), digitized online at a sampling rate of 2000 Hz and stored for analysis with commercially available software (Acqknowledge 4.1, Biopac Systems Inc.).

For each condition, neuromuscular evaluation was conducted twice in Pre-, Post-DHR, and Post-1D. After a specific KE or PF isometric warm-up in both Pre- and Post-1D (i.e., 3-min submaximal contractions performed at increasing force levels), participants performed a similar neuromuscular evaluation for KE and PF which first consisted of a 4-s MVC followed by two single potentiated twitches separated by 2 s on the relaxed muscles. This procedure was repeated a second time after 15 s of rest. Following a resting period of 30 s, the subjects performed a third 4-s MVC superimposed with Db100 and followed after 2 s by two potentiated doublets in the relaxed muscle, i.e., Db100 and Db10, delivered 2 s apart. After 15 s of rest, this procedure was repeated a second time. The amplitude of the potentiated Db10, Db100, and the amplitude of the potentiated twitch peak torque (T_W) that followed the two doublets as well as the ratio of paired stimulation peak forces at 10 Hz over 100 Hz (Db10:100) were analyzed for both KE and PF. Throughout the testing sessions, subjects were strongly encouraged during their MVC. On the contrary, they were asked to be as relaxed as possible during the peripheral fatigue measurements. For each variable, values were then averaged from the two series in Pre-, Post-DHR, and Post-1D.

The variability in VA was determined to assess central fatigue for KE and PF using a high-frequency doublet (100 Hz) superimposed on MVC. VA was calculated from the maximal force (F_{max}) attained during the MVC, the force just before the superimposed doublet (F_{before}), the peak force following the superimposed doublet ($Db100_{sup}$), and control Db100 on relaxed muscle (Giandolini et al., 2016a) as follows:

$$VA = \left[1 - \frac{(Db100_{sup} - F_{before}) \times \frac{F_{before}}{F_{max}}}{\text{control Db100}}\right] \times 100. \quad (1)$$

Running Economy and Cardiorespiratory Parameters

Breath-by-breath VO_2 values, V_E, and RER were averaged every 10 s by the metabolic cart during overall DHR and Post-1D run sessions. For analysis, RE (expressed as VO_2 for a given running velocity, in $mlO_2.min^{-1}.kg^{-1}$), V_E, and RER were averaged values from two time periods at the beginning (3–5 and 8–10 min) and the end of DHR (33–35 and 38–40 min) but also, during the 5-min running bout at V_{DHR} (3–5 min) in Post 1-D. Using the HR sensor of the metabolic cart, HR values were also determined during the same time periods.

Running Mechanics

Stride frequency was determined during the same time periods than for the RE measurements during DHR and in Post-1D (see above) using the app *Runmatic* recently validated (Balsalobre-Fernández et al., 2016) and installed on an iPhone 6 running iOS 11.0.3 (240 Hz high-speed camera, Apple Inc., Cupertino, CA, United States). To record the step periods, one operator

lay prone on the ground, 30 cm from the back of the treadmill (e.g., to analyze the back of the subjects' feet), and held the iPhone in a vertical position at the same level as the floor of the treadmill. Then, contact time (t_c) was calculated as the time between the first frame in which the foot contacts the treadmill and the first frame in which the foot takes off. Aerial time (t_a) was calculated as the time between the first frame in which the foot takes off from treadmill and the first frame in which the other foot makes contact with the treadmill. Finally, t_c and t_a (in s) were averaged throughout eight consecutive steps (i.e., four stride cycles) and used to calculate step frequency (f, in Hz). The standard errors of estimate of the app *Runmatic*, compared to an opto-electronic device (*Optojump Next*) were 0.0056 s for contact time and 0.0048 s for aerial time.

Soft-Tissue Vibrations

The current accelerometry method for quantifying soft-tissue vibrations has been previously validated in the context of running (Coza et al., 2010). Two lightweight tri-axial accelerometers (range = ± 200 g, mass = ± 5 g, TSD109, Biopac Systems, Inc., Goleta, CA, United States) were placed on the skin under the CON garments and CGs at the muscle belly of the *vastus lateralis* and *gastrocnemius medialis* of the right leg to quantify soft-tissue vibrations. Accelerometers were placed under CGs to maintain the permanent contact between the sites of skin and accelerometers. Accelerometers were attached using a double-sided adhesive and slightly plated with an adhesive tape to improve congruence with soft tissues without altering their motion. Their location was marked with indelible ink on the skin to enable reproducible accelerometer placement within- and between conditions. Acceleration signals were sampled at 1000 Hz, recorded for a 30-s interval at different time intervals of DHR and subsequently analyzed in Scilab 5.5.2 software (Scilab Enterprises, Orsay, France). To quantify the amount of soft-tissue vibrations, a time domain analysis was performed over the overall signals including both the stance and flight phases. The resultant acceleration (A_r) was calculated from the three acceleration components for each muscle. The root mean square of the A_r values (i.e., RMS A_r) was then calculated and averaged using a 10-ms time window. For analysis, RMS A_r for each muscle was finally averaged from two time periods at the beginning (4–5 and 9–10 min) and the end of DHR (34–35 and 39–40 min) to characterize the time effect on RMS A_r within-and-between DHR sessions.

Perceived Muscle Soreness

According to previous studies (Chen et al., 2009), perceived muscle soreness scores were assessed in Pre, Post-DHR, and Post-1D sessions, using a visual analogue scale consisting of a 100-mm continuous line anchored by "no pain" (0 mm) and "very, very painful" (100 mm). Subjects were asked to report the severity of global muscle soreness concerning the quadriceps and calves immediately after performing a five repetition sit–stand motion on each occasion.

Countermovement Jump Performance

All participants were familiarized with countermovement jump testing during the first visit and completed countermovement jumps with hands on their hips, starting from a static position. Then, subjects performed a countermovement downward immediately followed by a complete extension of the lower limbs. During the flight phase of the jump, participants were instructed to jump as high as possible, and take-off and land with the feet simultaneously contacting the ground with the ankle in full dorsiflexion. The countermovement jump height was calculated using the app *My Jump* recently developed and validated by (Balsalobre-fernández et al., 2015). This app was installed on the same iPhone 6 used for the running mechanics recordings. To monitor the countermovement jump with *My Jump*, the device was installed onto the ground facing the subject's feet (at ∼1.5 m). Take-off and landing frames of the video were used by the app for the calculation of the flight time and in turn countermovement jump height. Each athlete performed two countermovement jumps, separated by a 1-min passive rest period, in Pre (after a 10-min run warm-up), Post-DHR, and Post-1D sessions. The two countermovement jumps values were averaged for further statistical analysis. When compared to a force platform, Balsalobre-fernández et al. (2015) showed that a good reliability of the app for jump heights performed by different subjects (observer 1: α = 0.997, CV = 3.4%; observer 2: α = 0.988, CV = 3.6%). Furthermore, the Pearson product moment correlation coefficient showed almost perfect correlation between the app and the force platform measurements for jump height (r = 0.995, $P < 0.001$).

Statistical Analysis

All dependent variables among CON and CGs exercises over the DHR/Post-1D blocks were initially tested for the normality of distribution and the homogeneity of variances using Shapiro–Wilk and Levene tests. Separate two-way ANOVAs (condition [CON, CGs] × time [Pre, Post, or Post-1D] and time periods over DHR) with repeated measures were applied to all dependent variables. This allowed quantification of the acute (Pre-Post) and delayed (Pre-Post 1-D) fatigue induced by DHR on dependent variables. When significant main effects were observed, Bonferroni's test was used for *post-hoc* analysis. Within- and between exercise differences were also standardized from the use of Cohen's effect sizes (ES) and thresholds [>0.2 (*small*), >0.6 (*moderate*), >1.2 (*large*), and >2.0 (*very large*)] associated with 90% confidence limits (CL) to compare the magnitude of the difference of the change between pre- and post-DHR (immediate and post-1D) (Hopkins et al., 2009; Bieuzen et al., 2014). Probabilities that differences were higher, lower or similar to the smallest worthwhile difference (ES of 0.20) were evaluated qualitatively as follows: *possibly*, 25–74.9%; *likely*, 75–94.9%, *very likely*, 95–99.5%; *most (extremely) likely*, >99.5%. The true difference was assessed as *unclear* if the chance of both higher and lower values was >5% (Hopkins et al., 2009; Buchheit, 2016). The null hypothesis significance testing (NHST) was the primary method to discuss the current results and the qualitative approach (e.g., Cohen's ES and smallest worthwhile changes) was

used to further illustrate the differences between CON and CGs conditions, particularly when one clothing modality induced a significant effect over time. For all tests, an alpha of $P < 0.05$ was considered statistically significant. All qualitative analyses were conducted using modified statistical Excel spreadsheets.[2]

RESULTS

All participants successfully completed the two blocks of DHR-Post-1D sessions, excepted for one subject where PF MVC values could not be analyzed for technical reasons. All standardized effects are presented as ES ± 90% CL. In order to ensure the CGs or CON conditions were performed in a comparable physical state, MVC for KE and PF but also, body mass were selected as standardization variables across exercises (in Pre-DHR). No significant differences were observed between conditions for the MVC responses for KE ($P = 0.08$) and PF ($P = 1.00$) or body mass ($P = 1.00$).

Neuromuscular Function

The changes in neuromuscular responses within-and-between exercise blocks are presented in **Table 1** (i.e., Pre/Post-DHR) and **Table 2** (i.e., Pre-DHR/Post-1D). Small to very large alterations (i.e., significant decreases) in acute neuromuscular responses, excepted for VL and GAST M-waves but also, for PF Db10:100, were found for both CON and CGs conditions, whatever the studied muscle (**Table 1**). A significant time × condition interaction was identified immediately after DHR for the KE VA deficit ($P = 0.022$). The KE VA deficit was significantly higher after CON than CGs, with a "moderate" standardized difference (ES = 0.83 ± 0.76, **Table 1**). The PF VA deficit significantly increased in the CON condition ($P = 0.022$), whereas no significant change in this variable was observed in the CGs condition ($P = 0.398$). Substantial delayed effects were also identified on neuromuscular responses for KE after CON condition while minor delayed effects were reported following CGs condition (**Table 2**). A significant time × condition interaction was found for decrements in KE MVC ($P = 0.033$), KE Db10 ($P = 0.035$), and KE Db10:100 ($P = 0.042$) which were significantly lower at 24-h after CGs condition, compared to CON condition. These differences in delayed neuromuscular responses were not reported for PF, excepted for T_w values that were significantly lower following CON condition ($P = 0.036$). Given that the most beneficial effects of CGs were observed in Post-1D, the magnitude of Pre-DHR/Post-1D changes for all neuromuscular variables is presented in **Figure 2**. In addition, individual MVC responses for KE and PF across the two exercise blocks are displayed in **Figure 3**.

Soft-Tissue Vibrations

Figure 4A shows changes in RMS A_r over time for *vastus lateralis* and *gastrocnemius medialis* within CON and CGs conditions. A significant increase in RMS A_r was observed over time for the *vastus lateralis* in the CON condition (+11.6 ± 5.9%; ES = 0.69 ± 0.24; $P = 0.003$), while no significant difference in this variable was observed over time in the CGs condition (+6.6 ± 5.2%; ES = 0.33 ± 0.16, $P = 0.121$). The increase in RMS A_r was *likely* higher after CON than CGs conditions, with a "moderate" standardized difference between conditions (ES = –0.86 ± 0.71). No significant differences in RMS A_r were found for the *gastrocnemius medialis* within-and-between exercises ($P = 0.246$).

Running Economy, Cardiorespiratory Parameters, and Stride Frequency

The most significant changes in VO_2, V_E, HR, RER, and f were observed at the end of DHR in both CON and CGs conditions (**Figure 4**) while no significant differences in these variables were found in Post-1D, as compared to the beginning of each DHR (**Table 3**). At the end of DHR, a significant increase was observed in both conditions (i.e., time effect) for VO_2 (CON: +5.3 ± 6.0%; ES = 0.51 ± 0.31, $P = 0.049$ and CGs: +6.8 ± 5.6%; ES = 0.91 ± 0.41; $P = 0.012$), V_E (CON: +11.0 ± 11.3%; ES = 0.53 ± 0.27, $P = 0.013$ and CGs: +10.3 ± 9.6%; ES = 0.71 ± 0.36; $P = 0.013$), and HR (CON: +9.3 ± 5.5%; ES = 1.04 ± 0.35; $P = 0.002$ and CGs: +12.6 ± 7.4%; ES = 1.26 ± 0.39; $P < 0.001$). In addition, RER values were significantly lower over time only for CGs (–4.9 ± 4.4%; ES = –0.63 ± 0.31; $P = 0.004$), whereas no significant change in this variable was observed for CON (−3.1 ± 2.2%; ES = –0.40 ± 0.15; $P = 0.066$). Similarly, f values were significantly lower over time only for CGs (–2.2 ± 2.1%; ES = −0.69 ± 0.32; $P = 0.003$), whereas no significant change in this variable was observed for CON (–1.3 ± 2.7%; ES = –0.37 ± 0.39; $P = 0.119$).

Perceived Muscle Soreness and Jump Performance

The magnitude of changes in perceived muscle soreness reported after CON and CGs conditions and in Post-1D is presented in **Figure 5**. A significant time × condition interaction was identified for scores in perceived muscle soreness only at quadriceps level ($P = 0.026$) which were significantly lower in Post-1D following CGs compared to CON, whereas no significant time ($P = 0.069$) or time × condition interaction effects ($P = 0.117$) were found at calves level. In addition, a significant decrease in countermovement jump was observed for CON (−6.7 ± 7.1%; ES = –0.57 ± 0.32; $P = 0.003$) and a strong trend for a decrease was identified for CGs (–4.2 ± 8.4%; ES = –0.31 ± 0.26; $P = 0.073$) following DHR. The significant alterations in jump performance were also identified in Post-1D for CON (–7.7 ± 5.8%; ES = –0.68 ± 0.26; $P = 0.003$) and CGs (–5.1 ± 7.5%; ES = –0.39 ± 0.24; $P = 0.039$) conditions. The mean values in countermovement jump were 36.2 ± 4.0 and 35.2 ± 4.9 cm in Pre-DHR, 33.2 ± 3.4 and 33.6 ± 4.3 cm in Post-DHR, 33.3 ± 3.0 and 33.2 ± 3.3 cm in Post-1D for CON and CGs conditions, respectively.

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TABLE 1 | Magnitude of Post-Pre downhill running (DHR) changes in neuromuscular variables for knee extensors and plantar flexors within- and between control garments (CON) and compression garments (CGs) conditions.

Downhill running (Δ Post-Pre)	CON condition						CGs condition						Post-Pre condition change		
	Pre-DHR	Post-DHR	Post-Pre DHR				Pre-DHR	Post DHR	Post-Pre DHR				CGs–CON conditions		
	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	ES ± 90% CL	MBI	NHST (time × condition)
Knee extensors															
MVC (Nm)	222.9	183.6	−18.2	−0.76	Moderate****	$P < 0.001$	213.7	184.9	−13.9	−0.55	Small****	$P < 0.001$	0.21	Small*	$P = 0.163$
	±48.5	±48.3	±8.1	±0.16			±48.7	±49.3	±9.8	±0.19			±0.25		
VA (%)	93.8	86.5	−7.9	−1.37	**Large**** **	**$P = 0.001$**	92.4	90.0	−2.4	−0.34	Unclear	$P = 0.527$	0.83	**Moderate**** **	**$P = 0.022$**
	±4.9	±7.7	±5.1	±0.49			±6.5	±8.2	±8.4	±0.55			±0.76		
VL M-wave (mV)	8.2	6.9	−9.4	−0.67	Moderate**	$P = 0.743$	7.9	7.2	−6.6	−0.37	Small**	$P = 1.000$	0.22	Unclear	$P = 0.692$
	±1.3	±1.8	±18.9	±0.73			±1.6	±1.4	±14.7	±0.43			±0.82		
Tw (Nm)	48.9	31.2	−36.3	−3.20	Very large****	$P < 0.001$	46.6	28.8	−38.0	−2.77	Very large****	$P < 0.001$	0.00	Unclear	$P = 0.996$
	±5.2	±5.4	±9.0	±0.44			±6.0	±4.4	±6.9	±0.32			±0.53		
Db10 (Nm)	73.6	41.3	−43.7	−2.57	Very large****	$P < 0.001$	70.2	38.3	−45.1	−2.47	Very large****	$P < 0.001$	0.03	Unclear	$P = 0.843$
	±11.8	±9.2	±11.2	±0.40			±12.1	±7.4	±7.8	±0.34			±0.53		
Db100 (Nm)	75.6	62.3	−17.3	−1.10	Large****	$P < 0.001$	72.7	60.2	−16.9	−0.97	Large****	$P < 0.001$	0.07	Unclear	$P = 0.723$
	±11.3	±9.4	±8.2	±0.29			±12.0	±10.2	±7.3	±0.23			±0.37		
Db10:100 (%)	97.8	65.9	−32.0	−2.83	Very large****	$P < 0.001$	97.1	63.7	−33.7	−2.63	Very large****	$P < 0.001$	0.07	Unclear	$P = 0.673$
	±10.5	±8.8	±11.3	±0.57			±11.9	±6.7	±9.1	±0.47			±0.37		
Plantar flexors															
MVC (Nm)	132.1	111.5	−15.5	−0.63	Moderate****	$P < 0.001$	137.0	121.6	−10.6	−0.41	Small**	$P = 0.001$	0.16	Trivial*	$P = 0.225$
	±30.5	±29.0	±9.2	±0.21			±34.8	±31.2	±10.8	±0.22			±0.31		
VA (%)	97.4	90.5	−7.0	−1.87	Large***	$P = 0.022$	98.3	94.5	−3.9	−0.84	Moderate***	$P = 0.398$	0.78	Unclear	$P = 0.274$
	±3.4	±8.5	±8.2	±1.16			±4.2	±5.5	±3.7	±0.42			±1.16		
GAST M-wave (mV)	10.4	9.5	−9.2	−0.53	Moderate**	$P = 0.477$	10.6	9.5	−9.2	−0.54	Moderate**	$P = 0.245$	−0.10	Unclear	$P = 0.791$
	±1.6	±2.7	±23.9	±0.64			±1.9	±1.7	±15.7	±0.37			±0.72		
Tw (Nm)	18.8	15.7	−15.6	−0.62	Moderate****	$P < 0.001$	17.0	14.9	−10.7	−0.38	Small**	$P = 0.004$	0.20	Small*	$P = 0.152$
	±4.5	±3.8	±10.6	±0.24			±4.5	±4.1	±14.2	±0.24			±0.34		
Db10 (Nm)	33.9	27.7	−16.3	−0.47	Small***	$P = 0.005$	32.5	25.0	−18.8	−0.52	Small**	$P = 0.001$	−0.10	Unclear	$P = 0.513$
	±12.3	±10.0	±14.8	±0.23			±13.5	±10.4	±24.3	±0.34			±0.72		
Db100 (Nm)	36.8	33.0	−7.6	−0.26	Small*	$P = 0.037$	35.3	29.8	−11.9	−0.35	Small**	$P = 0.003$	−0.11	Unclear	$P = 0.348$
	±13.6	±11.1	±17.6	±0.26			±14.6	±12.0	±23.9	±0.30			±0.41		
Db10:100 (%)	92.3	84.0	−8.0	−0.89	Moderate**	$P = 0.270$	92.9	83.9	−8.3	−0.62	Moderate***	$P = 0.191$	−0.06	Unclear	$P = 0.892$
	±8.6	±10.7	±15.8	±0.83			±13.4	±7.0	±12.7	±0.40			±0.81		

*Values are expressed in percent change (%?) ± standard deviation (SD) and standardized effect size (ES) ± 90% confidence limits (CL). NHST, method of null-hypothesis significance testing ($P < 0.05$); MBI, method of magnitude based inferences; qualitative inferences are trivial (<0.20), small (0.20 to <0.60), moderate (0.60 to <1.20), large (1.20 to <2.00) and very large (>2.00): *possibly, 25 to <75%; **likely, 75 to <95%;***very likely, 95 to <99.5%, ****almost certain, >99.5%. MVC, maximal voluntary contraction; VA, maximal voluntary activation; Mwave, peak-to-peak amplitude; Tw, potentiated twitch torque; Db10, low-frequency doublet force; Db100, high-frequency doublet force and Db10:100, low-to-high doublet frequency ratio. Values are in bold when NHST (time × condition) is statistically significant ($P < 0.05$).*

TABLE 2 | Magnitude of Post-1D-Pre downhill running (DHR) changes in neuromuscular variables for knee extensors and plantar flexors within- and between control garments (CON) and compression garments (CGs) conditions.

Recovery phase (Δ Post-1D-Pre)	CON condition						CGs condition						Post-Pre condition change		
	Pre-DHR	Post-DHR	Post-Pre DHR				Pre-DHR	Post DHR	Post-Pre DHR				CGs–CON conditions		
	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	ES ± 90% CL	MBI	NHST (time × condition)
Knee extensors															
MVC (Nm)	222.9	201.5	−10.4	−0.41	**Small*** **	***P* = 0.002**	213.7	206.6	−2.6	−0.14	Trivial**	*P* = 0.707	0.29	**Small** **	***P* = 0.033**
	±48.5	±54.5	±7.9	±0.13			±48.7	±43.3	±7.22	±0.13			±0.18		
VA (%)	93.8	89.7	−4.4	−0.77	Moderate**	*P* = 0.463	92.4	89.8	−2.6	−0.37	Unclear	*P* = 1.000	0.29	Unclear	*P* = 0.622
	±4.9	±6.1	±4.2	±0.41			±6.5	±7.5	±8.8	±0.60			±0.73		
VL M-wave (mV)	8.2	7.7	−6.2	−0.35	Small*	*P* = 1.000	7.9	7.3	−5.8	−0.36	Small*	*P* = 1.000	0.07	Unclear	*P* = 0.836
	±1.3	±1.8	±14.0	±0.46			±1.6	±1.2	±16.4	±0.42			±0.63		
Tw (Nm)	48.9	46.2	−5.7	−0.49	Small**	*P* = 0.238	46.6	46.6	0.2	0.01	Unclear	*P* = 0.703	0.48	Small**	*P* = 0.123
	±5.2	±7.2	±9.6	±0.41			±6.0	±6.7	±8.3	±0.29			±0.48		
Db10 (Nm)	73.6	67.8	−8.5	−0.46	**Small*** **	***P* = 0.022**	70.2	69.7	−0.8	−0.03	Unclear	*P* = 1.000	0.44	**Small** **	***P* = 0.035**
	±11.8	±15.0	±9.0	±0.23			±12.1	±13.2	±8.1	±0.21			±0.32		
Db100 (Nm)	75.6	72.6	−4.1	−0.25	Small**	*P* = 0.175	72.7	72.0	−0.9	−0.06	Unclear	*P* = 1.000	0.19	Trivial*	*P* = 0.208
	±11.3	±12.5	±5.9	±0.17			±12.0	±12.1	±7.3	±0.20			±0.27		
Db10:100 (%)	97.8	93.1	−4.6	−0.42	**Small** **	***P* = 0.046**	97.1	97.2	0.2	0.00	Unclear	*P* = 1.000	0.42	**Small** **	***P* = 0.042**
	±10.5	±11.0	±8.1	±0.34			±11.9	±11.8	±6.4	±0.24			±0.42		
PLANTAR FLEXORS															
MVC (Nm)	132.1	123.5	−7.0	−0.26	Small*	*P* = 0.073	137.0	130.3	−4.1	−0.18	Trivial*	*P* = 0.247	0.06	Unclear	*P* = 0.639
	±30.5	±35.0	±11.2	±0.22			±34.8	±29.3	±7.9	±0.16			±0.27		
VA (%)	97.4	94.4	−3.0	−0.81	Moderate**	*P* = 0.319	98.3	94.3	−4.3	−0.91	Moderate***	*P* = 0.076	−0.28	Unclear	*P* = 0.580
	±3.4	±5.7	±6.2	±0.86			±4.2	±8.2	±5.0	±0.49			±0.94		
GAST M-wave (mV)	10.4	9.7	−7.4	−0.42	Small*	*P* = 1.000	10.6	9.5	−9.6	−0.55	Small**	*P* = 0.449	−0.22	Unclear	*P* = 0.630
	±1.6	±2.7	±19.2	±0.59			±1.9	±2.3	±19.6	±0.50			±0.77		
Tw (Nm)	18.8	16.8	−11.3	−0.40	Small**	*P* = 0.036	17.0	16.1	−3.5	−0.16	Trivial*	*P* = 0.917	0.21	Small*	*P* = 0.217
	±4.5	±5.3	±10.5	±0.21			±4.5	±4.3	±14.7	±0.21			±0.30		
Db10 (Nm)	33.9	30.7	−9.9	−0.24	Small*	*P* = 0.899	32.5	28.4	−7.0	−0.28	Small*	*P* = 0.414	−0.07	Unclear	*P* = 0.747
	±12.3	±15.3	±21.5	±0.31			±13.5	±10.1	±23.8	±0.31			±0.45		
Db100 (Nm)	36.8	33	−11.8	−0.26	Small*	*P* = 0.539	35.3	31.8	−5.0	−0.22	Small*	*P* = 0.737	0.03	Unclear	*P* = 0.896
	±13.6	±16.6	±18.9	±0.27			±14.6	±12.7	±25.5	±0.33			±0.44		
Db10:100 (%)	92.3	93.7	2.0	0.16	Unclear	*P* = 1.000	92.9	91.4	0.5	−0.10	Unclear	*P* = 1.000	−0.26	Unclear	*P* = 0.509
	±8.6	±9.0	±9.9	±0.50			±13.4	±12.4	±22.0	±0.66			±0.90		

*Values are expressed in percent change (%?) ± standard deviation (SD) and standardized effect size (ES) ± 90% confidence limits (CL). NHST, method of null-hypothesis significance testing (P < 0.05); MBI = method of magnitude based inferences; qualitative inferences are trivial (<0.20), small (0.20 to <0.60), moderate (0.60 to <1.20), large (1.20 to <2.00), and very large (>2.00): *possibly, 25 to <75%; **likely, 75 to <95%;***very likely, 95 to <99.5%, ****almost certain, >99.5%. Abbreviations: MVC, maximal voluntary contraction; VA, maximal voluntary activation; Mwave, peak-to-peak amplitude; T_w, potentiated twitch torque; Db10, low-frequency doublet force; Db100, high-frequency doublet force; Db10:100, low-to-high doublet frequency ratio. Values are in bold when NHST (time × condition) is statistically significant (P < 0.05).*

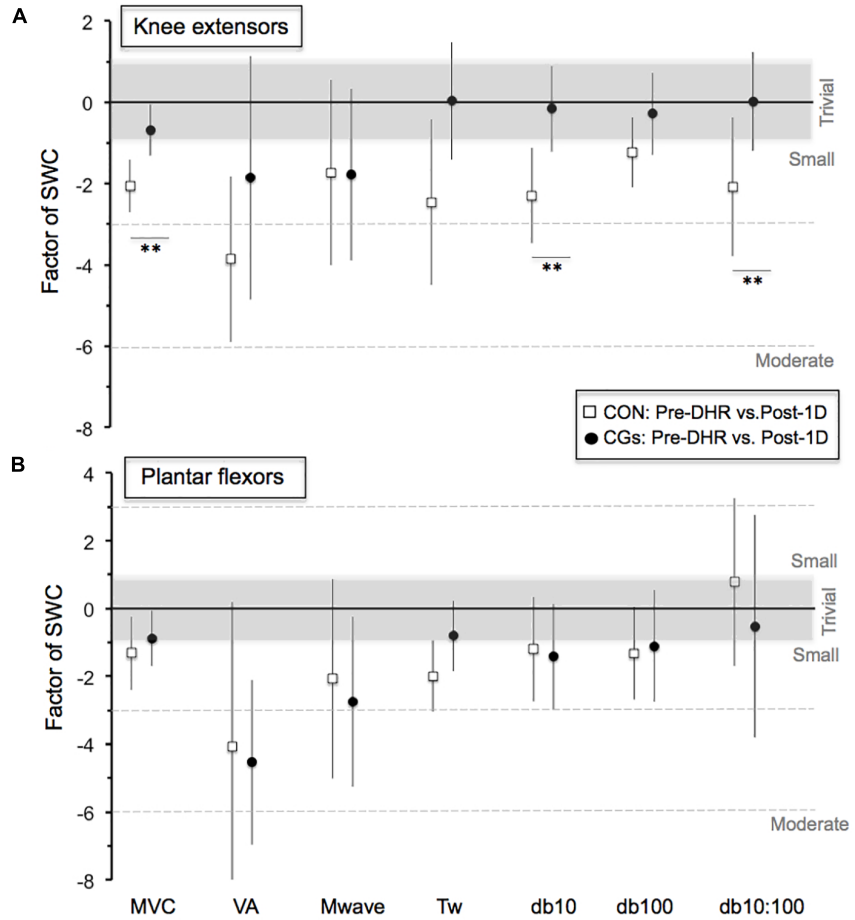

FIGURE 2 | Magnitude of changes in the neuromuscular responses (Pre-DHR/Post-1D) for knee extensors **(A)** and plantar flexors **(B)** for control (CON) and compression garments (CGs) exercises. The standardized differences are expressed as a factor of the smallest worthwhile change [SWC = ES (Cohen's *d*) of 0.2]. Bars indicate the 90% CLs. The number of asterisk (*) indicates the likelihood for the between-exercise differences to be substantial, with * indicating a *possible* and ** a *likely* difference. In the CGs exercise, the magnitude of changes in MVC, Db10, and Db10:100 for knee extensors was 1.5, 2.2, and 2.1 times greater than the SWC, respectively. Abbreviations: MVC, maximal voluntary contraction; VA, maximal voluntary activation; Mwave, peak-to-peak amplitude; Tw, potentiated twitch torque; Db10, low-frequency doublet force; Db100, high-frequency doublet force and Db10:100, low-to-high doublet frequency ratio.

DISCUSSION

We hypothesized that wearing high-pressure CGs during an intense downhill run would attenuate soft-tissue vibrations, acute and delayed alterations in muscle function and improve RE. Our hypotheses have been partially confirmed, as the most important findings of this study are (i) an attenuation of soft-tissue vibrations during DHR (only for the *vastus lateralis*) and a reduced VA deficit (only for KE) in the CGs condition, (ii) a deterioration in RE for both CGs and CON conditions, and (iii) a faster recovery of MVC and peripheral parameters at 24 h post-CGs with lesser muscle soreness (only for KE).

Acute Effects of Wearing Compression Garments

A reduction in MVC is a well-acknowledged and reliable index for assessing muscle damage within a whole muscle group (Damas, 2016). The magnitude of MVC decline appears to be directly related to the number of muscle fibers with myofibrillar disruption and/or excitation-contraction coupling failure (Raastad et al., 2010; Peake et al., 2017). *Small* to *moderate* decreases in MVC were observed immediately after DHR either in CGs or CON condition for KE (–13.9 ± 9.8 and –18.2 ± 8.1%, respectively) and PF muscles (–10.6 ± 10.8 and –15.5 ± 9.2%, respectively, **Table 1**). The significant decrements in MVC, whatever the condition, were lower than those measured in previous downhill studies using a 30-min treadmill exercise (∼ –20% for KE) (Chen et al., 2007) or a 6.5 km downhill trail run (−18.6% for KE and −25.4% for PF) (Giandolini et al., 2016a). The observed discrepancies between studies are likely due to the differences in DHR training status of the subjects. Indeed, the subjects recruited in the present study had a high trail running background and/or practice and hence, all were highly accustomed to DHR before the starting of laboratory sessions. Overall, the acute MVC responses to the high DHR intensity (∼4.2 $m.s^{-1}$) might be specific to the training status of our

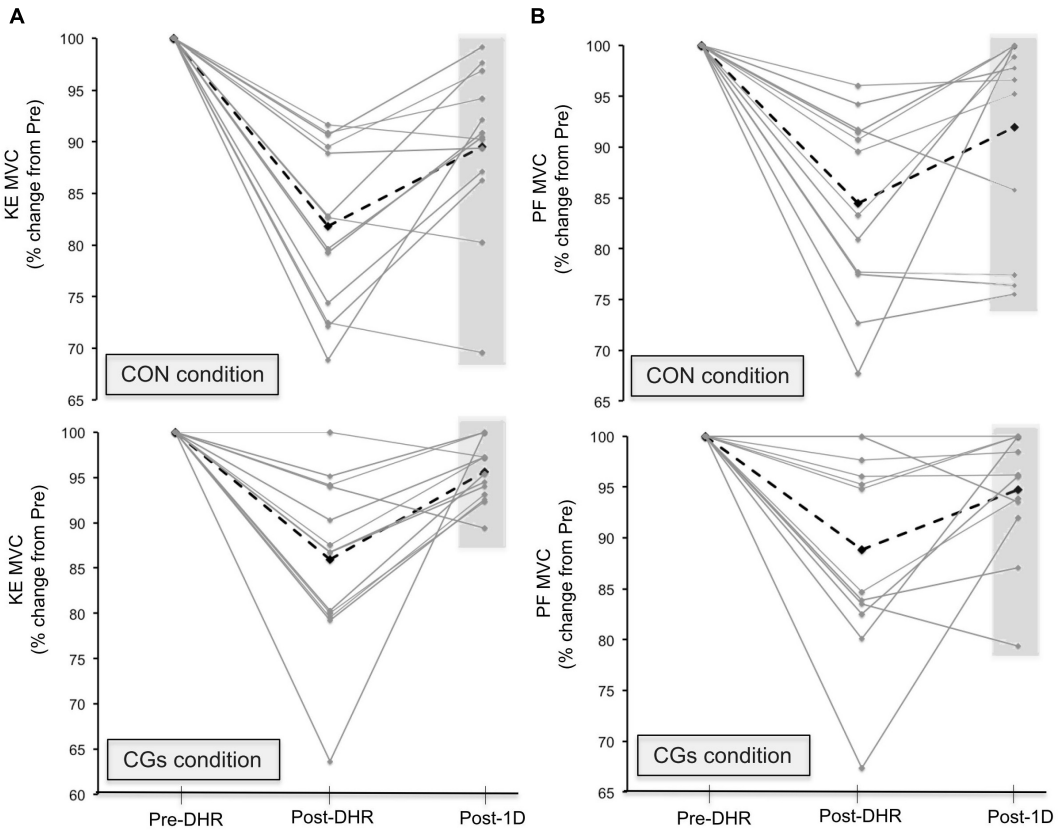

FIGURE 3 | Individual responses in maximal voluntary contraction (MVC) torques for knee extensors [KE **(A)**] and plantar flexors [PF **(B)**] muscles in Pre-downhill running (DHR), immediate Post-DHR, and following 1 day recovery (Post-1D). Values are expressed in percentage change from Pre-DHR. The black dotted line indicates the average MVC value for a given condition. The gray area indicates subjects that may be "responders" or "non-responders" to CGs exercises in Post-1D sessions. A great percentage of positive responders to KE MVC recovery was identified in the CGs exercise. Abbreviations: CON, control garments; CGs: compression garments.

population and thus provide valuable insights on the evaluation of muscle function following DHR in trained subjects.

Maximal voluntary contractions loss for KE and PF following DHR is usually related to alterations in both central and peripheral factors (Giandolini et al., 2016b). The current findings indicate small to large alterations in VA and peripheral variables (M-wave or Db10:100) for KE and PF, independently of the experimental condition, with greater changes in the peripheral components (**Table 1**). These data are consistent with those reported in previous laboratory and ecological downhill studies (Martin et al., 2004; Giandolini et al., 2016a). Thus, eccentric exercise such as DHR is known to induce severe lower limb tissue damage and low-frequency fatigue (i.e., decreased Db10:100), particularly for KE. Larger decreases in Db10:100 for KE (from –32.0 to –33.7%) compared to PF (from –8.0 to –8.3%) were observed in the present study, which are in line with those recently observed following a 6.5-km downhill trail run (Giandolini et al., 2016a). Several mechanisms are proposed to characterize peripheral fatigue, including impairments in sarcolemmal action potential conduction and excitability (Piitulainen et al., 2008, 2010), depressed Ca^{2+} release from the sarcoplasmic reticulum (Hill et al., 2001; Martin et al., 2005) but also decreased in Ca^{2+} sensitivity and/or force produced by active cross-bridges (Place et al., 2010). Although using CGs has been shown to reduce muscle activation during prolonged level running, which might improve muscle function (Hsu et al., 2017), results of the present work do not support a beneficial effect of CGs on peripheral fatigue for KE and PF. The delay time for measuring muscle function (7–10 min after exercise termination) constitutes a methodological limitation, which may have counteracted the potential benefits of CGs on the extent of peripheral fatigue. In this regard, Froyd et al. (2013) demonstrated a rapid recovery of peripheral variables within the first 8 min of the recovery period, thus underestimating not only the extent of peripheral fatigue in our study (and previous reports) but also the potential beneficial effect of CGs.

Surprisingly, the most noticeable acute effect of wearing CGs was observed on the VA deficit. Indeed, the KE VA deficit was significantly lower following the CGs condition, compared to the CON condition (–2.4 vs. –7.9%, respectively, **Table 1**). It is well documented that central drive is controlled by a combination of factors including excitatory and inhibitory reflex inputs from muscles, joints, tendons, and cutaneous afferents (Millet et al., 2012; Amann et al., 2015). Hence, an attenuated VA deficit,

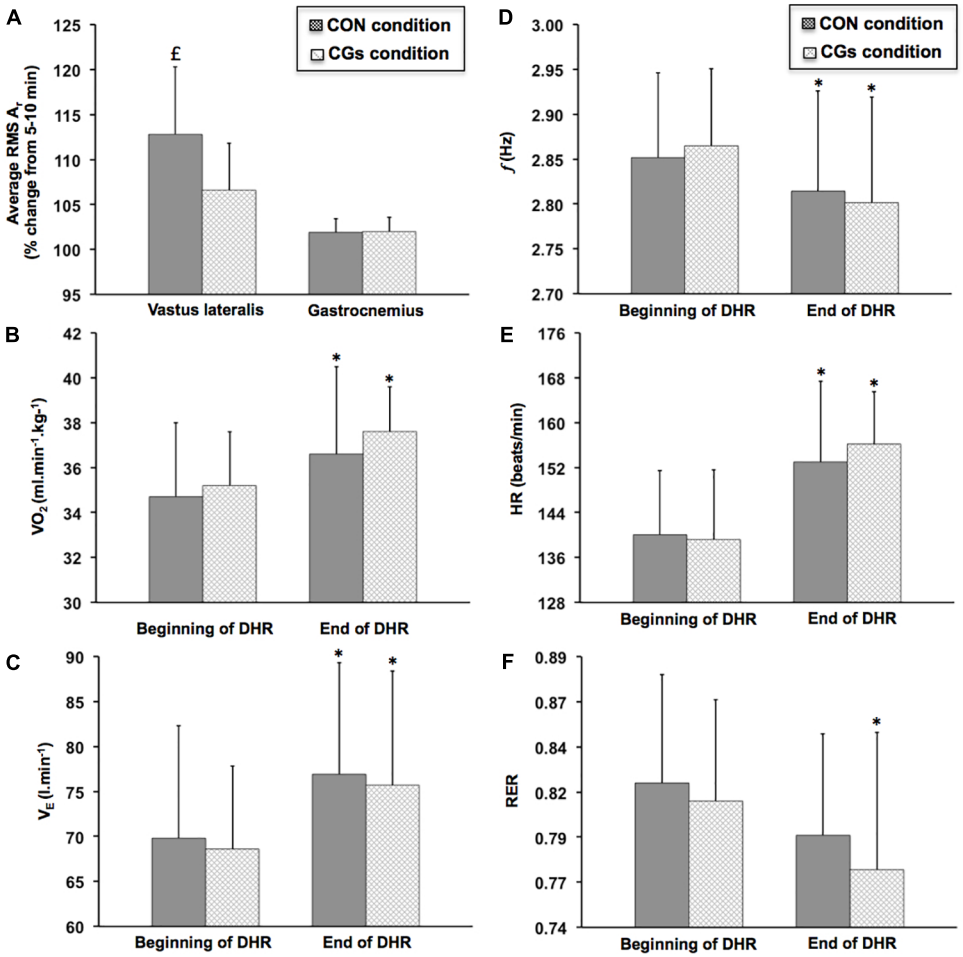

FIGURE 4 | Changes in average resultant acceleration for *vastus lateralis* and *gastrocnemius medialis* [RMS A_r **(A)**], oxygen uptake [VO_2 ***(B)***], ventilation [V_E **(C)**], step frequency [f **(D)**], heart rate [HR **(E)**], and respiratory exchange ratio [RER **(F)**] during the 40-min downhill runs performed with control garments (CON) and compression garments (CGs). RMS A_r was expressed as a function of time. VO_2, V_E, f, HR, and RER were averaged from two time periods at the beginning (3–5 and 8–10 min) and the end of DHR (33–35 and 38–40 min). *Significantly different than the beginning of DHR for a given condition ($P < 0.05$). £Significantly different than the CGs condition ($P < 0.05$).

as reported following the CGs condition, could be attributed to a higher output from the motoneuron pool resulting not only from decreased inhibitory actions (group III/IV muscle afferents) but also through facilitation of Ia afferents inputs onto alpha motoneurons (Souron et al., 2017). In a recent literature review, Souron et al. (2017) reported that Ia afferents, which innervate muscle spindles, are the most responsive receptors to local vibratory stimuli, especially when muscles are stretched (as during DHR). Interestingly, a significant time effect on RMS A_r values was identified, indicating an increase in RMS A_r and thereby, soft-tissue vibrations over time for the *vastus lateralis* only in the CON condition (**Figure 4A**). Although no significant interaction effect was observed, the change in RMS A_r was *likely* higher after CON than CGs conditions with a "moderate" standardized difference between conditions (ES = –0.86 ± 0.71). Wearing CGs at the quadriceps level may have exerted dynamic immobilization reducing muscle oscillation and improving joint stability, and in turn, enhancing neural input (Kraemer et al., 2001; Doan et al., 2003). Although the analysis was limited to the *vastus lateralis*, the decrease in soft-tissue vibrations only in the CGs condition, that reflects an attenuation of impact forces (Friesenbichler et al., 2011), might contribute to the reduced VA deficit in the CGs condition. While vibrations were not local but naturally extended to overall quadriceps during DHR, we suggest that the decrease in soft-tissue vibrations for the *vastus lateralis* only in the CGs condition influences the feedback from muscle spindle afferents and reduces central drive alteration. Future mechanistic approach is necessary to better understand the mechanisms underlying the relationship between soft-tissue vibrations, muscle fatigue and CGs.

To date, laboratory studies quantifying soft-tissue vibrations were limited to level treadmill running during which no evaluation of muscle fatigue was carried out (Friesenbichler et al., 2011; Khassetarash et al., 2015). During level running,

TABLE 3 | Changes in oxygen uptake (VO_2), ventilation (V_E), heart rate (HR), respiratory exchange ratio (RER) and step frequency (*f*) between the 3–5 min interval of downhill running (DHR) in the control garments (CON) or compression garments (CGs) conditions and the 3–5 min interval of the running bout in Post-1D following CON and CGs.

Recovery phase Δ Post-1D-DHR (3–5 min)	CON condition						CGs condition						Post-1D-DHR condition change			
	DHR	Post-1D	Post-1D-DHR				DHR	Post-1D	Post-1D-DHR				CGs–CON conditions			
	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	Mean ± SD	Mean ± SD	%Δ ± SD	ES ± 90% CL	MBI	NHST	ES ± 90% CL	MBI	NHST (time × condition)	
VO_2 (ml.min^{-1}.kg^{-1})	34.5 ± 3.5	35.4 ± 3.1	2.8 ± 4.4	0.23 ± 0.22	Small*	$P = 1.000$	35.3 ± 2.4	36.1 ± 2.7	2.3 ± 9.0	0.27 ± 0.63	Unclear	$P = 1.000$	−0.06 ± 0.53	Unclear	$P = 0.839$	
V_E (l.min^{-1})	69.8 ± 12.5	67.9 ± 7.9	−1.3 ± 11.3	−0.14 ± 0.35	Unclear	$P = 1.000$	68.6 ± 9.2	68.8 ± 10.1	−0.7 ± 11.9	0.02 ± 0.39	Unclear	$P = 1.000$	0.18 ± 0.53	Unclear	$P = 0.573$	
HR (beats.min^{-1})	140.1 ± 11.5	135.6 ± 13.1	−3.2 ± 4.5	−0.36 ± 0.28	Small**	$P = 0.713$	139.2 ± 12.3	138.4 ± 10.6	−1.2 ± 6.4	−0.14 ± 0.39	Unclear	$P = 1.000$	−0.29 ± 0.35	Small*	$P = 0.525$	
RER	0.82 ± 0.06	0.78 ± 0.03	−4.0 ± 8.6	−0.60 ± 0.63	Moderate**	$P = 0.454$	0.81 ± 0.06	0.80 ± 0.03	−0.9 ± 0.03	−0.16 ± 0.45	Unclear	$P = 1.000$	0.48 ± 0.79	Unclear	$P = 0.328$	
f (Hz)	2.86 ± 0.10	2.91 ± 0.13	1.9 ± 3.5	0.52 ± 0.49	Small**	$P = 0.120$	2.86 ± 0.09	2.88 ± 0.10	0.7 ± 1.6	0.22 ± 0.25	Small*	$P = 1.000$	−0.34 ± 0.58	Unclear	$P = 0.239$	

*Values are expressed in percent change (%?) ± standard deviation (SD) and standardized effect size (ES) ± 90% confidence limits (CL). NHST, method of null-hypothesis significance testing ($P < 0.05$); MBI, method of magnitude based inferences; qualitative inferences are trivial (<0.20), small (0.20 to <0.60), moderate (0.60 to <1.20), large (1.20 to <2.00), and very large (>2.00); *possibly, 25 to <75%; **likely, 75 to <95%; ***very likely, 95 to <99.5%, ****almost certain, >99.5%.*

it has been suggested that muscles actively participate to the shock and vibration attenuation, according to the paradigm of "muscle tuning" proposed by (Nigg and Wakeling, 2001). In this paradigm, pre-activation and muscle activation intensities are adapted in accordance with the impact magnitude at ground contact in order to control soft-tissue vibrations (Wakeling et al., 2001; Boyer and Nigg, 2004). However, higher values in peak vertical impact and loading rate were observed during DHR exercises compared to uphill or level running exercises (Mizrahi et al., 2000; Gottschall and Kram, 2005), with an accentuated involvement of "muscle tuning" in such conditions. In the current study, we suggest that the protective mechanism of "muscle tuning" is accentuated in the CGs condition, especially on muscle mass substantially involved in shocks absorption (e.g., KE muscles). Indeed, a significant variation in soft-tissue vibrations (e.g., RMS A_r) was observed over time for the *vastus lateralis* only in the CON condition whereas no significant difference in RMS A_r was found for the *gastrocnemius medialis* within-and-between exercises (**Figure 4A**). It has been previously reported a greater eccentric work (in relation with higher volume and/or mass) for KE compared to PF muscles during DHR (Buczek and Cavanagh, 1990), which might be associated with higher shock absorption, thus locally affecting the magnitude of soft-tissue vibrations. Interestingly, when focused on the *vastus lateralis*, the difference between DHR conditions was *likely* beneficial in favor of CGs, as shown by the lower increase in RMS A_r over time (–5%) and the large ES (–0.86 ± 0.71) for this condition. Considering these results, wearing CGs during DHR might constitute a mechanical strategy to accentuate the muscle damping of soft-tissue vibrations especially for KE.

However, the beneficial effects of CGs on soft-tissue vibrations or VA were not associated with improved perceived muscle soreness scores and countermovement jump responses following DHR. Muscular power and strength as well as muscle soreness are the most common markers used to assess EIMD following eccentric exercises (Hill et al., 2014). It has been demonstrated that muscle soreness, reflecting connective tissue damage and inflammation in the extracellular matrix (Peake et al., 2017), appears to be independent of other markers such as MVC (Nosaka et al., 2002). In the present study, the lack of differences in perceived muscle soreness scores between sessions immediately after DHR might be explained by the high DHR intensity sustained for both conditions (thus affecting immediately perceived muscle soreness responses) and/or the short delay for measuring perceived muscle soreness after DHR (∼2 min). Indeed, perceived muscle soreness are known to increase in the hours following eccentric exercises and peak after 1–3 days (Cheung et al., 2003). Otherwise, although significant decreased (or strong tendency) countermovement jump height were found after DHR, no difference in jump performance was identified between sessions, confirming previous results obtained after simulated trail running races (Vercruyssen et al., 2014; Kerhervé et al., 2017). Considering these elements, MVC appears to be the most sensitive marker to assess acute effects of DHR on muscle damage (Damas, 2016).

Finally, altered RE, V_E, RER, and HR responses were observed over time and regardless of conditions (**Figure 4**), suggesting

a relationship between these cardiorespiratory parameters. As previously reported by Chen et al. (2007), it is likely that the changes in HR, V_E, and RER are indicative of an altered RE (i.e., significant increase in the VO_2 response) among DHR sessions. For instance, HR values were significantly higher over time (from +9.3 to +12.6%, **Figure 4E**) that might be related to the VO_2 drift but also, to the potential increase of core body temperature experienced during DHR (Westerlind et al., 1992). Time course of RE responses during DHR are relatively scarce in literature (Dick and Cavanagh, 1987; Westerlind et al., 1992; Gavin et al., 2015), with no reports in trained runners accustomed to eccentric work. In the current investigation, we reported a 5.3–6.8% increase in RE (**Figure 4B**) at the end of DHR for both conditions. These RE alterations are lower than those reported in previous studies (Dick and Cavanagh, 1987; Westerlind et al., 1992) indicating substantial increases in RE (expressed as VO_2) over time (>+10%), probably due to the low training status of the subjects. Moreover, stride pattern can affect RE during fatiguing and non-fatiguing running (Moore, 2016). For instance, we reported a significant decrease in f (**Figure 4D**) in the CGs condition that might be related to the significant increase in RE in this condition. These findings are in agreement with previous work showing a similar RE-stride pattern relationship in trained subjects running for 1 h at the fastest possible speed (Hunter and Smith, 2007). This suggests that subjects of the present work modify their RE responses while adjusting an optimal stride pattern with fatigue. In addition, several hypotheses may be proposed to explain the altered RE over time for both DHR exercises, including an increased motor unit recruitment to maintain prolonged DHR exercises and/or preferential type II fiber recruitment (Dick and Cavanagh, 1987; Douglas et al., 2017) but also, substantial normal impact force and parallel braking force peaks (Gottschall and Kram, 2005). Therefore, RE responses in the present study are consistent with recent investigations reporting changes in RE responses independently of wearing CGs either during level running of short duration or prolonged trail running in well-trained populations (Stickford et al., 2015; Vercruyssen et al., 2017). Based on a recent study focusing on the relationship between downhill training and chronic RE responses (Shaw et al., 2018), RE does not appear to be the most sensitive index to evaluate the efficacy of an intervention in already well-trained subjects.

Delayed Effects of Wearing Compression Garments

This study is the first to demonstrate that the completion of a DHR exercise while wearing CGs may exert meaningful differences between acute and delayed neuromuscular responses. In contrast with acute effects of CGs described above, peripheral alterations were strongly reduced for KE compared to PF muscles at 24 h post-CGs (**Table 2**), suggesting that the use of CGs during strenuous DHR may constitute a strategy for runners to enhance subsequent recovery of KE muscles. All subjects wore CGs only during DHR, thus excluding the potential recovering effect of wearing CGs in the hours following eccentric exercises (Hill et al., 2014). Reductions in KE MVC were –2.6% at 24 h post-CGs (vs. –10.4% at 24 h post-CON), and were substantially lower that those reported at 24-h following 30–45 min DHR exercises (–17.0%) in physically active subjects (Malm et al., 2004; Maeo et al., 2017) or after a 6.5 km downhill trail run session (–8.5% at post-48 h) in trained runners (Giandolini et al., 2016a). These discrepancies in MVC responses between studies at 24 h post-DHR may be mainly explained by differences in training status. All participants in the present work had a high DHR training status and thus, a better recovery capacity for this exercise modality (Douglas et al., 2017; Hyldahl et al., 2017). For instance, the percentage of positive responders to MVC recovery was particularly elevated for KE (**Figure 3**) and might represent a specificity of our population.

This delayed effect of CGs on the reduction in MVC loss is consistent with those previously reported (Bieuzen et al., 2014), indicating *likely* and *possibly* beneficial effects of CGs on MVC losses at 1 and 24 h post-trail running, respectively. These authors have related the delayed benefits of CGs to the reduction in muscle oscillation and/or mechanical stress induced during trail running. In support of this hypothesis, the reduction in soft-tissue vibrations observed only in the CGs condition for the *vastus lateralis* may have contributed to the improvement of muscle recovery, notably by reducing peripheral alterations. Interestingly, a rapid recovery in Db10 and Db10:100 variables for KE was also observed at 24 h post-CGs (**Table 2**), suggesting a reduced failure in excitation–contraction coupling mechanisms. Such restoration of these neuromuscular variables was not observed at 24 h post-CGs for PF, excepted for T_w values. From a mechanistic perspective, future research is warranted to evaluate, within the recovery phase, the vibration damping properties which may vary with fatigue (Friesenbichler et al., 2011; Khassetarash et al., 2015) and its relationship with EIMD.

Previous theories have been proposed to describe the extent of EIMD, including sarcomeres disruption (e.g., the "popping sarcomere" hypothesis") or damage to the excitation–contraction coupling system (Morgan and Proske, 2004; Proske and Morgan, 2001). In a recent review, Douglas et al. (2017) have reported that overstretched sarcomeres induce ultrastructural myofibrillar disruption, overloading sarcolemma and t-tubules structures and in turn, excitation–contraction coupling dysfunction. In addition, extensive EIMD may lead to inflammatory syndrome, triggering nociceptor stimulation (group III and IV afferents) and subsequently, muscle soreness (Proske and Morgan, 2001; Peake et al., 2017). Considering these statements, we assume that wearing CGs during an intense and prolonged DHR may have "mechanically" preserved KE muscles, which are strongly exposed to muscle damage and peripheral fatigue during DHR (Giandolini et al., 2016b; Maeo et al., 2017). This "protective effect" exerted by the use of high-pressure CGs might be effective especially in the hours following exercise where EIMD symptoms begin to spread intensively within skeletal muscles (Peake et al., 2017). This hypothesis is consistent with perceived muscle soreness scores reported for quadriceps (**Figure 5**), that were significantly lower at 24 h post-CGs, whereas no significant changes in perceived muscle soreness were found between conditions after DHR. While countermovement jump performances did not change within the recovery phase,

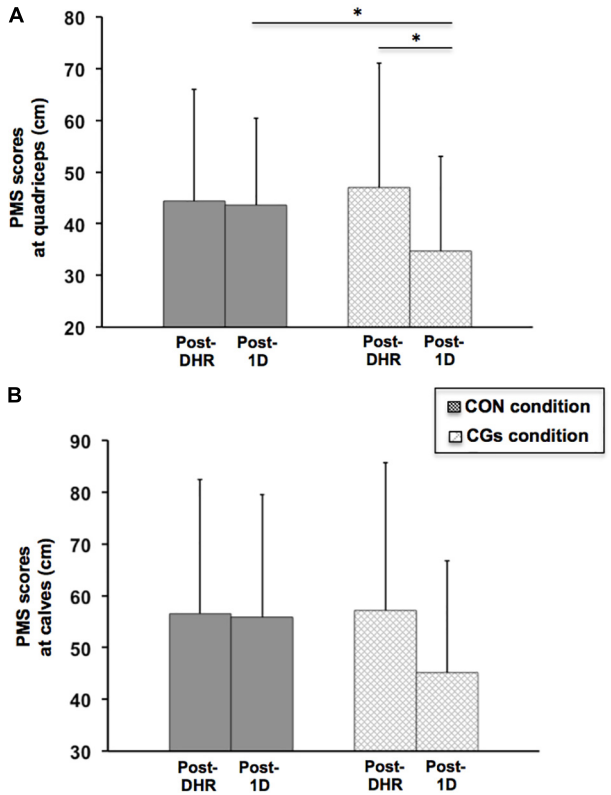

FIGURE 5 | Perceived muscle soreness (PMS) scores at quadriceps **(A)** and calves **(B)** levels in the control garments (CON) and compression garments (CGs) conditions, after DHR, and during the recovery process (Post-1D). *Significantly different from Post-DHR for a given condition but also from the same period when compared to the CON condition ($P < 0.05$).

perceived muscle soreness index seems to be more sensitive in the hours following DHR and might be related to lesser peripheral alterations. Based on reviews considering CGs as a strategy for enhancing recovery from muscle damage and inflammation (Brown et al., 2017; Peake et al., 2017; Dupuy et al., 2018), we suggest that wearing high-pressure CGs (especially for KE) during DHR contribute to the enhanced muscle recovery process by exerting an exercise-induced "mechanical protective effect". Further research is warranted to know whether this "protective effect" may be potentiated by the use of CGs during the recovery phase, notably on KE muscles.

The reduction of EIMD and peripheral fatigue at 24 h post-CGs was not associated with responses in RE and cardiorespiratory parameters (**Table 3**) that were unchanged, whatever the condition. These findings are consistent with RE responses reported 24 h after the completion of a 30-min DHR exercise in physically active males (Mizuno et al., 2016). However, in this investigation, CGs were not used during DHR (only during the 24 h recovery period), making the comparison with our data difficult. Regardless of the use of CGs, it has been reported that DHR induced an alteration in RE at 48 h post-exercise in well-trained runners and triathletes (Braun and Dutto, 2003), which lasted for 3 days in soccer players (Chen et al., 2007) and 5 days in untrained subjects (Chen et al., 2009). In these studies, altered RE responses were associated with increases in EIMD markers (e.g., MVC, creatine kinase activity) or changes in stride mechanics (e.g., increased stride frequency), highlighting a relationship between RE and muscle damage in populations unaccustomed to DHR exercises. Although our experimental design was quite different than those used in these previous investigations, the present work indicates no significant differences in RE and cardiorespiratory responses in Post-1D among conditions, as compared to the first 5-min of DHR, and shows that during the recovery phase, these variables returned rapidly to values obtained at the beginning of DHR in highly trained runners. On the opposite, f values were *significantly* higher in Post-1D, as reported in previous work (Chen et al., 2007). Considering acute and delayed RE responses, we postulate that RE is a too "robust" indicator to be sensitive to certain external strategies, such as wearing CGs, in already well-trained subjects (Shaw et al., 2018).

Some methodological limitations must be considered such as the delay time for measuring muscle fatigue (7–10 min after DHR), possibly counteracting not only the potential benefits of CGs on acute central and peripheral adaptations (Froyd et al., 2013) but also the lack of mechanistic explanation of the "protective effect" observed in the CGs condition and its consequences on muscle damage and peripheral fatigue during the recovery phase. Within this framework, the temporal analysis of soft-tissue vibrations only during DHR and limited to the *vastus lateralis* as well as the lack of calculation of damping characteristics did not allow to infer on the potential relationship between vibrations and muscle function in the CGs condition and the recovery phase. It has been shown that soft tissue vibrations tend to increase as fatigue develops from a level running protocol (Friesenbichler et al., 2011). In this regard, a methodology based on the complex analysis of vibratory properties (Enders et al., 2012) might be useful to determine whether the musculoskeletal system is able to further dampen the increased vibration amplitude with the use of CGs, and how vibratory properties may act, in turn, on muscle damage and peripheral fatigue during and after exercise. Additionally, it would be interesting in future investigations to use histological analyses of muscle biopsies (Valle et al., 2013) or transverse relaxation time-weighted magnetic resonance imaging (Maeo et al., 2017) to better understand the impact of wearing CGs on the extent of muscle damage (e.g., sarcomeres disruption) and inflammatory edema following DHR. Finally, although a great majority of beneficial effects was reported on KE in the hours following DHR, it remains however uncertain whether such benefits are primarily linked to the use of thigh compression during DHR or if the "protective effect" results in the combined effects of calves and quadriceps compression. Using different apparel strategies, further work could isolate the responses of each muscular compartment compressed on muscle damage and/or muscle fatigue during running and within the recovery phase.

CONCLUSION AND PERSPECTIVES

This study shows that the use of high-pressure CGs during DHR induces beneficial effects on soft-tissue vibrations, acute and delayed neuromuscular responses, and muscle soreness, in well-trained off-road runners. The attenuation of soft-tissue vibrations only in the CGs condition might contribute, at least in part, to the reduced VA deficit immediately after DHR and, to the improved muscle function during the recovery phase. This study suggests that the use of CGs exerts an exercise-induced "mechanical protective effect," that might constitute an external strategy for runners, especially to tolerate a high training load or to optimize recovery process within multi-stage races. Given that our findings were observed in highly trained trail runners, we assume that the observed effects with the use of CGs would be even greater in less trained or recreational subjects. Future studies are required to better understand the extent to which wearing CGs may alter the degree of muscle damage or reduce decrements in central and peripheral fatigue-related variables for KE muscles. Moreover, a detailed analysis of the contribution of wearing CGs during DHR to inflammatory mechanisms would be interesting, since they are involved in cellular signaling allowing adaptation to muscle regeneration following training (Peake et al., 2017). It would thus be necessary to ensure that a usual wearing of CGs during training does not interfere with muscular adaptation, especially in recreational or moderately trained subjects.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception, analysis and interpretation, drafting the paper, and gave their final approval of the manuscript.

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OPINION
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Happiness vs. *Wellness* During the Recovery Process in High Performance Sport

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In the last June 2017, Dr. Martin Buchheit alerted the Sport's community with an editorial entitled "Houston, we still have a problem," (Buchheit, 2017) published in one of the most impact scientific journal the field of sports sciences. In that document, he explained that some of the most used methods in elite sports with a high level of evidence (1a) are not linking of athletes. However, other methods, without any evidence yet, are usually used among them. Although these used strategies present a high placebo effect among elite athletes, the implementation is a controversial topic in sports competition. Besides the physician staff have the control about its use, it is their responsibility to show leadership to create a satisfying and productive working environment for the future colleagues (Kimura, 2016), but these practices have not been studied yet.

In particular, some of these techniques and products used in order to enhance the recovery process are described in the scientific literature. For example, regarding ergogenic aids, only 5 supplements with 1a level of evidence improve performance according to the Australian Institute of Sport (caffeine, betalanine, bicarbonate, creatine, and nitrate (http://www.ausport.gov.au/ais/nutrition/supplements), although professional teams spend a lot of money using products without evidences (Bishop, 2010). With relation to the dip in frozen water, we have gained time and comfort. Currently athletes dip during 10 min at 11–15°C, compared with old technologies in with dips at 0°C during longer periods of time (Anderson et al., 2017). On the other hand, massage is also a technique widely used in the world of sports and that sportsmen like, but meta-analysis studies did not show effect on sports recovery (Poppendieck et al., 2016). Recently, trips are one of the hot topics in sports science. Every time the athletes compete more frequently and must make more trips during periods of high competitive density. A priori being able to guarantee an adequate recovery with hygiene of the circadian rhythm is of vital importance throughout a season. Therefore, the fact of sleeping the day before and after the game at the destination should be a common practice in order to ensure recovery, since the reduced amount and quality of sleep are mainly evident after a game in elite players (Fullagar et al., 2016). However, athletes prefer to break their biological rhythm, arrive at dawn to the place of origin, in order to sleep at home with their family. In that way objective data showed trends toward longer sleep length at home (Baulk and Fletcher, 2012). In this case accelerating the come back home despite not being the most convenient is proposed in the part of the season where there are no important periods and as we are getting closer to competitions of interest having to spend the night in the hotel.

In all these situations the same reflection occurs when implementing or not the different recovery methods, in relation to the level of evidence in order to obtain a wellness, or on the contrary, if the athlete really prefers, with what we would obtain an evident happiness.

Athlete self-report measures include perceptions of wellbeing (e.g., fatigue) and psychological variables (e.g., mood) which are influenced by both training and non-training stressors (Kellmann, 2010).

According to Corbin (2009) "emotional wellness is a person's ability to cope with daily circumstances and to deal with personal feelings in a positive, optimistic, and constructive manner".

Erickson refers to the so-called Wellness Wheel; a model which "portrays a balance between six dimensions of life and health—physical, social, environmental, emotional, spiritual, and intellectual." Consequently, it is vital, for sportsmen, to keep all the wellness dimensions in balance in order to produce better performance (Erickson, 2012).

In contrast, Happiness is of great importance to most people and has been found to be a highly valued goal in most societies (Fisher, 2010).

Happiness underlying factors are considerable from two dimensions: endogenic factors (biological, cognitive, personality, and ethical sub-factors) and exogenic factors (behavioral, socio-cultural, economical, geographical, life events, and aesthetics sub-factors). Among all endogenic factors, biological sub-factors are the significant predictors of happiness (Dfarhud et al., 2014). Besides, improving staff happiness may contribute to increase in moral and counter burnout (Baruch et al., 2013). In that way, investigation proposes that for many people happiness is being able to make the practices of everyday life work, such that positive feelings control over negative feelings follow-on from daily hassles (Olsson et al., 2013).

Although one of the most important elements of performance and exercise is rest (Counting on the placebo effect as an interesting phenomenon to consider) and a significant predictor of successful performance (Uphill et al., 2014), confirming the previous statement of Totterdell (Totterdell, 1999); players perform better when they are in positive moods. For that reason, the balance between "happiness and wellness" in high sports performance, is presented as an exciting challenge in the coming years, where the cost-benefit should be the main measurement parameter, considering that happiness is strongly correlated with perceived good health (Sabatini, 2014), although to date for the best of our knowledge, there is no scientific evidence about it. Probably, in the initial phases of the season, we could give greater importance to happiness, because the happiness was the attainment of a worthy life (Stearns, 2012), and as we approach the important competitions generate a culture of wellness.

Time will tell, but we must remember that once again, we think this applies to all jobs within social services as we are working with people and impacting on their lives. Therefore, we could say: "Houston we still have a problem" but we could add., "Remember that "We are physicians, but first and foremost, human beings"" (Kimura, 2016).

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Photobiomodulation by Led Does Not Alter Muscle Recovery Indicators and Presents Similar Outcomes to Cold-Water Immersion and Active Recovery

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Purpose: The aim of the present study was to investigate the effectiveness of photobiomodulation therapy (PBMT) on muscle recovery based on inflammation (interleukin-10 [IL-10]; tumor necrosis factor-α [TNFα]), muscle damage markers (creatine kinase [CK]; lactate dehydrogenase [LDH]), delay onset muscle soreness (DOMS), and countermovement jump performance (CMJ) after two sprint interval training (SIT) sessions compared with a placebo condition (part-I), as well as to compare the effectiveness of PBMT with active recovery (AR) and cold-water immersion (CWI) (part-II).

Methods: Part-I was conducted as a double-blind, randomized and placebo-controlled study and part-II as a parallel-group study. Thirty-six men participated in the studies (12 participants in part-I and 36 participants in part-II). Volunteers performed two SITs interspaced by 24-h (SIT_1 and SIT_2) to mimic the effect of accumulating 2 consecutive days of SIT. In part-I, only after SIT_2, PBMT [Total energy: 600J (300J per leg in 5 spots); wavelength: 660–850 nm] or placebo interventions were performed, while in part-II PBMT (part-I data), AR (15-min; 50% of the maximal aerobic power), or CWI (10-min; 10°C) were carried out, also after SIT_2. Blood samples were collected before (i.e., baseline), and 0.5, 1, 24, 48, and 72-h after SIT_2, while CMJ and DOMS were measured before, 24, 48, and 72-h after SIT_2.

Results: In part-I, there were no interactions between PBMT and placebo conditions for any blood markers ($P \geq 0.313$), DOMS ($P = 0.052$), and CMJ ($P = 0.295$). However, an effect of time was found with increases in LDH, CK, and IL-10 ($P \leq 0.043$) as well as a decrease in DOMS at 72-h compared with 24-h ($P = 0.012$). In part-II, there were no interactions between the PBMT, AR, and CWI groups for any markers at the same moments ($P \geq 0.189$) and for the peak and integral values ($P \geq 0.193$), for DOMS ($P = 0.314$) and CMJ ($P = 0.264$). However, an effect of time was found with an increase in CK and IL-10 ($P = 0.003$), while DOMS decreased at 48 and 72-h compared with 24-h ($P = 0.001$).

Conclusion: In summary, PBMT had no effect on inflammation, muscle damage, CMJ performance, or DOMS after two consecutive sprint interval training sessions compared to placebo, CWI, and AR strategies.

Keywords: low-level light therapy, high-intensity interval training, inflammation, interleukin-10, tumor necrosis factor-alpha, creatine kinase, L-lactate dehydrogenase

INTRODUCTION

Sprint interval training (SIT) is a time-efficient method of providing cardiorespiratory and muscular adaptations with a lower training volume (Gibala et al., 2012), in addition to which, it has also recently been suggested as an additional tool in the treatment of disease (Gibala et al., 2012). However, execution of this mode of training seems to be associated with a higher possibility of damage and inflammatory processes in muscular tissue, evidenced by increases in systemic biochemical markers and cytokine concentrations (Antosiewicz et al., 2013; Harnish and Sabo, 2016). Considering that during training planning, interest in the monitoring/measurement of the recovery status is growing (i.e., measurement of responses of autonomic nervous system by heart rate variability, training impulse, or RPE-session) with a focus on choosing the ensuring training load/stress (Heidari et al., 2018), the use of recovery modalities after exercise, aiming to speed up the process of tissue repair, may be a valid strategy to associate with SIT sessions (Barnett, 2006).

Traditionally, active recovery (AR) and cold-water immersion (CWI) have been widely used to accelerate muscular recovery after intense exercise sessions (Barnett, 2006). However, despite their popularity, the beneficial effects of CWI and AR have recently been questioned (Barnett, 2006; Roberts et al., 2015). In this way, photobiomodulation therapy (PBMT), a type of light therapy that utilizes non-ionizing and non-thermal light sources in the visible and infrared spectrum, eliciting photophysical and photochemical events on biological tissue (Anders et al., 2015), has attracted attention in the area of sports and health sciences. Some isolated studies have suggested its effects in reducing muscle damage markers (Baroni et al., 2010; De Marchi et al., 2012; de Paiva et al., 2016), attenuating or anticipating inflammatory responses (Amadio et al., 2015; Zagatto et al., 2016), and reducing some symptoms of inflammation such as delayed onset muscle soreness (DOMS) and loss of muscle function (Borges et al., 2013). However, despite these findings, the effects of PBMT on overall human muscle recovery (i.e., considering perceptive, physiological, and functional aspects) are contradictory and the actual effectiveness remains uncertain.

The majority of studies with PBMT have investigated its effects using isolated contractions and exercise-induced muscle damage protocols (Baroni et al., 2010; Borges et al., 2013; de Paiva et al., 2016), and only a few after a common high-intensity exercise session such as SIT. Additionally, some studies have compared the effects of PBMT with cryotherapy methods (de Paiva et al., 2016; De Marchi et al., 2017), however, without precise temperature control, a determinant parameter for its effectiveness (Machado et al., 2016), and no studies have compared PBMT with an AR protocol, a widely used method after exercise sessions. The possible beneficial effects of PBMT on overall muscle recovery may contribute to fortifying this method as an additional tool in the exercise routine.

Therefore, the aim of the present study was to investigate the effectiveness of PBMT on muscle recovery in view of systemic inflammation (interleukin-10 [IL-10] and tumor necrosis factor-α [TNFα]), muscle damage (creatine kinase [CK] and lactate dehydrogenase [LDH]), DOMS, and muscle performance (countermovement jump performance [CMJ]) after SIT, and to compare PBMT with AR and CWI interventions. The hypothesis of the study was that PBMT would decrease CK and LDH blood concentrations, accelerate systemic inflammatory responses, and reduce DOMS and loss in CMJ performance.

MATERIALS AND METHODS

The study was conducted in two parts. Part-I was performed to compare the PBMT with the placebo (PLA) condition in a double-blind design, while part-II aimed to compare PBMT with AR and CWI with parallel groups.

Participants

The minimum sample size for a statistical power of 90 % (alpha: 0.05; allocation ratio: 1) was 10 participants in each group. The sample size was calculated based on the findings of De Oliveira et al. (2018), using the TNFα results and assuming an effect size of 1.4 (*d* value). Thus, a total of thirty-six healthy men participated in the present investigation, of which twelve participated in both part-I and part-II, with an addition of twenty-four volunteers in part-II (i.e., total of thirty-six in part-II, allocated into three groups of twelve participants each).

Prior to beginning the study, volunteers were informed about the procedures, risks, and benefits involved in the tests and then signed the consent form. All experimental procedures were approved by the Human Research Ethics Committee from the School of Sciences, São Paulo State University – UNESP

Abbreviations: Δ%, percentage of difference to baseline; AR, active recovery; BW, bout work; CK, creatine kinase; $CK^{\Delta\%}$, creatine kinase concentration percentage of difference to baseline; CMJ, countermovement jump; CMJ^{Δ}, countermovement jump height normalized by baseline; C_{PBMT}, photobiomodulation therapy condition; C_{PLA}, placebo condition; CWI, cold-water immersion; DOMS, delay onset muscle soreness; $DOMS^{\Delta}$, delay onset muscle soreness perception normalized by baseline; G_{AR}, active recovery group; G_{CWI}, cold-water immersion group; GXT, graded exercise test; IL-10, interleukin-10; $IL\text{-}10^{\Delta\%}$, interleukin-10 concentration percentage of difference to baseline; LDH, lactate dehydrogenase; $LDH^{\Delta\%}$, lactate dehydrogenase concentration percentage of difference to baseline; LED, light emitting diode; MAP, maximal aerobic power; MP, mean power; PBMT, photobiomodulation therapy; PP, peak power; SIT, sprint interval training; TNFα, tumor necrosis factor-α; $TNF\alpha^{\Delta\%}$, tumor necrosis factor-αconcentration percentage of difference to baseline; TT, total work; VAS, visual analog scale; $\dot{V}O_2$, oxygen uptake;$\dot{V}O_{2peak}$, peak oxygen uptake.

(protocol number: 1.139.070) and conducted in accordance with the Declaration of Helsinki.

Participants were untrained, healthy individuals, without any vascular disease, metabolic disorders, recent muscle-skeletal or joint injuries (i.e., in the previous 6 months), and had not used nutritional or pharmacological substances for at least 3 months. Volunteers who were regularly absent from the trials, initiated the use of nutritional and/or pharmacological substances during the evaluations, or presented muscle injury were excluded from the study.

Experimental Design

Participants arrived at the laboratory in the morning after fasting (≈8-h). One hour before the evaluations an individual breakfast was offered to the volunteers composed of 30% of daily caloric expenditure (Mifflin et al., 1990).

The graded exercise test (GXT) and SIT were performed on an electromagnetic cycle ergometer (Excalibur Sport, Lode, Netherlands). Before all tests, a 5-min warm-up at 30% of the maximal aerobic power (MAP) reached in the GXT was performed.

The study was divided into two sequenced and dependent parts (I and II).

Part-I was conducted in a double-blind, randomized, and placebo-controlled design. Firstly, a GXT was performed to determine the peak oxygen uptake ($\dot{V}O_{2peak}$) and the MAP. Five days after the GXT, two SIT sessions (SIT_1 and SIT_2) were performed, interspaced by 24-h of recovery (double SIT), to potentiate the stress on active muscle. The double SIT (i.e., set of 2 SIT sessions) was performed twice before each experimental condition (i.e., PBMT and placebo), separated by 5-days. Immediately after the double SIT (i.e., only post the SIT_2), PBMT was applied in mode on (PBMT condition – C_{PBMT}) or off (PLA condition – C_{PLA}) in randomized and counterbalanced order. To ensure blinding in each experimental condition, participants were blindfolded and wore headphones to eliminate light and sound signals. A person not involved in any parts of the study applied the PBMT and randomization.

Part-II was conducted as a parallel-group trial, with GXT and SIT sessions identical to those reported in part-I; however, immediately after the double SIT (i.e., only post SIT_2), the recovery interventions were composed of active recovery (AR; group AR – G_{AR}) or cold-water immersion (CWI; group CWI- G_{CWI}). Posteriorly, the C_{PBMT} data recorded from part-I were used to compare with G_{AR} and G_{CWI} in part-II.

Venous blood sample collections were realized in the medial cubital vein at rest (i.e., baseline) and 0.5, 1, 24, 48, and 72-h after each recovery condition (part-I) or intervention (part-II) using vacutainer tubes of 10 and 4 mL (BD, Juiz de Fora, MG, Brazil) for inflammatory and muscle damage marker measurements. Capillary blood samples (25 μL) were collected from the earlobe 3, 5, and 7-min after GXT, and before, between intervals (i.e., 3rd-min after each Wingate test) and 5 and 7-min after each SIT session for measurement of lactate concentrations.

In parts I and II, CMJ performance and DOMS were evaluated at rest (i.e., baseline), and 24, 48, and 72-h after interventions.

Figure 1 presents the experimental design of studies-I and II.

Graded Exercise Test

Graded exercise test was performed to determine the $\dot{V}O_{2peak}$ and MAP, starting at 75 W, with a 25 W increment every 2-min until exhaustion (Howley et al., 1995; Ozyener et al., 2001), measured at 670-m above sea level. During GXT, respiratory responses were registered breath-by-breath using a gas analyzer (Quark

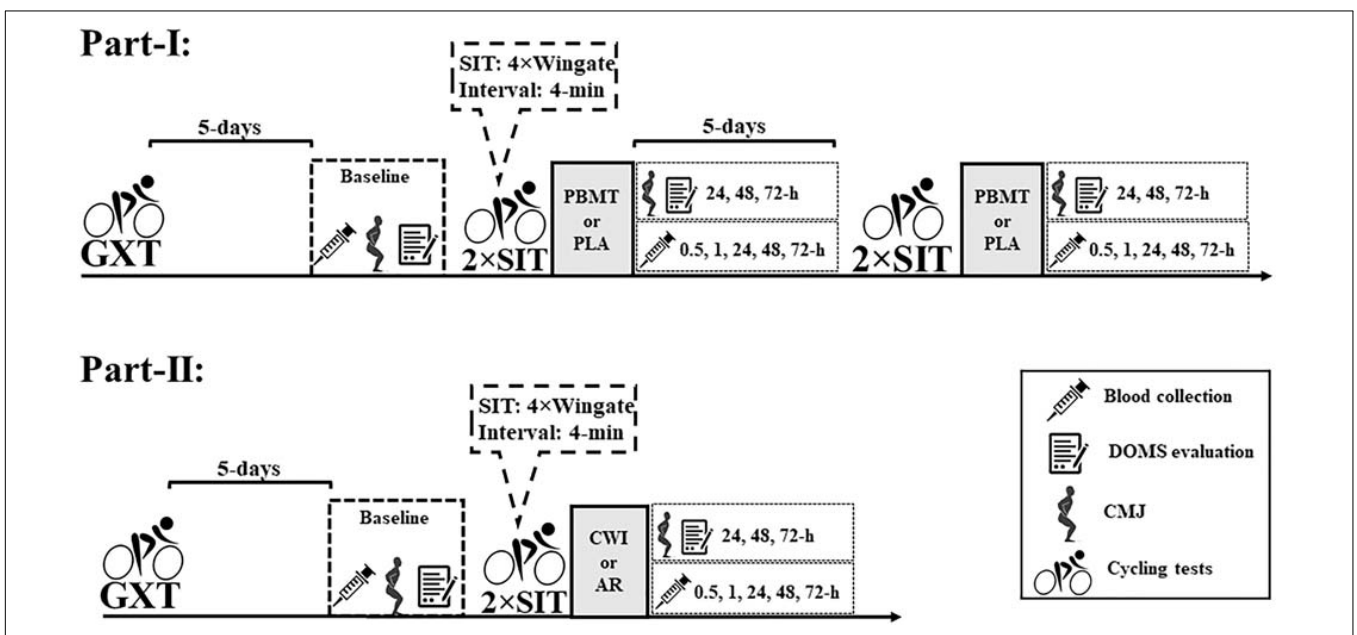


FIGURE 1 | Experimental design of studies I and II. DOMS, delay onset muscle soreness; CMJ, countermovement jump; PBMT, photobiomodulation therapy; CWI, cold-water immersion; AR, active recovery; PLA, placebo; GXT, graded exercise test; 2 × SIT, double sprint interval training.

CPET, COSMED, Italy), previously calibrated in accordance with the manufacturer's instructions. Data were smoothed every 10-points and interpolated every 1-s using the software OriginPro 8.0 (OriginLab Corporation, United States). For $\dot{V}O_{2peak}$ determination, the oxygen uptake ($\dot{V}O_2$) mean of the final 20-s of each stage was determined and the $\dot{V}O_{2peak}$ was assumed as the highest $\dot{V}O_2$ mean reached in the GXT. MAP was recorded and considered the highest exercise power performed during the test.

Sprint Interval Training Session

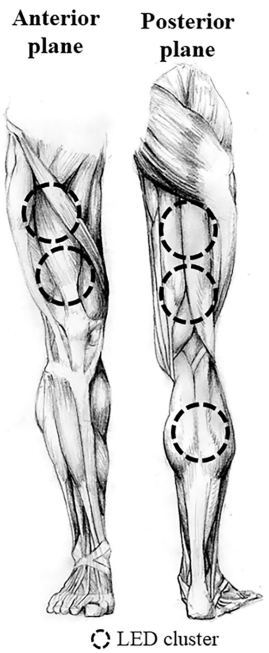
The double SIT comprised two SIT sessions interspaced by 24-h, mimicking the accumulated effects of two consecutive training days. Each SIT constituted four Wingate tests (i.e., 30-s at 0.7 $Nm \cdot kg^{-1}$) with a 4-min recovery between bouts (Burgomaster et al., 2005). In the first minute of recovery time an active recovery at 30% of MAP and ≈75–80 rpm was performed to minimized is comfort, while the additional recovery time (i.e., 3-min) was composed of passive recovery (Gibala et al., 2012). The SIT protocol was controlled using Wingate 1.11 software (Lode, Netherlands) which enabled measurement of bout work (BW), peak power (PP), and mean power (MP). Workload performed during the double SIT was assumed as the sum of all work performed during the Wingate tests (total work – TT). In a previous study performed in our laboratory, the SIT showed good reliability of TT 7-days after a first session (ICC = 0.89) (Malta et al., 2018). The SIT performance parameters were measured to ensure that the volunteers were submitted to the same exercise effort in both study parts.

CMJ and DOMS Measurements

To evaluate the symptoms of inflammation (i.e., muscle functional limitation and DOMS), the CMJ and DOMS were measured. CMJ was composed of 3 maximal jump trials interspaced by 1-min of passive recovery. Volunteers were instructed to remain with hands on hips and flex the knees quickly to 90° to jump. To assess the jump height, a jump platform was used (Jump test, Cefise, Brazil) and the highest jump was considered. This configuration of CMJ test was chosen as it does not influence blood cytokines or muscle damage markers. In addition, DOMS perception was assessed using a VAS consisting of a 100 mm line, on which the "0" represents "no pain" and "100" "very painful" (Carlsson, 1983). For greater leg pain perception, the scale was applied during low-intensity pedaling (Borges et al., 2013). In the present study, CMJ and DOMS values are presented as change related to baseline (Δ), being described using the variable acronym plus the "Δ" (i.e., CMJ^{Δ} and $DOMS^{\Delta}$).

Recovery Methods

Photobiomodulation therapy was applied using a cluster multi-diode containing 104 LED (THOR-LX2, THOR Photomedicine Ltd., United Kingdom). The PBMT protocol had an overall duration of 2.5-min, with application in both legs simultaneously. LED irradiation was performed in two regions of the quadriceps muscle, two regions of the biceps femoris, and one region between the soleus and gastrocnemius muscles, following the distribution axis of the muscle fibers. The interventions were performed using the spot method, with direct contact (i.e., 90° angle) of the equipment on the skin surface. The technical



Parameters	Specifications
Number of LEDs	104 (56 diodes of 660 nm and 48 diodes of 850 nm)
Wavelength	mixed, of 660 and 850 nm
Frequency	0-1500 Hz
Optical output	10 mW (660 nm) and 30 mW (850 nm)
Cluster area	69 cm^2
LED area	0.2 cm^2
Power density	50 mW/cm^2 (660 nm) and 150 mW/cm^2 (850 nm)
Energy	60 J at each point (0.3 J from each red LED and 0.9 J from each infrared LED)
Energy density	1.5 J/cm^2 from each red LED and 4.5 J/cm^2 from each infrared LED
Total energy delivered	600 J (300 J per leg)
Treatment time	30 s at each point

FIGURE 2 | Technical parameters of PBMT and location of LED irradiation. LED, light emitting diode.

parameters of PBMT and location of LED irradiation are shown in **Figure 2**.

Active recovery and CWI were applied only in part-II. AR was performed on a cycle ergometer, immediately after the double SIT, with a duration of 15-min and intensity corresponding to 50% of MAP (Wigernaes et al., 2001). During AR, a cadence of ≈75 rpm was maintained. The partial CWI, to the waist, was performed immediately after the double SIT with the volunteers sitting in an immersion bath containing 200 L of water, at 10°C for 10-min (Machado et al., 2016). The temperature was set using an auto-cooling system, controlled by a digital thermostat (TIC17RGTi, ES Full Gauge, United States), triggered when the water temperature increased 0.1°C.

Blood Sample Analysis

Capillary blood samples were deposited into microtubes containing 50 μL of sodium fluoride at 1% and analyzed in an electrochemical analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments, United States) for determination of lactate concentrations. According to the manufacturer, the equipment has a measurement error of ±2%.

Venous blood samples were centrifuged for 10-min at 1306 *g* and 4°C (Vision scientific, VS-15000FNII, SKR) for extraction of plasma and serum. Plasmatic cytokines (IL-10 and TNFα) were assessed using ELISA commercial kits (Affymetrix, eBioscience, United States; Lot: IL-10 4295480; TNFα 4298657). Plates were read using a spectrophotometer, SpectraMax Plus 384 (Molecular Devices, United States). Serum CK and LDH concentrations were assessed through a kinetic method using a random-access analyzer (A-15, Biosystems S.A., Spain) and commercial kits (Biosystems S.A., Spain; Lot: CK 13869; LDH 09998).

Considering the great individual variation in CK, LDH, IL-10, and TNFα blood concentrations, in the present study the blood markers are presented as percentage difference to baseline (Δ%), being described using the variable abbreviation plus the "Δ%" (i.e., $CK^{\Delta\%}$, $LDH^{\Delta\%}$, $IL\text{-}10^{\Delta\%}$, and $TNF\alpha^{\Delta\%}$). The integrals of CK, LDH, IL-10, and TNFα (i.e., area under the curve considering its concentration and the evaluation time) were calculated using the software OriginPro 8.0 (OriginLab Corporation, United States) to test whether exposure to inflammation and muscle damage were affected by recovery methods (Krzanowski and Hand, 2009). In addition, peak concentration of CK, LDH, IL-10, and TNFα (i.e., highest value obtained between 0.5 and 72-h) were calculated to measure the magnitude of increase.

Statistical Analysis

Statistical analyses were performed using the software package SPSS version 23.0 (IBM Corp., Armonk, NY, United States). Data are presented as means and standard deviations (mean ± SD). The normality of the data was confirmed using the Shapiro-Wilk test. In both parts (e.g., I and II), to compare the $CK^{\Delta\%}$, $LDH^{\Delta\%}$, $IL\text{-}10^{\Delta\%}$, and $TNF\alpha^{\Delta\%}$ blood concentrations, $DOMS^{\Delta}$ and CMJ^{Δ} performance between moments (i.e., main time effect) and between and within-conditions and groups (i.e., interaction time*groups) a two-way repeated measure ANOVA was used and a SIDAK *post hoc* was applied when necessary. Mauchly's sphericity test was used in all ANOVA analyzes, and in cases of sphericity violation, the F and significance corrected by Greenhouse-Geisser were assumed. Only in part-I, the paired *t*-test was used to compare the means of SIT total work, integral, and peak blood concentration of $CK^{\Delta\%}$, $LDH^{\Delta\%}$, $IL\text{-}10^{\Delta\%}$, and $TNF\alpha^{\Delta\%}$ (i.e., $C_{PBMT} \times C_{PLA}$). In addition, the *t*-test was also used to compare peak lactate concentrations which were measured two times for each condition (i.e., C_{PBMT} $SIT_1 \times SIT_1$ C_{PLA} and C_{PBMT} $SIT_2 \times SIT_2$ C_{PLA}). In part-II, the ANOVA one-way test was used to compare peak lactate concentrations, MAP, $\dot{V}O_{2peak}$, and integral and peak blood markers between groups (i.e., C_{PBMT}, G_{AR}, and G_{CWI}). In both parts (e.g., I and II), SIT total work and peak lactate concentration were compared to verify similar exercise-induced workload and metabolic stress. In all cases, a significance level of 5% was assumed.

RESULTS

Part-I and II: GXT and SIT Outcomes

In part-I, the $\dot{V}O_{2peak}$ and MAP reached in the GXT were 40.0 ± 5.7 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ and 210.4 ± 29.1 W, respectively. The double SIT total work (i.e., SIT_1 and SIT_2 work sum) for C_{PBMT} and C_{PLA} were 96.4 ± 13.4 and 99.5 ± 13.9 kJ, respectively. In addition, the peak lactate concentrations reached in SIT for C_{PBMT} and C_{PLA} were 14.1 ± 2.7 and 14.4 ± 2.0 (SIT_1), 13.9 ± 2.9, and 14.2 ± 2.0 $mmol{\cdot}L^{-1}$ (SIT_2), respectively. No differences between C_{PLA} and C_{PBMT} were verified in double SIT total work [$P = 0.941$, $t_{(11)} = -0.075$] or peak lactate concentration reached after SIT sessions [SIT_1: $P = 0.479$, $t_{(9)} = -0.738$; SIT_2: $P = 0.666$; $t_{(11)} = -0.444$].

In part-II, there were no significant differences between the anthropometric characteristics of the volunteers [$P \geq 0.136$; $F_{(2,33)} \leq 2.123$] (**Table 1**). The $\dot{V}O_{2peak}$ reached in GXT for

TABLE 1 | Anthropometric characteristics of the volunteers.

	Part-I	Part-II		ANOVA one-way		
		G_{CWI}	G_{AR}	*P*-value	*F*	Df
Age (years)	25.7 ± 5.1	23.7 ± 4.4	24.2 ± 5.5	0.606	0.509	2, 33
Height (cm)	177.3 ± 3.0	175.5 ± 4.1	179.7 ± 7.0	0.229	1.541	2, 33
Weight (kg)	76.3 ± 7.4	73.9 ± 7.8	73.6 ± 10.0	0.597	0.524	2, 33
BMI (kg/m^2)	24.2 ± 1.7	25.4 ± 4.2	22.8 ± 2.9	0.136	2.123	2, 33

G_{CWI}, group submitted to cold-water immersion; G_{AR}, group submitted to active recovery; BMI, body mass index; Df, degrees of freedom.

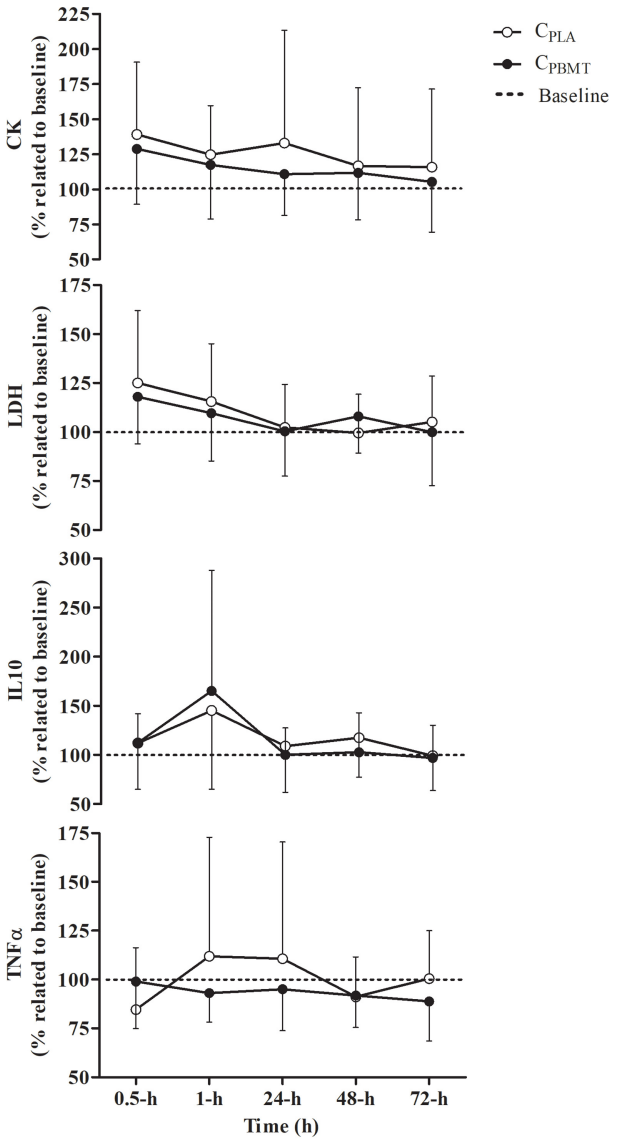


FIGURE 3 | Blood concentration of IL-10$^{\Delta\%}$, TNFα$^{\Delta\%}$, CK$^{\Delta\%}$, and LDH$^{\Delta\%}$ (mean ± SD) for C_{PBMT} and C_{PLA}. IL-10$^{\Delta\%}$, interleukin 10 expressed as percentage difference to baseline; TNFα$^{\Delta\%}$, tumor necrosis factor alpha expressed as percentage difference to baseline; CK$^{\Delta\%}$, creatine kinase expressed as percentage difference to baseline; LDH$^{\Delta\%}$, lactate dehydrogenase expressed as percentage difference to baseline; C_{PBMT}, photobiomodulation therapy condition; C_{PLA}, placebo condition.

G_{AR} and G_{CWI} were 41.9 ± 5.0, 38.1 ± 6.5 mL·kg^{-1}·min^{-1}, while MAP values were 218.8 ± 45.4 and 214.6 ± 45.8 W, respectively. No significant differences were verified between groups (i.e., C_{PBMT}, G_{AR}, and G_{CWI}) in $\dot{V}O_{2peak}$ [P = 0.306; $F_{(2,33)}$ = 1.229] and MAP [P = 0.883; $F_{(2,33)}$ = 0.125] reached in the GXT. The SIT total work for G_{AR} and G_{CWI} were 103.1 ± 25.8, and 99.6 ± 21.8 kJ. In addition, the peak lactate concentrations reached in SIT for G_{AR} and G_{CWI} were 14.7 ± 1.2 and 14.3 ± 0.9 mmol·L^{-1} (SIT_1), and 14.0 ± 1.4 and 13.5 ± 1.4 mmol·L^{-1} (SIT_2), respectively. No differences between C_{PBMT}, G_{AR}, and G_{CWI} were verified in double SIT total work [P = 0.874; $F_{(2,33)}$ = 135] and peak lactate concentration reached after the SIT session [SIT_1: P = 0.859; $F_{(2,31)}$ = 0.152 and SIT_2: P = 0.889; $F_{(2,33)}$ = 0.118].

Additional performance parameters (i.e., BW, PP, and MP) and lactate kinetics before and during, and the peak reached after double SIT sessions for parts I and II are shown in **Supplementary Figures S1**, **S2**, respectively.

Part-I: Recovery Outcomes

There was a time effect showing kinetic changes in some blood markers, independent of the treatment, but no effect for TNFα$^{\Delta\%}$ blood concentration [P = 0.668; $F_{(4,32)}$ = 0.324]. The LDH$^{\Delta\%}$ increased at 0.5-h compared to 24, 48, and 72-h [P = 0.000; $F_{(4,36)}$ = 7.035; *post hoc* $P \leq 0.030$]. The CK$^{\Delta\%}$ decreased over time at 72-h compared with 0.5-h [P = 0.043; $F_{(4,40)}$ = 2.716; *post hoc* P = 0.021]. The IL-10$^{\Delta\%}$ also decreased over time at 24 and 72-h compared with 1-h [P = 0.035; $F_{(4,40)}$ = 3.944; *post hoc* $P \leq 0.048$]. Contrarily, there were no interactions (i.e., interaction time*groups) between C_{PBMT} and C_{PLA} for any blood markers [$P \geq 0.313$; $F_{(4,32)} \leq 1.327$] (**Figure 3**). The part-I absolute values of CK, LDH, IL-10, and TNFα blood concentrations are presented in **Supplementary Table S1**.

Table 2 presents the peak and integral values of CK$^{\Delta\%}$, LDH$^{\Delta\%}$, IL-10$^{\Delta\%}$, and TNFα$^{\Delta\%}$ reached in C_{PBMT} and C_{PLA}. For all blood markers, there were no significant differences between C_{PBMT} and C_{PLA} for peak [$P \geq 0.104$; $t_{(11)} = -1.774$] or integral values [$P \geq 0.370$; $t_{(8)} = -0.950$].

For DOMS$^{\Delta}$, a time effect was found for DOMS$^{\Delta}$ that decreased at 72-h compared with 24-h [P = 0.012; $F_{(2,22)}$ = 7.263; *post hoc* P = 0.043], however, there were no interactions (i.e., interaction time*groups) between C_{PBMT} and C_{PLA} at the same moments as for DOMS$^{\Delta}$ [P = 0.052; $F_{(2,22)}$ = 4.298] and for CMJ$^{\Delta}$ [P = 0.295; $F_{(2,22)}$ = 1.289] (**Figure 4**).

Part-II: Recovery Outcomes

A time effect was found for CK$^{\Delta\%}$ and IL-10$^{\Delta\%}$, but not for LDH$^{\Delta\%}$ and TNFα$^{\Delta\%}$ blood concentrations. CK$^{\Delta\%}$ increased at 0.5, 1, and 48-h compared with 72-h, and increased at 0.5-h compared with 24-h [P = 0.003; $F_{(8,104)}$ = 5.393; *post hoc* $P \leq 0.023$]. In addition, IL-10$^{\Delta\%}$ increased over time at 1-h compared with 24 and 48-h [P = 0.003; $F_{(8,108)}$ = 7.568; *post hoc* P = 0.048]. However, there were no interactions (i.e., interaction time*groups) between C_{PBMT}, G_{AR}, and G_{CWI} for all markers at the same moments [$P \geq 0.189$; $F_{(8,80)} \leq 1.568$] (**Figure 5**). The part-II absolute values of CK, LDH, IL-10, and TNFα blood concentrations are presented in **Supplementary Table S2**.

Table 3 presents the peak and integral values of CK$^{\Delta\%}$, LDH$^{\Delta\%}$, IL-10$^{\Delta\%}$, and TNFα$^{\Delta\%}$ reached in C_{PBMT}, G_{AR}, and G_{CWI}. For all blood markers, there were no significant differences between C_{PBMT}, G_{AR}, and G_{CWI} for peak [$P \geq 0.193$; $F_{(2,31)} \leq 1.734$] and integral values [$P \geq 0.224$; $F_{(2,28)} \leq 1.578$].

Similar results were found for DOMS$^{\Delta}$ and CMJ$^{\Delta}$. There was a time effect for DOMS$^{\Delta}$ [i.e., DOMS$^{\Delta}$ decreased at 48 and 72-h compared with 24-h] [P = 0.001; $F_{(4,62)}$ = 11.478; *post hoc* $P \leq 0.005$], but not for CMJ$^{\Delta}$ [P = 0.253; $F_{(4,62)}$ = 1.404], while no interaction was found between groups for either parameter

TABLE 2 | Comparison between C_{PBMT} and C_{PLA} for peak and integral values of CK, LDH, IL-10, and TNFα.

	C_{PLA}		C_{PBMT}		*t*-test			
	Peak (Δ%)	Integral (Δ%·h)	Peak (Δ%)	Integral (Δ%·h)	Peak		Integral	
					P-value (t)	Df	*P*-value (t)	Df
CK	170.6 ± 81.0	8330.5 ± 3308.6	139.7 ± 40.7	7902.5 ± 2170.3	0.10 (−1.774)	10	0.47 (−0.756)	10
LDH	132.1 ± 32.6	7442.6 ± 1469.7	129.1 ± 23.2	7427.3 ± 1332.8	0.28 (−0.358)	10	0.93 (−0.091)	9
IL-10	172.9 ± 70.2	8314.5 ± 2648.1	184.5 ± 110.7	7956.7 ± 2642.4	0.75 (0.323)	10	0.72 (−0.370)	10
TNFα	127.1 ± 57.5	6687.8 ± 1169.0	111.3 ± 23.1	6498.7 ± 983.0	0.29 (−1.119)	9	0.37 (−0.950)	8

IL-10, interleukin 10; TNFα, tumor necrosis factor alpha; CK, creatine kinase; LDH, lactate dehydrogenase; C_{PBMT}, condition photobiomodulation therapy; C_{PLA}, condition placebo; Δ%, percentage alteration related to baseline; Δ%·h, percentage alteration related to baseline per hour; Df, degrees of freedom.

[P = 0.314; $F_{(4,62)}$ = 1.215 and P = 0.264; $F_{(4,62)}$ = 1.343, respectively] (**Figure 6**).

All raw data of inflammation and muscle damage markers, delayed onset muscle soreness, and countermovement jump performance are shown in **Supplementary Data Sheet S1**.

DISCUSSION

Some recovery strategies have been used to accelerate muscle recovery in sport routines (Barnett, 2006), however, despite their wide use, several doubts remain in the literature about the effectiveness of these methods. To the best of our knowledge, this is the first study to investigate the effects of PBMT on muscle recovery before SIT sessions using systemic blood markers, muscle performance, and DOMS, and compare it with CWI and AR. The main finding of the current study was the lack of effect of PBMT on muscle recovery compared with C_{PLA}, as well as the fact that PBMT did not demonstrate better effects than AR or CWI. Therefore, our initial hypothesis was refuted. The time effects found (**Supplementary Figures S3**, **S4**) in the current study only show changes in variable behavior independent of the experimental treatment and therefore do not demonstrate effectivity of any isolated intervention.

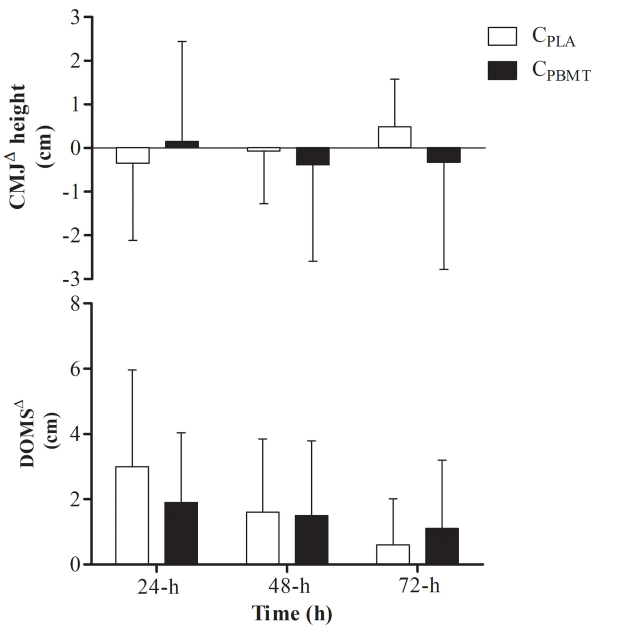


FIGURE 4 | $DOMS^{\Delta}$ performance and $DOMS^{\Delta}$ for G_{PBMT} and G_{PLA} at 24, 48, and 72-h after double SIT session. $DOMS^{\Delta}$, delay onset muscle soreness expressed as difference to baseline; CMJ^{Δ}, countermovement jump performance expressed as difference to baseline; C_{PBMT}, condition photobiomodulation therapy; C_{PLA}, condition placebo.

Initially, it should be mentioned that there were no differences in workload or metabolic stress induced by the double SIT session in both studies (I and II), indicating that all volunteers presented similar damage induction in all conditions. Consequently, the double SIT elicited increases in CK (≈57%), LDH (≈42%), IL-10 (≈86%), and TNFα (≈24%) blood concentrations in both studies and this increase was not different between conditions.

The process of muscle recovery may be monitored through systemic inflammatory marker kinetics such as cytokines (e.g., interleukins), and is usually accompanied by a decrease in muscle exercise performance and an increase in DOMS (Peake et al., 2017). Interleukins such as IL-10 and TNFα play an important role in the muscle recovery process and their concentration may give an indication of the inflammatory status (Petersen and Pedersen, 2005). In this way, it has been hypothesized that PBMT applied before or after exercise sessions may alter these inflammatory responses due to a decreased effect of reactive oxygen and nitrogen species on cell membranes (Powers and Jackson, 2008), increased activity of satellite cells (Ben-Dov et al., 1999; Shefer et al., 2002), and increased ATP levels (Ferraresi et al., 2015), resulting in better inflammation control and resolution.

Ferraresi et al. (2012) reported evidence of the supposed effect of PBMT on inflammation, which is supported mainly by animal model studies and *in vitro* assay results, focusing predominantly on rehabilitation. Among the few studies that investigated systemic inflammation after exercise with humans, Zagatto et al. (2016) verified only trivial to moderate effect sizes of PBMT on IL-10, IL-1β, and TNFα in young athletes after water polo training sessions, while Aver Vanin et al. (2016) verified a reduction only in IL-6 blood concentration after exercise-induced muscle damage. Therefore, it is clear that there is little evidence to support the beneficial effect

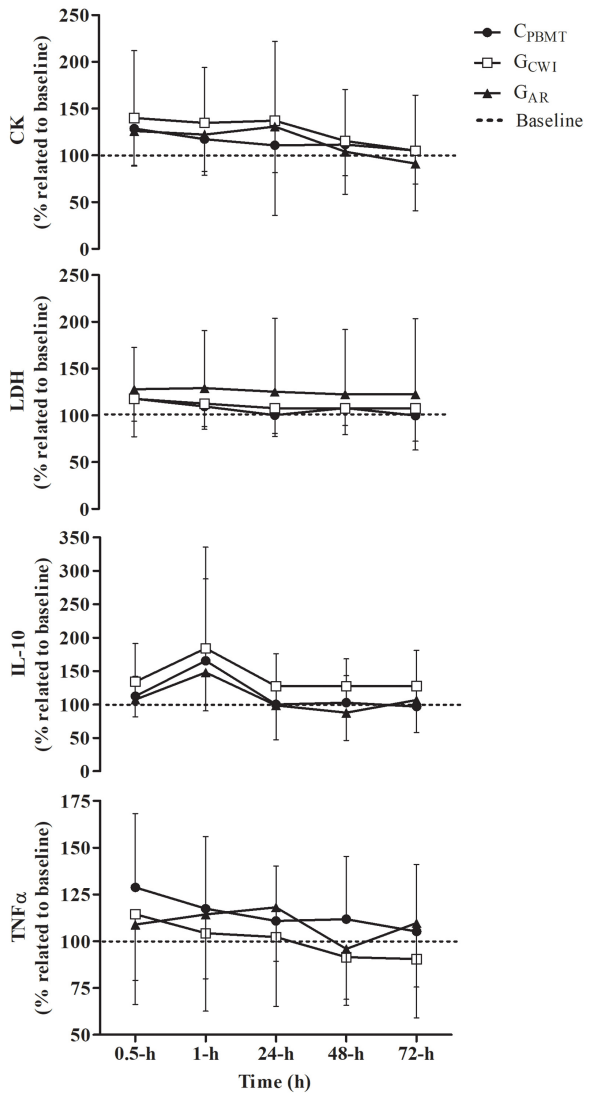


FIGURE 5 | Blood concentration of IL-10$^{\Delta\%}$, TNFα$^{\Delta\%}$, CK$^{\Delta\%}$, and LDH$^{\Delta\%}$ (mean ± SD) for C_{PBMT}, G_{AR}, and G_{CWI}. IL-10$^{\Delta\%}$, interleukin 10 expressed as percentage difference to baseline; TNFα$^{\Delta\%}$, tumor necrosis factor alpha presented as percentage difference to baseline; CK$^{\Delta\%}$, creatine kinase expressed as percentage difference to baseline; LDH$^{\Delta\%}$, lactate dehydrogenase expressed as percentage difference to baseline; C_{PBMT}, condition photobiomodulation therapy; G_{CWI}, group submitted to cold-water immersion; G_{AR}, group submitted to active recovery.

of PBMT on inflammation triggered by exercise in humans and our findings indicate that when performed after high-intensity efforts, PBMT as applied in the present investigation has no significant effect on systemic inflammation (see **Figure 2**).

Decreases in blood CK concentration through PBMT is well reported in the literature (Aver Vanin et al., 2016; De Marchi et al., 2017) while its effect on LDH has been little investigated. Although there is no specific evidence that this mechanism actually occurs, decreases in blood CK and LDH concentrations are often related to the supposed effect of PBMT on hydroxyl radical production in muscle cells, thus reducing the damage caused in the sarcolemma and extravasation of intracellular content to the blood flow (Ferraresi et al., 2012). However, in the present study, PBMT was not able to decrease CK and LDH blood concentrations, and neither were the CWI and AR which presented similar results to PBMT. It should be noted that our results were consistent (i.e., neither damage marker changed) and corroborate with other investigations that also did not verify changes in LDH concentration using PBMT (De Marchi et al., 2012; Zagatto et al., 2016).

The conflicting results in the literature may be explained by the different doses or energy used (Ferraresi et al., 2012). In this way, many authors have made efforts to clarify the dose response effect of PBMT on exercise performance (Dellagrana et al., 2018) and muscle recovery indices (Aver Vanin et al., 2016), however, the optimal dose is still unclear. In the present investigation a dose of 600 J was applied, which is higher than other recent studies (Aver Vanin et al., 2016; Zagatto et al., 2016). However, considering that several lower limb muscles are active during cycling (Hug and Dorel, 2009), and that several application points were required to radiate the entire area, our doses were high mainly due to the application area. Therefore, when the dose per diode irradiation area was relativized the values were ≈ 1.5–4.5 J/cm^2, close to the dose proposed by Ferraresi et al. (2012) for decreasing muscle damage (≈ 1.0–2.5 J/cm^2).

Recent studies have observed beneficial effects of PBMT on lower and upper limb isometric maximum voluntary contraction (Aver Vanin et al., 2016; De Marchi et al., 2017) and upper limb one-repetition maximum tests (Felismino et al., 2014) after exercise-induced damage protocols. However, in the present study there were no significant effects of PBMT on CWJ performance compared to C_{PLA}, G_{CWI}, and G_{AR}. These results are in agreement with our inflammatory marker findings, which may produce loss in muscle performance (Peake et al., 2017). In the present study CMJ was used due to its efficacy to detect performance loss after high-intensity exercise sessions (Claudino et al., 2017) and the insignificant effect of the jump, a brief effort, on muscle damage parameters.

In the present study, PBMT was not able to decrease DOMS after the double SIT while CWI and AR were not better than PBMT. Therefore, the results of the present study agree with our findings on inflammation, muscle damage, and performance. However, our results do not corroborate with recent studies that verified beneficial effects using PBMT or when associating PBMT with cryotherapy (de Paiva et al., 2016; De Marchi et al., 2017). It is worth mentioning that the cryotherapy method performed by these authors had no accurate temperature control (ice bag intervention). Machado et al. (2016) in a recent review verified that CWI effectiveness on DOMS is dependent on the temperature (≈ 11–15°C). Therefore, more studies with humans and a well-controlled CWI temperature are necessary to clarify these conflicting results.

TABLE 3 | Comparison between C_{PBMT}, G_{CWI}, and G_{AR} for peak and integral values of CK, LDH, IL-10, and TNFα.

	C_{PBMT}		G_{CWI}		G_{AR}		ANOVA one-way			
							Peak		Integral	
	Peak (Δ%)	Integral (Δ%·h)	Peak (Δ%)	Integral (Δ%·h)	Peak (Δ%)	Integral (Δ%·h)	*P*-value (F)	Df	*P*-value (F)	Df
CK	139.7 ± 40.7	7902.5 ± 2170.3	162.8 ± 78.5	8316.5 ± 3627.9	157.7 ± 94.3	8560.5 ± 5189.3	0.74 (0.310)	2, 26	0.93 (0.079)	2, 32
LDH	129.1 ± 23.2	7427.3 ± 1332.8	146.9 ± 46.1	7869.8 ± 1536.9	185.0 ± 113.4	7995.2 ± 2470.1	0.19 (1.734)	2, 31	0.78 (0.781)	2, 25
IL-10	184.5 ± 110.7	7956.7 ± 2642.4	219.2 ± 139.1	10112.2 ± 3580.7	185.9 ± 93.8	7672.1 ± 3857.5	0.72 (0.338)	2, 32	0.22 (1.578)	2, 28
TNFα	111.3 ± 23.1	6498.7 ± 983.0	127.9 ± 38.6	7044.5 ± 1623.8	127.3 ± 50.8	7650.0 ± 2019.0	0.56 (0.587)	2, 30	0.32 (1.202)	2, 26

IL-10, interleukin 10; TNFα, tumor necrosis factor alpha; CK, creatine kinase; LDH, lactate dehydrogenase; C_{PBMT}, condition photobiomodulation therapy; G_{CWI}, group submitted to cold-water immersion; G_{AR}, group submitted to active recovery; Δ%, percentage alteration related to baseline; Δ%·h, percentage alteration related to baseline per hour; Df, degrees of freedom.

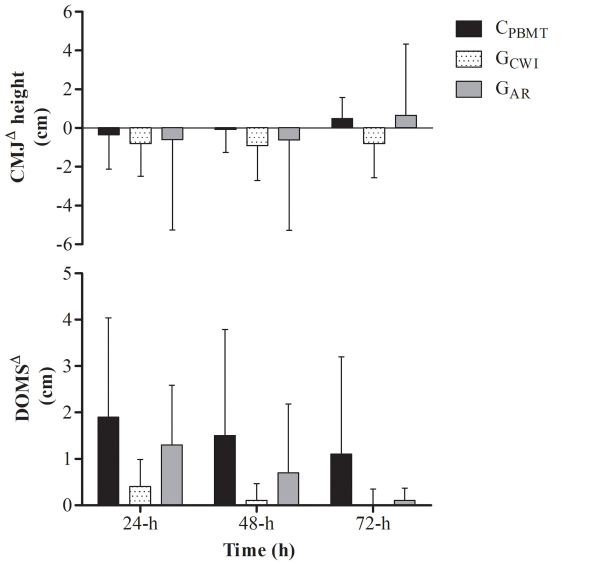


FIGURE 6 | $DOMS^{\Delta}$ performance and $DOMS^{\Delta}$ for C_{PBMT}, G_{AR}, and G_{CWI} at 24, 48, and 72-h after double SIT session. $DOMS^{\Delta}$, delay onset muscle soreness expressed as difference to baseline; CMJ^{Δ}, countermovement jump performance expressed as difference to baseline; C_{PBMT}, condition photobiomodulation therapy; G_{CWI}, group submitted to cold-water immersion; G_{AR}, group submitted to active recovery.

The main limitation of the present study is that muscle biopsies were not performed to determine intramuscular recovery parameters. However, in view of the number of blood collections (i.e., 11 in part-I and 6 in part-II) this type of procedure proved unfeasible.

Therefore, our results indicate that PBMT use after acute high-intensity efforts has no effect on muscle recovery. In addition, although the literature suggests the potential changes generated by CWI on muscle recovery mainly due to decreased DOMS (Machado et al., 2016), in the present investigation the CWI was not different to PBMT in any recovery index. Similarly, although AR is a popular recovery method in sports routines (Barnett, 2006), this method also was not different from PBMT, which had no effect on recovery. However, it should be mentioned that some findings of the present study do not agree with previous studies, providing evidence that further studies on this same theme are needed to elucidate the real effects of PBMT.

In summary, PBMT had no effect on inflammation, muscle damage, CMJ performance, or DOMS and was not better than CWI or AR on these recovery indices.

AUTHOR CONTRIBUTIONS

EM, FSL, FM, and AMZ conceived and designed the experiments. EM and AMZ performed the experiments. EM, FSL, ASZ, and AMZ analyzed the data. All authors contributed to reagents, materials, and analysis tools and wrote the manuscript.

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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.2018.01948/full#supplementary-material

FIGURE S1 | Performance parameters and lactate kinetics concentration before, during and after the double SIT session. SIT, sprint interval training; BW, bout work; PP, peak power; Wingate test; TW, total work; PP, peak power; MP, mean power; C_{PBMT}, photobiomodulation therapy condition; C_{PLA}, placebo condition.

FIGURE S2 | Performance parameters and lactate kinetics concentration before, during and after the double SIT session. SIT, sprint interval training; BW, bout work; PP, peak power; Wingate test; TW, total work; PP, peak power; MP, mean power; C_{PBMT}, condition photobiomodulation therapy; G_{CWI}, group submitted to cold-water immersion; G_{AR}, group submitted to active recovery.

FIGURE S3 | Main time effect of blood concentration of IL-$10^{\Delta\%}$, TNF$\alpha^{\Delta\%}$, $CK^{\Delta\%}$, and $LDH^{\Delta\%}$ (mean ± SD) in part-I and part-II. IL-$10^{\Delta\%}$, interleukin 10 expressed as percentage difference to baseline; TNF$\alpha^{\Delta\%}$, tumor necrosis factor alpha expressed as percentage difference to baseline; $CK^{\Delta\%}$, creatine kinase expressed as percentage difference to baseline; $LDH^{\Delta\%}$, lactate dehydrogenase expressed as percentage difference to baseline. $^{a}p < 0.05$ compared with 0.5-h. $^{c}p < 0.05$ compared with 24-h. $^{d}p < 0.05$ compared with 48-h. $^{e}p < 0.05$ compared with 72-h.

FIGURE S4 | Main time effect of $DOMS^{\Delta}$ performance and CMJ^{Δ} (mean ± SD) in part-I and part-II. $DOMS^{\Delta}$, delay onset muscle soreness expressed as difference to baseline; CMJ^{Δ}, countermovement jump performance expressed as difference to baseline. $^{d}p < 0.05$ compared with 48-h. $^{e}p < 0.05$ compared with 72-h.

TABLE S1 | Blood concentration of IL-10, TNFα, CK, and LDH (mean ± SD) expressed in absolute values for C_{PBMT} and C_{PLA}. IL-10, interleukin 10; TNFα, tumor necrosis factor alpha; CK, creatine kinase; LDH, lactate dehydrogenase; CPBMT, photobiomodulation therapy condition; CPLA, placebo condition; Df, degrees of freedom.

TABLE S2 | Blood concentration of IL-10, TNFα, CK, and LDH (mean ± SD) expressed in absolute values and the effect size of C_{PBMT}, G_{AR}, and G_{CWI} at baseline, 30 min, 1, 24, 48, and 72 h. L-10, interleukin 10; TNFα, tumor necrosis factor alpha; CK, creatine kinase; LDH, lactate dehydrogenase; CPBMT, photobiomodulation therapy condition; GCWI, group submitted to cold-water immersion intervention; GAR, group submitted to active recovery; Df, degrees of freedom.

DATA SHEET S1 | Raw data of inflammation and muscle damage markers, delayed onset muscle soreness, and countermovement jump performance.

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frontiers in Physiology

ORIGINAL RESEARCH
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Effectiveness of Grounded Sleeping on Recovery After Intensive Eccentric Muscle Loading

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Purpose: We set out to investigate the effectiveness of grounded sleeping on the time course of recovery with respect to muscle soreness and athletic performance after intensive eccentric muscle loading.

Methods: Twenty-two healthy participants were recruited for this study and randomly assigned to an experimental group (GRD, grounded sleeping, n = 12) or control group (UGD, sham-grounded sleeping, n = 10) to evaluate the effects of 10 days recovery with GRD vs. UGD following a single intensive downhill treadmill intervention in a triple-blinded (participant, tester, and data analyst) manner. To operationalize recovery a test battery was performed at baseline and on days 1, 2, 3, 5, 7, and 10 post-intervention: (1) perception of muscle soreness (VAS), (2) creatine kinase blood levels (CK), (3) maximum voluntary isometric contraction (MVIC) for both legs, (4) counter movement jump (CMJ) and drop jump (DJ) performance. Furthermore, in four participants blood was sampled for detailed analysis of complete blood counts and serum-derived inflammation markers.

Results: The downhill treadmill running intervention led to distinct changes in all measured parameters related to fatigue. These changes were detectable already 5-min post intervention and were not fully recovered 10 days post intervention. GRD led to less pronounced decrease in performance (CMJ, MVIC) and less increase with respect to CK compared with UGD (all $P < 0.05$). Detailed blood samples demonstrated that grounded sleeping modulates the recovery process by (a) keeping a constant hemoconcentration, as represented by the number of erythrocytes, and the hemoglobin/hematocrit values; and (b) by the reduction of muscle damage-associated inflammation markers such as, IP-10, MIP-1α, and sP-Selectin.

Conclusion: The downhill running protocol is a feasible methodology to produce long term muscle soreness and muscular fatigue. GRD was shown to result in faster recovery and/or less pronounced markers of muscle damage and inflammation. GRD might be seen as a simple methodology to enhance acute and long-term recovery after intensive eccentric exercises.

Keywords: creatine kinase (CK), downhill running, inflammation, muscle soreness, muscle strength

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INTRODUCTION

Recovery following intense training bouts is crucial for both professional and recreational athletes. It is well understood that exercise-induced muscle damage (EIMD) usually follows novel, unaccustomed repetitive movements and/or strenuous eccentric contractions (Proske and Morgan, 2001; Proske et al., 2004; Mackey et al., 2008). This kind of external load induces muscle damage that is associated with delayed onset muscle soreness (DOMS) (Hough, 1902).

Exercise-induced muscle damage is often associated with temporary substantial drops in performance, local muscle soreness and enhanced risk for musculoskeletal injuries. In professional sports, athletes have to recover within a few days to regain their performance levels. In spite of both the frequency and monetary consequences of DOMS in elite athletes, the de facto underlying mechanisms and their impacts on performance and treatment strategies remain vague (Cheung et al., 2003). A number of conventional therapeutic strategies are applied to alleviate clinical symptoms of DOMS. Nonetheless, Cheung et al. (2003) and Seidel et al. (2012) concluded that, in fact, no treatment strategy consistently supported or enhanced muscle recovery, which indicates that there is actually a lack of compelling evidence-based and practical strategies to help prevent and/or alleviate DOMS.

Grounding, earthing, or grounded sleeping, is a process in which the athlete becomes grounded via an electrically conducted device. The person is grounded in an indirect way that corresponds to being barefoot with direct, continuous contact with the earth. Nowadays, it is almost impossible to be earthed, due to urbanization, insulating footwear or bituminization. There are various grounding systems available that permit contact with the surface of the Earth. This indirect way of earthing or grounding is based on trivial conductive systems like sheets, mats, wrist or ankle bands, adhesive patches that can be used during sleeping or working, or inside footwear. These devices get coupled to the Earth by a typical cord slotted into a grounded wall outlet (Oschman et al., 2015). Referring to Chevalier et al. (2006); Oschman (2007), and Oschman et al. (2015) the main hypothesis about earthing is based on the connection to the surface of the Earth, which is satiated with free electrons. This indirect or direct contact with the Earth enables "mobile" electrons to migrate into the body. Oschman (2007) suggests that these free electrons act as antioxidants in the organism and could neutralize reactive oxygen species (ROS). ROS are byproducts of mitochondrial metabolism of oxygen and delivered by the oxidative burst as part of the inflammation response. Harman (1956, 2009) postulated that these reactive chemical species are linked to the aging process, originally known as free-radical theory of aging, and in the 1970s was extended to the mitochondrial theory of aging. Over the past several years, an emerging body of evidence is starting to indicate that ROS are associated with tumorigenesis, cancer and chronic inflammatory systemic diseases (Reddy and Clark, 2004; Waris and Ahsan, 2006; Gupta et al., 2012). Consequently, Oschman (2007) suggests that the earthing based mobile electrons could also prevent or diminish inflammation.

Actually, evidence-based research regarding the effectiveness of grounding is lacking. The narrative review of Chevalier et al. (2012) included grounding studies that indicated improvements in sleep (Ghaly and Teplitz, 2004), indices of DOMS (Brown et al., 2010; Brown et al., 2015), autonomic tone (Sinatra, 2011) and reduction in blood viscosity (Chevalier et al., 2013; Brown and Chevalier, 2015). Due to the potentially reduced blood viscosity, enhanced blood flow velocity, improved sleep quality and decreased muscle damage, it is suggested that grounding could be implemented as a viable, effective recovery tool after strenuous exercise. However, currently, the evidence of treatment strategies to accelerate recovery after EIMD is still inconsistent (Connolly et al., 2003).

Therefore, the aim of this study was to investigate the effectiveness of grounded sleeping on the time course of DOMS and athletic performance after intensive downhill running. We hypothesized that grounded sleeping would alleviate exercise induced muscle and accelerate the recovery of athletic performance after strenuous downhill running.

MATERIALS AND METHODS

Participants

Twenty-two healthy sport science and physiotherapy students (mean ± SD; n = 22, 10 women, 12 men, age: 23.8 ± 3.2 years, weight: 67.2 ± 7.6 kg, height: 174.2 ± 6.3 cm) were recruited for this study. All Participants were free of musculoskeletal disorders, cardiovascular diseases, dietary supplements or prescription medicines that could potentially affect muscle recovery. All participants received detailed oral and written information about the procedures and the possible risks, and gave their written consent to participate. The study received approval from the local Ethics Committee of the University of Salzburg (Kapitelgasse 4, 5020 Salzburg, GZ 13/2017) and was conducted in accordance with the Declaration of Helsinki. Participants were given grounding equipment for compensation. Participants were randomly assigned to the experimental group (GRD, grounded sleeping, n = 12) or the control group (UGD, sham-grounded sleeping, n = 10). No difference in any baseline values between groups were detected (**Table 1**).

Study Design

A triple-blinded (participant, tester, and data analyst) randomized controlled design was used to evaluate the effects of 10 days recovery including grounded sleeping (GRD) or sham-grounded sleeping (UGD) after a single intensive downhill treadmill running intervention designed to induce DOMS. To operationalize recovery a test battery was performed before the intervention (baseline) and on days 1, 2, 3, 5, 7, and 10 post intervention. This test battery included: perception of muscle soreness via a visual analog scale (VAS), creatine kinase blood levels (CK), maximum voluntary isometric contraction (MVIC) for both legs, counter movement jump (CMJ) and drop jump (DJ) performance (see **Figure 1**). Furthermore, as a pilot study in four participants (GRD, n = 2; and UGD, n = 2), blood was sampled

TABLE 1 | Physical characteristics of the participants in the grounded sleeping group (GRD) versus the sham-grounded group (UGD) (mean ± SD).

Group	Height (m)	Body mass (kg)	Age (years)	CMJ-Height (cm)	DJ-Coefficient (height/time)	MVIC-R (*N*)	MVIC-L (*N*)	CK (U/L)	VAS (6–20)
GRD	177 ± 8	71 ± 10	23.9 ± 4.1	29.2 ± 6.0	140 ± 43	2029 ± 749	1986 ± 780	261 ± 168	4.6 ± 4.3
UGD	174 ± 7	67 ± 9	23.7 ± 2.3	27.4 ± 7.4	115 ± 28	1713 ± 507	1596 ± 539	154 ± 99	3.7 ± 4.0

SJ, squat jump; DJ, drop jump; MVIC-R, MVIC-L, maximum isometric voluntary contraction right-left leg; CK, creatine kinase; VAS, visual analog scale.

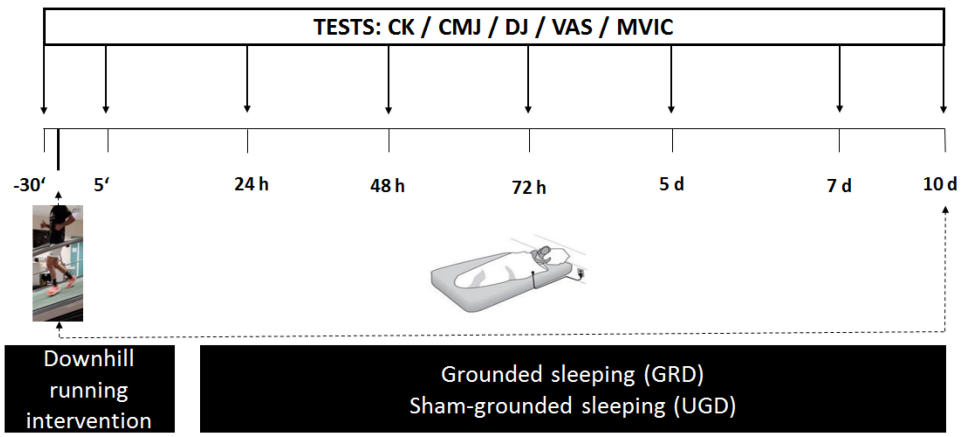

FIGURE 1 | Schematic illustration of the procedure with a timeline: pretest (–30 min prior to intervention), intervention (downhill running), posttests (+5 min, 24 h, 48 h, 72 h, 5 days, 7 days, and 10 days post intervention) consisting determination of blood creatine kinase content (CK), visual analog scale (VAS) related to muscle soreness, drop jump (DJ) performance, counter movement jump performance (CMJ) and a maximum voluntary isometric contraction (MVIC) of both the left and right leg.

for detailed analysis of complete blood counts and serum-derived inflammation markers.

Instruments and Procedures

Downhill Running

All participants underwent a 20-min exercise bout of intensive downhill running (−25% slope, 12 km · h^{-1}) on a motorized treadmill (Saturn 300/100 rs, h/p/cosmos sports & medical GmbH, Germany). For determination of the physiological and psychological loading of the intervention, heart rate (Suunto Ambit 3.0, Helsinki, Finland), blood lactate (Biosen S-Line EFK Diagnostik, Germany) in the 1st, 3rd, and 5th minute post intervention and rating of perceived exertion (BORG, 6–20) in the last minute of running were collected.

Grounded or Sham-Grounded Sleeping

During the 10-day post intervention, the GRD group slept grounded with a conductive sheet connected with a grounded wall outlet, while the UGD group slept sham-grounded. Both groups received identical sheets provided by BTZ (Badisches Therapie Zentrum, Baden-Baden, Germany) prior to the start of the study. These sheets (sized 90 cm × 200 cm) are made of 100% cotton with conductive silver fibers woven into the fabric. The sheet attaches to a grounded cord that connects at the other end to the ground of the wall socket. There is no direct connection to electricity; therefore, grounding via an indirect manner (e.g., cable) is safe and does not pose any danger to the individuals. To establish the control situation and subsequent blinding, the grounding plugs were manipulated and masked by an independent person. Neither the investigator, nor the participants were aware of this modification of the grounded system. The participants were instructed to sleep grounded at home on their sheets, ensuring as much skin contact as possible. Additionally, the participants were consistently reminded that it would be best if they slept either naked, or at most, wear no more than underwear. The participants were allowed to sleep with a pillow that was not grounded. In the event that a participant was not to sleep at home on any of the 10 days, they were advised to take the sheet with them.

A pilot study (n = 10) about the effects of earthing with the conductive sheets (grounded vs. sham-grounded) was performed prior to start of the main study. Participants were asked to lay down on a massage bed and relax on both the grounded139 or sham-grounded sheet for 5-min each wearing only underwear. Electrostatic charge was measured on the skin at the region of the vastus lateralis via an electrostatic voltmeter (ESVM 1000, Wolfgang Warmbier, Germany). The pilot study revealed values of −0.2 ± 0.1 V vs. −81.9 ± 25.6 V ($P < 0.001$) in the grounded vs. sham-grounded situation clearly demonstrating the effects of grounding via the conductive sheet.

Counter-Movement-Jump and Drop-Jump

The CMJ was executed without arm-swing and the DJ was performed from a 40 cm dropping height. Both jump modes were performed on a force plate (BP600900, AMTI, United States) to

evaluate the maximum jumping height (CMJ and DJ) and the ground contact time and coefficient between jump height and ground contact time during the DJ. The best of three attempts were used for further analysis.

Maximum Voluntary Isometric Contraction (MVIC)

The MVIC of the left and the right leg was assessed in a self-constructed unilateral horizontal leg press with an integrated load cell (Hottinger Baldwin Messtechnik GmbH, Germany). The participants were seated with 110° knee flexion angle (180° equivalent to full knee extension) and were allowed to grab the handles. The individual set up of the knee angle was documented with a goniometer to ensure identical settings at baseline and at follow-ups. The best of three attempts was used for further analysis. The stronger leg at baseline was defined as the dominant leg. The researchers motivated verbally during the execution.

Creatine Kinase (CK)

The CK enzyme is one of the most common objective reported indices of DOMS that is associated with muscle damage (Eston et al., 1994; Urhausen et al., 1995; Brown et al., 2015; Saw et al., 2016). For analysis of CK a capillary blood draw of 32 μl was taken and analyzed with the Reflotron Sprint system (Roche Diagnostics GmbH, Germany).

Perception of the Muscle Soreness (VAS)

The VAS was used to evaluate the muscle soreness of the lower extremity. The length of the VAS was 100 mm, and the participants had to fill in the scale at the beginning of each test day. The VAS is commonly used to assess the muscle soreness and is a valid and reliable test (Bijur et al., 2001; Gallagher et al., 2001).

Blood Samples

In four subjects venous blood was collected at each time point. All blood draws were performed by an experienced and certified phlebotomist. The analysis of blood counts (erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, leucocytes, monocytes, granulocytes, platelets and lymphocytes) was performed using a Celltac MEK-6500 (EuroLAB, Hallein, Austria). Serum-derived inflammation markers including sE-selectin, GM-CSF, sICAM-1/CD54, IFNα, IFNγ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A/CTLA-8, IP-10/CXCL10, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, sP-selectin, and TNF alpha were analyzed with a Luminex® MAGPIX® System (Luminex Corporation, Austin, TX, United States) using a 20-Plex Human ProcartaPlex Panel™ (Thermo Fisher Scientific, Waltham, MA, United States). The C-reactive protein (CRP) value was determined from full blood using a EUROLyser CUBE-S (EUROLyser Diagnostica GmbH, Salzburg, Austria). Raw data were normalized to percentage difference based on the baseline value (day 0), equation: 100*(value-baseline)/baseline.

Statistical Analysis

All data exhibited a Gaussian distribution verified by the Shapiro–Wilk's test and accordingly, the values are presented as means (±SD). The effects of the downhill treadmill running intervention on the various test parameters were analyzed with paired sampled *t*-tests between baseline and 5-min post intervention values. All post intervention test data where grounded or sham-grounded overnight sleeping has already taken place (days 1–10) were compared as how they changed from the baseline levels in per cent (day 0). A 2 × 6 ANOVA with repeated measures was used to compare both groups (GRD versus UGD) over the six-time points (days 1, 2, 3, 5, 7, and 10). The blood data were analyzed using the GraphPad Prism 7 software (La Jolla, CA, United States). Statistical analysis of blood values and inflammation markers was performed using an unpaired *t*-test with Welch's correction. Alpha level of significance was set to 0.05. In addition, the values obtained were evaluated by calculating the effect size (η_p^2) and statistical power. The Statistical Package for the Social Sciences (Version 24.0; SPSS Inc., Chicago, IL, United States) was used for statistical analysis.

RESULTS

In both groups the intensive downhill treadmill protocol led to high physiological (e.g., heart rate of 200 bpm) and psychological loading (RPE between 18 and 19) with slightly higher response in UGD compared with GRD with respect to peak blood lactate (5.1 versus 3.2 mmol · L^{-1}, $P = 0.047$) and RPE (18.9 vs. 18.3, $P = 0.033$) (**Table 2**). The intervention led to distinct reductions in jump performance (CMJ jump height, DJ jump height, DJ coefficient, all $P < 0.001$), MVIC during leg extension (both legs $P < 0.001$) and increases in VAS associated with muscle soreness ($P < 0.001$). CK levels were increased in both GRD ($P = 0.003$) and UGD ($P = 0.024$).

The time course of the absolute values in measured variables across the 10-days recovery period is presented in **Table 3**. With respect to the CMJ jump height there was a main effect of time ($P < 0.001$, $\eta_p^2 = 0.50$, power = 1.0) and group, with a systematically lower reduction in GRD compared with UGD (−8.2 ± 5.4% vs. −14.3 ± 5.4%, $P = 0.017$, $\eta_p^2 = 0.25$, power = 0.70) but no interaction in the time course of recovery between the two groups ($P = 0.79$). Lowest CMJ performance was achieved on the 1st day post intervention (**Figure 2**).

For the DJ jump height, ground contact time and jump coefficient there was a main effect of time (all $P < 0.001$, $\eta_p^2 = 0.28$ to 0.88, power = 1.0). Both for jump height and ground contact time, no main effect for group was found. Lowest performance in the DJ was detected on day 2 (height and coefficient) and 3 (ground contact time) post intervention. For the jump coefficient a trend toward a group effect ($P = 0.06$) with a lower reduction in

TABLE 2 | Physical and psychological exertion during the treadmill downhill running intervention in the grounded sleeping (GRD) and sham-grounded sleeping (UGD) group (mean ± SD).

	LA_{peak} (mmol · L^{-1})	HR_{max} (bpm)	HR_{mean} (bpm)	RPE (6–20)
GRD	3.2 ± 1.3	201 ± 12	177 ± 12	18.3 ± 0.8
UGD	5.1 ± 2.9*	201 ± 8	177 ± 11	18.9 ± 0.7*

*LA_{peak}, peak lactate within the first 5-min post intervention; HR_{max}, maximal heart rate; HR_{mean}, mean heart rate; RPE, rate of perceived exertion (6–20 Borg scale); *$P < 0.05$, different to GRD.*

TABLE 3 | Measured test parameters during the 10 days recovery period after the strenuous downhill running intervention in the grounded sleeping (GRD) and sham-grounded sleeping (UGD) group (mean ± SD).

	Group	BL (–30')	D_0 (+5')	D_1 (24 h)	D_2 (48 h)	D_3 (72 h)	D_5	D_7	D_{10}
CMJ height (cm)	UGD	27.4 ± 7.4	22.9 ± 6.7***	22.2 ± 6.2^{7,10}	22.1 ± 5.9^{7,10}	22.9 ± 6.0^{1}	24.2 ± 6.2^{1,2}	24.6 ± 6.2^{0,1,2}	25.1 ± 6.5^{1,2}
	GRD	31.3 ± 7.5	27.9 ± 7.3***	27.2 ± 7.2^{7,10}	27.6 ± 7.8^{7,10}	29.0 ± 8.3^{7}	29.4 ± 7.5	30.1 ± 8.3^{1,2,3}	30.2 ± 7.8^{1,2}
DJ height (cm)	UGD	28.3 ± 5.2	22.2 ± 5.4^{***7,10}	21.2 ± 4.9^{5,7,10}	20.5 ± 3.9^{5,7,10}	23.0 ± 4.6	25.9 ± 4.2^{1,2}	25.9 ± 4.5^{0,1,2}	26.8 ± 5.1^{0,1,2}
	GRD	34.2 ± 7.8	28.2 ± 7.5^{***5,7,10}	28.1 ± 7.4^{5,7,10}	28.8 ± 7.9^{5,7,10}	30.8 ± 9.6	32.0 ± 9.6^{0,1,2}	33.4 ± 9.0^{0,1,2}	32.7 ± 8.4^{0,1,2}
DJ GCT (ms)	UGD	250 ± 27	272 ± 42*	276 ± 50	288 ± 47^{7,10}	292 ± 53^{10}	274 ± 43	270 ± 42^{2}	253 ± 41^{2,3}
	GRD	253 ± 53	274 ± 70	262 ± 70	276 ± 61	274 ± 74	262 ± 59	259 ± 67	256 ± 59
DJ coeff (cm/s)	UGD	115 ± 28	85 ± 29^{**10}	80 ± 27^{7,10}	74 ± 21^{5,7,10}	82 ± 26^{5,7,10}	98 ± 29^{2,3}	99 ± 27^{1,2,3}	110 ± 31^{0,1,2,3}
	GRD	140 ± 43	110 ± 42^{***5,7,10}	116 ± 45^{7}	112 ± 41^{5,7,10}	122 ± 51^{7}	129 ± 50^{0,2}	137 ± 51^{0,1,2,3}	134 ± 45^{0,1,2}
MVIC-R (N)	UGD	1713 ± 507	1235 ± 496^{***5,7,10}	1279 ± 371^{5,7,10}	1334 ± 427^{7,10,}	1331 ± 404^{5,7,10}	1483 ± 480^{0,1,3}	1524 ± 494^{0,1,2,3}	1566 ± 466^{0,1,2,3}
	GRD	2029 ± 749	1574 ± 595^{***2,3,5,7,10}	1737 ± 712^{7}	1812 ± 685^{0}	1830 ± 700^{0}	1847 ± 641^{0,7}	1931 ± 656^{0,1,7}	1885 ± 592^{0}
MVIC-L (N)	UGD	1596 ± 539	1168 ± 417^{***3,5,7,10}	1234 ± 382^{5,7,10,}	1314 ± 399	1356 ± 382^{0}	1451 ± 443^{0,1}	1528 ± 434^{0,1}	1520 ± 507^{0,1}
	GRD	1986 ± 780	1565 ± 648^{***1,2,3,5,7,10}	1690 ± 714^{0,7}	1784 ± 748^{0}	1799 ± 740^{0}	1846 ± 727^{0}	1892 ± 624^{0,1}	1890 ± 600^{0}
CK (U · L^{-1})	UGD	154 ± 99	201 ± 122^{**5,7}	641 ± 403^{5,7}	548 ± 383^{5,7}	638 ± 507	1132 ± 765^{0,1,2,10}	653 ± 671^{0,1,2,10}	608 ± 422^{5,7}
	GRD	260 ± 168	318 ± 237^{2}	874 ± 503^{1}	482 ± 291	595 ± 570	710 ± 418	465 ± 233	315 ± 273
VAS (mm)	UGD	4 ± 4	37 ± 21^{***2,10}	51 ± 15^{7,10}	69 ± 17^{0,5,7,10}	55 ± 17^{5,7,10}	26 ± 19^{2,3,10}	11 ± 15^{1,2,3}	2 ± 2^{0,1,2,3,5}
	GRD	5 ± 4	40 ± 29^{***7,10}	53 ± 26^{5,7,10}	65 ± 20^{3,5,7,10}	45 ± 22^{3,5,7,10}	18 ± 13^{1,2,3,7,10}	4 ± 4^{0,1,2,3,5}	2 ± 2^{0,1,2,3,5,7}

BL, baseline examination 30 min prior to the intervention; D_0, 5' post intervention; D_1, D_2, D_3,…, D_{10}, day 1, day 2, day 3,…, day 10 post intervention; CMJ, counter movement jump; DJ, drop jump; VAS, visual analog scale related to muscle soreness; MVIC, maximal voluntary isometric contraction of the right (R) or left (L) leg; *, **, ***, $P < 0.05$, $P < 0.01$, $P < 0.001$ significant difference between baseline (BL) and D_0 (5-min post intervention), $^{0,1,2,\ldots,10}$, significantly to the respective day (0, 1, 2,…,10) post intervention.

GRD compared with UGD (−12.2 ± 10.6% vs. −21.4 ± 10.6%) but no interaction between time x group ($P = 0.18$) was found.

For the MVIC in the dominant leg, a main effect of time ($P < 0.001$, $\eta_p^2 = 0.49$, power = 1.0) and group ($P < 0.03$, $\eta_p^2 = 0.22$, power = 0.61) with a less pronounced reduction in performance in GRD compared with UGD (−9.5 ± 16.8% vs. −17.3 ± 38.3%) was found. Day-to-day analysis revealed group × time interactions within the first 3 days with a more pronounced recovery in GRD compared with UGD ($P < 0.05$) (**Figure 3**). For the non-dominant leg, only a main effect of time was found ($P < 0.001$, $\eta_p^2 = 0.49$, power = 1.0) with no group differences. Lowest strength performance for both legs was found immediately after the intervention (day 0).

With respect to CK levels main effects of time ($P < 0.001$, $\eta_p^2 = 0.30$, power = 0.98) and group ($P = 0.007$, $\eta_p^2 = 0.31$, power = 0.81) and an interaction effect time × group ($P = 0.001$, $\eta_p^2 = 0.26$, power = 0.95) were found, demonstrating a lower increase GRD compared with UGD (310 ± 120% vs. 760 ± 380%) paralleled with a more pronounced increase in UGD at days 3, 5, and 7. Highest CK levels were measured on day 5 post intervention (**Figure 4A**). Individual response analysis revealed that within the GRD group none of the participants demonstrated a large increase in CK levels (i.e., change of >20% from baseline), while in UGD this was the case in 40% of the participants ($n = 4$). Furthermore in GRD even 25% ($n = 3$) demonstrated no increase (e.g., <3%) in CK levels while this was not the case in any participant of UGD (**Figure 4B**).

Regarding VAS only a significant effect of time ($P < 0.001$, $\eta_p^2 = 0.55$, power = 1.0) with no main effect of group ($P = 0.13$) or interaction effect ($P = 0.46$) was found. VAS was highest at day 2.

The effects of grounded sleeping on the post-exercise recovery process of four participants were further analyzed at the cellular and molecular levels. With regard to the differential blood count (**Figure 5**, **Supplementary Figure S1**, and **Supplementary Tables S1, S2**), the most obvious divergence between both groups was observed for erythrocyte counts and for hemoglobin and hematocrit values, which significantly increased from recovery days 2–7 in the UGD group ($P = 0.007$, $P = 0.029$, and $P = 0.017$, respectively). These alterations were accompanied by a decrease in the average volume of red blood cells (MCV, $P = 0.024$). In contrast, these parameters remained at baseline level in the GRD group. No difference was found between the two groups regarding the average mass of hemoglobin per red blood cell (MCH), indicating that the ratio of hemoglobin to erythrocytes was not altered during the recovery process following intense exercise activity. A slight initial boost in the number of leucocytes (10^3 cells per µl) was observed for the GRD group between days 3 and 7 of the recovery phase ($P = 0.079$). Between recovery days 3 and 5, a similar trend was observed for granulocytes ($P = 0.038$), which represent the vast majority of white blood cells (Lacelle and Cameron, 2015). Interestingly, a significant decrease of blood-derived monocytes was detected in the recovery phase between days 2 and 7 in GRD group ($P = 0.039$). In both UGD and GRD groups, the amount of platelets (10^3/mm^3) decreased in the recovery phase following

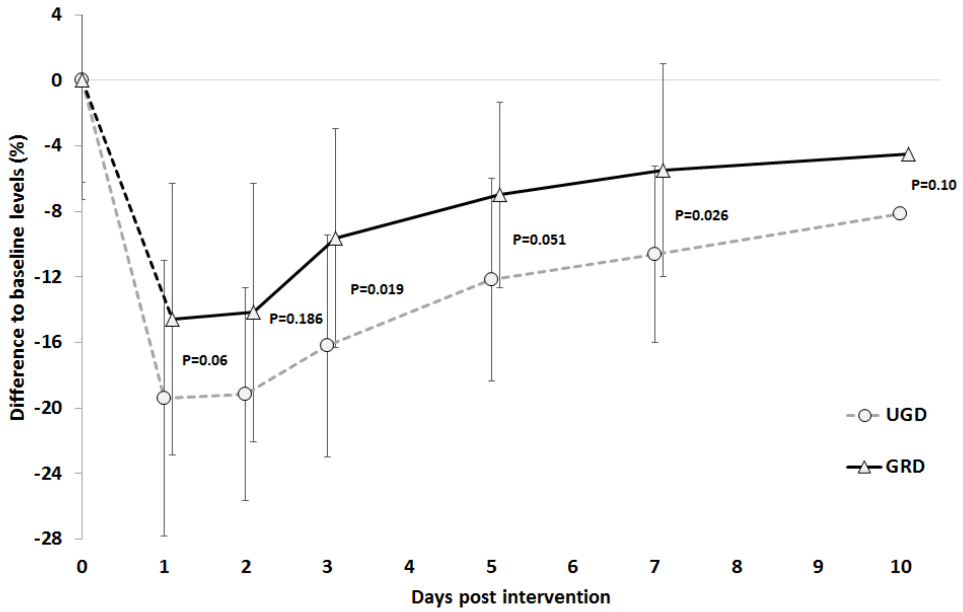

FIGURE 2 | Time course of the % reductions with respect to baseline levels in the counter movement jump (CMJ) across the 10 days post intervention period. UGD, sham-grounded sleeping group; GRD, grounded sleeping group (mean ± SD).

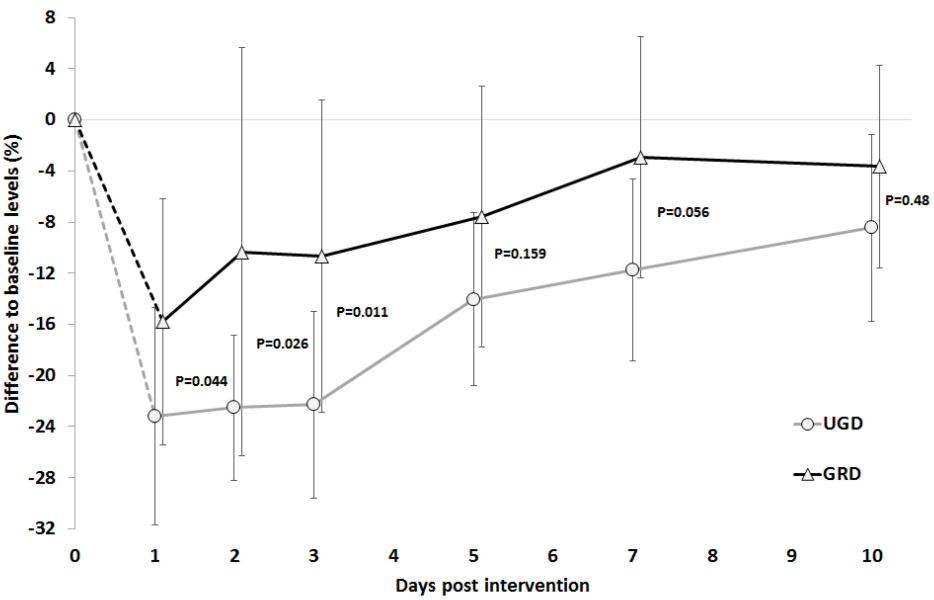

FIGURE 3 | Time course of the % reductions with respect to baseline levels in the isometric maximal strength for the dominant leg across the 10 days post intervention period. UGD, sham-grounded sleeping group; GRD, grounded sleeping group (mean ± SD).

intensive eccentric muscle loading. However, the amount of platelets in the UGD group returned to baseline levels earlier (between days 5 and 10, $P = 0.01$) than in the GRD group, suggesting that a prolonged recruitment of platelets to injured muscle tissue may have occurred. No significant alterations between both groups were observed for the mean corpuscular hemoglobin concentration (MCHC) and the overall lymphocyte count.

The C-reactive protein (CRP) value was monitored throughout the study and used as an indicator of unrelated inflammatory conditions (e.g., viral or bacterial infections), which could affect the evaluated blood parameters. The CRP values remained unaltered in all four subjects (**Supplementary Tables S3, S4**).

At the molecular level, we found alterations in serological markers of inflammation, particularly for soluble cell adhesion molecules (sCAM) and chemokines (**Figure 6**,

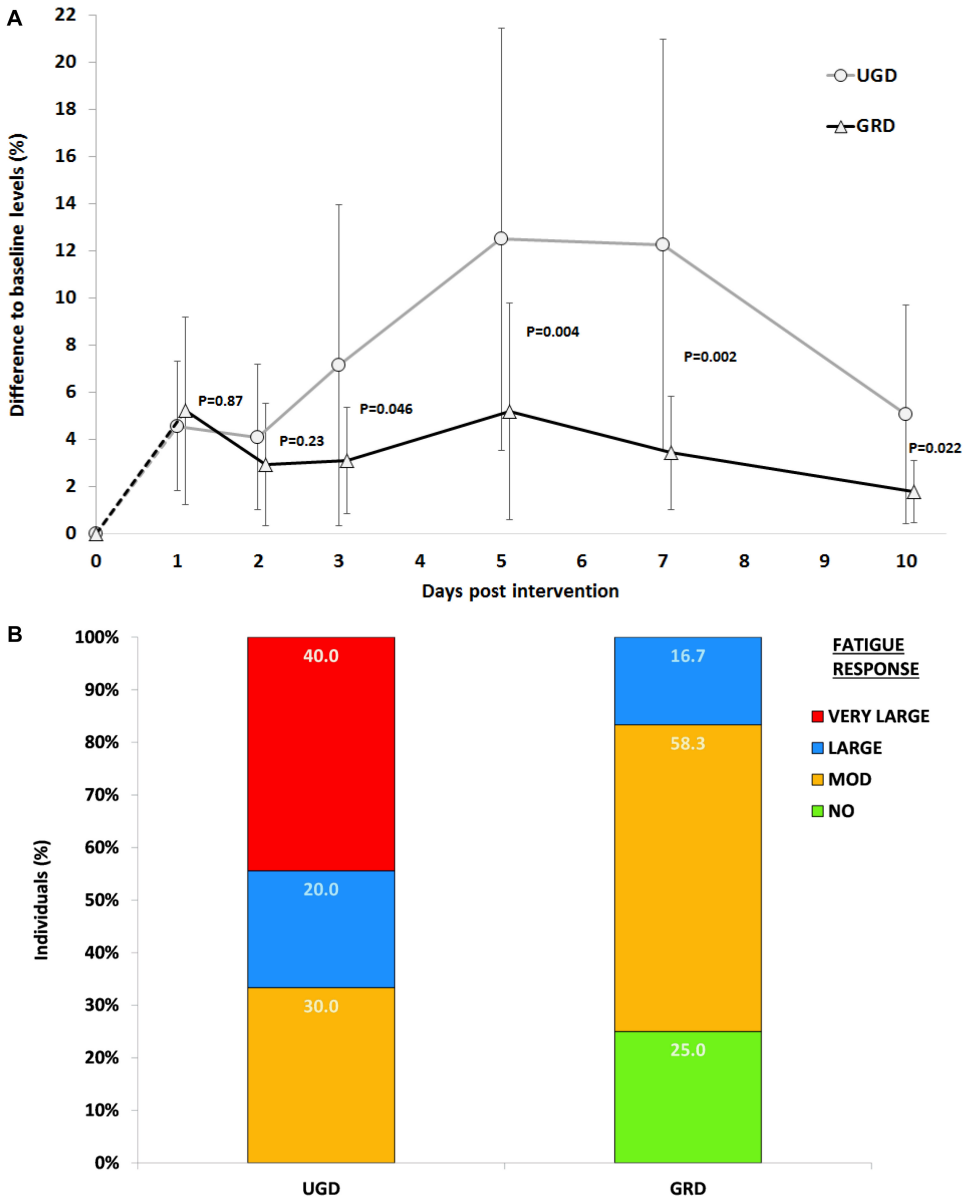

FIGURE 4 | (A) Time course of the % increases with respect to baseline levels for CK-blood levels across the 10 days post intervention period. UGD, sham-grounded sleeping group; GRD, grounded sleeping group (mean ± SD). **(B)** Individual response analysis for increases in CK levels with respect to GRD (grounded sleeping) and UGD (sham-grounded sleeping). Percent differences are categorized as non-response: <3%, moderate response: 3–10%, large response: 10–20%, and very large response >20%.

Supplementary Figure S2, and **Supplementary Tables S3, S4**). The intercellular adhesion molecule 1 (ICAM-1), which is expressed on epithelial cells and involved in cell adhesion and co-stimulation of macrophages, monocytes and granulocytes, either remained at baseline level or was higher in the GRD compared to the UGD group. From days 1 to 5, a clear decrease of the sICAM-1 levels (approximately 10%) was observed in the UGD group (P = 0.005). Furthermore, the cell adhesion molecule, sP-selectin, was downregulated in the GRD compared to the UGD and remained below baseline (approximately 20% reduction; P = 0.003) during the whole study period. In general, inflammation-associated chemokines, such as the interferon gamma-induced protein 10 (IP-10) and the macrophage inflammatory proteins (MIP-1α and MIP-1β), showed lower values (P = 0.014, P = 0.004, and P = 0.359, respectively) in the GRD group. No significant alterations were observed for the monocyte chemoattractant protein 1 (MCP-1), which is involved in the recruitment of monocytes, and the sCAM sE-selectin. It was not possible to detect chemokines/cytokines GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A/CTLA-8, and TNFα in the sera of the healthy athletes within the assay's limit of detection.

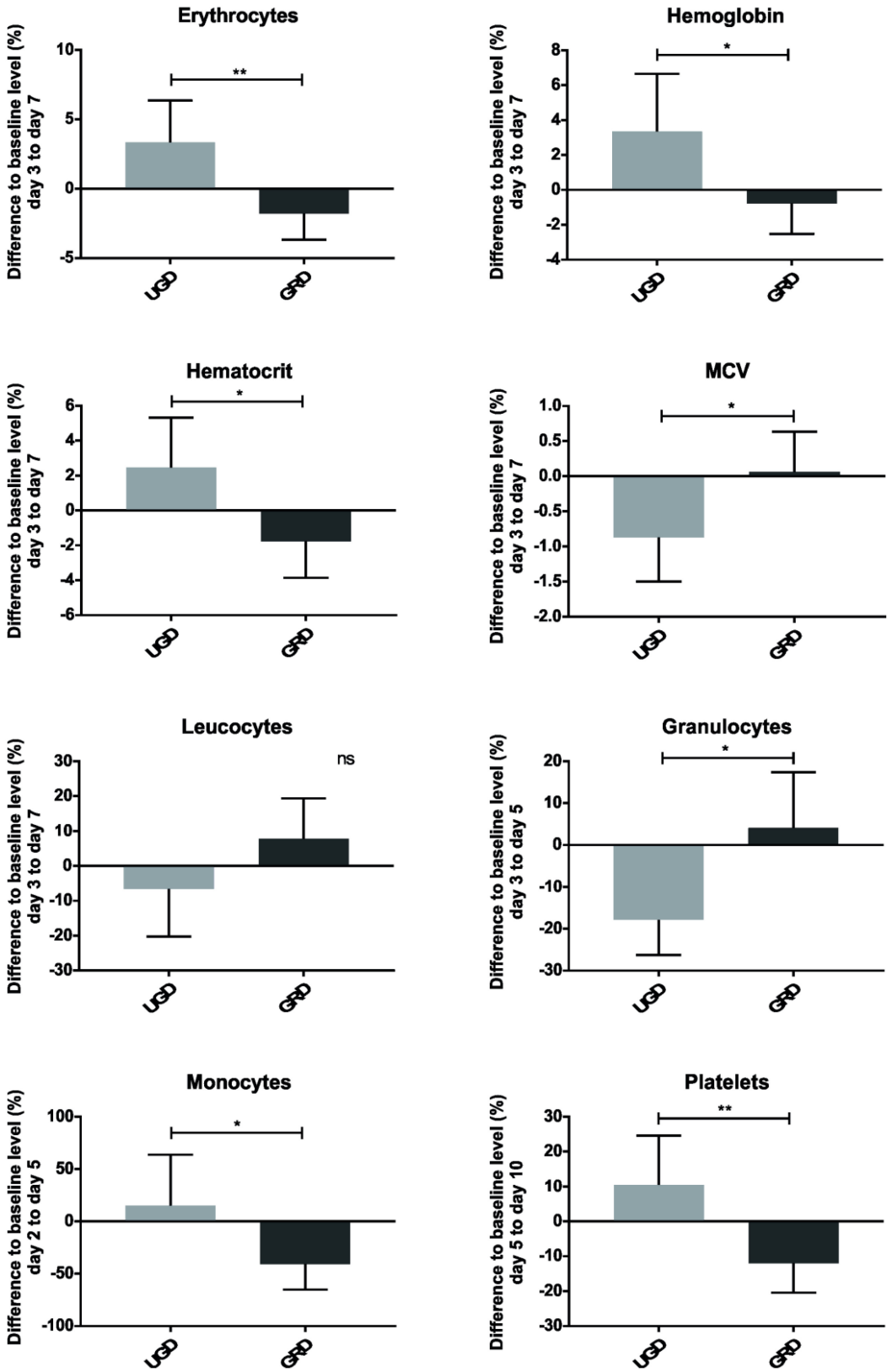

FIGURE 5 | Parameters of the differential blood count observed over a defined time, including erythrocytes, hemoglobin, hematocrit, MCV, leucocytes, granulocytes, monocytes and platelets; represented as mean with standard deviation of the defined days of each group; $^{ns}P > 0.05$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

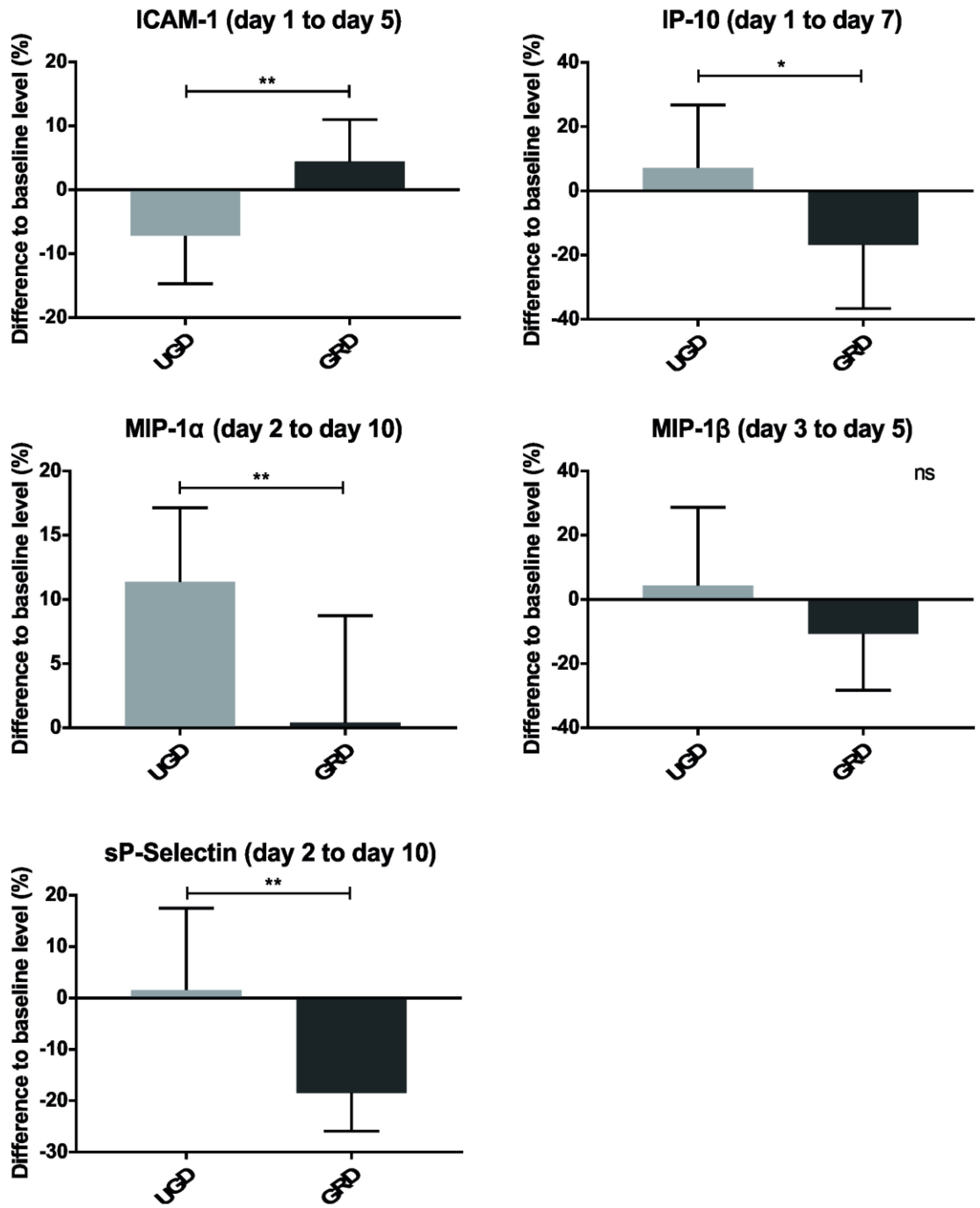

FIGURE 6 | Inflammation markers observed over a defined time, including sICAM-1, IP-10, MIP-1α, MIP-1β, and sP-selectin; represented as mean with standard deviation of the defined days of each group; $^{ns}P > 0.05$, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

DISCUSSION

The main findings of the current study were sixfold: (1) the downhill treadmill running intervention led to distinct changes in measured parameters related to fatigue and were already detectable 5 min post intervention; (2) grounded sleeping led in various variables to less pronounced decrease in performance compared with sham-grounded sleeping; (3) grounded sleeping led to a much lower increase of CK compared with UGD during the whole recovery; (4) 10 days post intervention in none of the measured parameters full recovery has been stated; (5) highest CK levels were found on day 5, while for jump and strength performance the highest decrease was the case on day 1 and for VAS the highest increase happened on day 2 post

intervention, and (6) detailed blood samples demonstrated that grounded sleeping modulates the recovery process by (a) keeping a constant hemoconcentration, as represented by the number of erythrocytes, and the hemoglobin/hematocrit values, and (b) by the reduction of muscle damage-associated inflammation markers such as IP-10, MIP-1α, and sP-Selectin.

Repetitive eccentric muscle activations like downhill running have repeatedly been shown to be responsible for DOMS. DOMS is associated with release of muscle proteins like CK into the blood stream, as well as with prolonged decreases in muscle performance capacity (Hoppeler, 2016). During eccentric exercises, the activated muscles are stretched which can create mechanical damage to sarcomeres, inflammation, disruption of the sarcolemma, as well as damage by reactive oxygen species (Lovering and Brooks, 2014). The applied 20-min downhill running protocol within the current study led to distinct decreases in performance and increases in measures of muscle damage and VAS, within the majority of analyzed parameters. No measured parameter returned to baseline levels after 10 days of recovery. Therefore, the applied downhill running intervention can be seen as a well-chosen method to provoke muscle damage resulting in distinct decrements in performance. This protocol can, therefore, be seen as suitable for analyzing the effectivity of different types of recovery interventions. In this specific case to analyze if grounded sleeping is able to enhance the ability to recover more quickly, or to dampen the decrements in performance when compared with an ungrounded situation. It is worth noting that Cheung et al. (2003) and Seidel et al. (2012) concluded that, in fact, no treatment strategy consistently supported or enhanced muscle recovery, which indicates that there is actually a lack of compelling evidence-based and practical strategies to help prevent and/or alleviate DOMS. Furthermore, the evidence of treatment strategies to accelerate recovery after EIMD is still inconsistent (Connolly et al., 2003).

However, the current study revealed that GRD showed lower decrements in performance with respect to CMJ, maximal leg strength (MVIC), a trend for DJ coefficient and less pronounced in increase in CK when compared with UGD. The less pronounced decrease in measures of performance (strength, jump performance) and less increase in CK levels within the GRD group might be attributed toward potentially reduced blood viscosity (Chevalier et al., 2013; Brown and Chevalier, 2015), enhanced blood flow velocity, improved sleep quality (Ghaly and Teplitz, 2004) and decreased muscle damage (Brown et al., 2010; Brown et al., 2015) as it is clearly demonstrated by the blood analyses of the present study. Referring to Chevalier et al. (2006); Oschman (2007), and Oschman et al. (2015) the main hypothesis about earthing is based on the connection to the surface of the Earth, which is satiated with free electrons. This indirect or direct contact with the Earth enables "mobile" electrons to migrate into the body. Consequently, Oschman (2007) suggests that the earthing based mobile electrons could also prevent or diminish inflammation, which could also be the reason for the unaffected hemoconcentration as well as the dampened CK response.

The observed increase in the number of erythrocytes and in the hemoglobin and hematocrit values in the course of intensive eccentric muscle loading in the UGD group could be due to volumetric variation. Post-exercise hemoconcentration is typically resulting from water loss (Bloomer and Goldfarb, 2004; Del Coso et al., 2008). Hemoconcentration is also a possible explanation for the increased CK levels in the UGD group (Bassini-Cameron et al., 2007). Our preliminary results suggest that grounded sleeping might prevent the phenomenon of hemoconcentration via the reduction of blood viscosity and improved blood circulation (Chevalier et al., 2013). No alteration in the lymphocyte counts in the blood between both groups was expected since lymphocytes are mostly associated with adaptive immune responses to foreign antigens originating from bacteria, virus, and parasites (Paul and Seder, 1994).

Upon muscle injury, damaged myofibres and other muscle cells at lesion sites undergo necrosis, which in turn are removed by various infiltrating immune cells, mostly mast cells and neutrophils (Bentzinger et al., 2013). These early stages of muscle regeneration are further characterized by activation of the complement system and release of pro-inflammatory cytokines and chemokines such as, TNF-α, IFN-γ, IP-10, and sICAM-1 (Chazaud et al., 2003; Cheng et al., 2008). In response to INF-γ and TNF-α, several cell types secrete interferon gamma-induced protein 10 (IP-10) that can be measured in the blood. The significant decrease in IP-10 observed in the GRD group strongly suggests that grounded sleeping downregulates INF-γ-induced inflammatory responses as well as IP-10-associated (NK) cell-mediated cytolysis. Furthermore, a decrease in the levels of the anti-angiogenic IP-10 might favor angiogenesis and the subsequent influx of blood to the regenerating muscle (Gotsch et al., 2007).

Following these early events in muscle regeneration, muscle stem cells are activated and other immune cells, especially macrophages and T cells, are recruited to the injured muscle tissue. Accordingly, a significant increase in the number of macrophages was observed on day two post-injury (Yang and Hu, 2018). These observations are in line with our findings showing that both chemokines MIP-1α and MIP-1β, which are produced by macrophages (Petray et al., 2002), are increased in the UGD group starting at day 2 post-injury. In contrast, our pilot study showed a decrease in the levels of these chemokines in the GRD group, suggesting a lower attraction of macrophages and thus local inflammation.

Our assumption that grounded sleeping dampens inflammation is further supported by a general decrease of inflammation markers observed in the GRD group. Of note, the sCAM sP-selectin (Schrijver et al., 2017) remained below the baseline level in the GRD group throughout the observation period, suggesting that grounded sleeping has a beneficial and long-lasting effect on this inflammation marker. sP-selectin is found on activated endothelial cells and platelets and is a crucial factor for leukocyte recruitment to lesion sites (Cleator et al., 2006). In the GRD group, a downregulation of activation of sP-selectin on endothelial cells and platelets could result in diminished leukocyte recruitment at sites of damaged muscle tissue.

Our findings that another sCAM, sICAM-1, was found to decline in the UGD compared to the GRD group was not in line with other studies showing high plasma sICAM-1 levels after strenuous exercise. However, sICAM-1 levels seem to be altered

depending on the type of exercise as no alterations were observed after bicycle ergometer exercise (Akimoto et al., 2002).

CONCLUSION

Taken together, grounded sleeping was shown to result in faster recovery and/or less pronounced markers of muscle damage and inflammation. Our preliminary results with respect to the detailed blood analysis strongly support the view that grounded sleeping modulates key events in the early stages of muscle regeneration at both cellular and molecular levels. Based on the investigated immunological parameters, the modulatory effects of grounded sleeping seem to dampen inflammatory responses triggered by EIMD. GRD might be seen as a simple methodology to enhance acute and long-term recovery after intensive exercises within the training process or following intensive competitions. Based on the results of the pilot study, more research is necessary to clearly point out the mechanisms behind possible effects of grounded sleeping on the cellular and molecular levels. Additionally, the magnitude of being grounded, that is how many "mobile" electrons migrate into the body while GRD vs. UGD sleeping, needs to be investigated further. Finally, whether or not grounded sleeping affects sleep patterns (e.g., sleep quality) also needs to be addressed in future studies. An improvement in the sleeping quality by grounded sleeping might result in alterations in performance and changes in stress markers in athletes.

AUTHOR CONTRIBUTIONS

EM, TS, PP, LA, and FF-B conception and designed the experiments. PP and LA performed the experiments. PP, TS, and LA analyzed data. TS, PP, EM, and LA prepared the manuscript. All authors read and approved the final manuscript.

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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.2019.00035/full#supplementary-material

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Effects of Endurance Running Training Associated With Photobiomodulation on 5-Km Performance and Muscle Soreness: A Randomized Placebo-Controlled Trial

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This study aimed to investigate the influence of endurance running training associated with PBM on endurance performance variables and muscle soreness in untrained men. Thirty untrained men were distributed randomly into a placebo (PLA) group and photobiomodulation group (PBMG) and they performed 8 weeks of running training. The PBMG had the PBM performed before all training sessions. The PBM was applied using LED equipment with 56 diodes of red light (660 nm) and 48 diodes of infrared light (850 nm). The application was performed in 5 points per leg, with a dose of 60 J at each point and a total energy delivered per leg of 300 J. Peak running velocity, time limit tests and 5-km performance were assessed pre and post-training; muscle soreness was evaluated before all training sessions. The V_{peak} increased and 5-km running time (t_{5-km}) decreased ($P < 0.001$) in both groups. In addition, the magnitude based-inference analysis showed a *possibly positive* effect on V_{peak} and t_{5-km} and for PBMG compared to PLA group. Furthermore, there was a moderate ES of 0.82 on attenuation in muscle soreness in the third week of endurance running training. Therefore, although the magnitude-based inference analysis demonstrated a *possibly positive* effect on V_{peak} and t_{5-km} and for PBMG compared to PLA group and a moderate ES on attenuation in muscle soreness in the last weeks of endurance running training, no significant difference were found between PBMG and PLA interventions.

Keywords: LED therapy, exercise training, aerobic exercise, athletic performance, performance-enhancing effects

INTRODUCTION

Various methods have been used to optimize muscle recovery after exercise to facilitate the adaptations resulting from the endurance training (Vanin et al., 2018). The use of light-emitting diodes (LED), as well as low-level lasers, which comprise a photobiomodulation (PBM) modality, has shown many positive effects in accelerating the recovery process after exercise (Leal Junior et al., 2015; Ferraresi et al., 2016a; Vanin et al., 2018). In addition, PBM can be considered an ergogenic aid that positively affects oxidative metabolism by improving mitochondrial function (Ferraresi et al., 2015, 2016a; Vanin et al., 2018).

Recent human studies that associated PBM and acute aerobic exercise showed positive effects on physiological variables and endurance performance in the experimental condition (e.g., PBM) compared to placebo (PLA) (Dellagrana et al., 2018; Lanferdini et al., 2018a; Mezzaroba et al., 2018). For example, Dellagrana et al. (2018) found that all PBM doses tested (15, 30, and 60 J per site) positively affected running economy, rate of perceived exertion (RPE), velocity at maximal oxygen uptake (vVO_{2max}), peak running velocity (V_{peak}), and total time to exhaustion in recreational runners. In addition, Mezzaroba et al. (2018) investigated physically active men and reported that PBM applied prior to running enhanced maximum and submaximal VO_2, increased the peak velocity (V_{peak}), and reduced heart rate (HR), and Rating of Perceived Exertion (RPE) during incremental tests.

Although a longitudinal biological effect of PBM has been suggested, few studies in humans have examined the influence of PBM on human performance biomarkers with respect to endurance training (Paolillo et al., 2011, 2013; Vieira et al., 2012; Miranda et al., 2018). Paolillo et al. (2011, 2013) demonstrated significant improvements in post-exercise recovery parameters such as heart rate (HR) and muscle power of the lower limb in the PBM group in runners treated with LED during training compared to a control group. Miranda et al. (2018) also associated PBM (applied before and/or after each training session) with running training and reported positive effects on time to exhaustion and VO_2 in healthy volunteers compared to the PLA group. However, these studies did not evaluate endurance running performance parameters such as 5-km running performance and V_{peak}.

Another positive effect of PBM is on delayed onset muscle soreness (DOMS); PBM attenuated the increase in DOMS after a strength exercise session (Antonialli et al., 2014) and after an aerobic time trial (Machado et al., 2017). However, only Ferraresi et al. (2016b) evaluated DOMS longitudinally during a strength training program with PBM applied after all sessions and found that DOMS scores were lower in the PBM condition than in the PLA group 24 h after training sessions. Nevertheless, the effect of PBM associated with endurance running training on muscle soreness evaluated by DOMS after running training is not known and must be investigated, since PBM being considered a recovery method during exercise and will likely be used during the training season as a chronic intervention.

In this study, we aimed to investigate the influence of endurance running training associated with PBM on endurance performance variables and muscle soreness in untrained men. We hypothesized that the group with PBM application would present improved running performance and attenuated muscle soreness compared to the PLA group.

MATERIALS AND METHODS

Participants

The sample size was calculated from *a priori* analysis for a group by time interaction comparison (F test, Anova for repeated measures, within-between interaction) according to an effect size of 0.52 (obtained from a pilot study), power of 80% and significance level of 5%. We used the software Gpower 3.1 (Düsseldorf, Germany) for the calculation. The *priori* power analysis revealed a minimal sample of 10 participants per group (n = 20). Volunteers were excluded if they used regular pharmacological agents or nutritional supplements, were smokers, were diagnosed with diabetes, hypertension, or asthma, presented any cardiovascular disorder, presented a body mass index $\geq$ 30 $kg \cdot m^{-2}$, or were engaged in other regular systematic physical training. Furthermore, only the participants who completed at least 90% of the training sessions were included in the final evaluation (Buchheit et al., 2010). Thus, 30 young and untrained men (aged between 20 and 35 years) volunteered to participate in this study. Participants were considered untrained if they had not engaged in running training or any other regular and systematic exercise training. **Table 1** shows the characteristics of the study participants (mean ± *SD*).

This study was carried out in accordance with the recommendations of Permanent Committee on ethics in research with human beings (COPEP). The protocol was approved by the Local Human Research Ethics Committee (#623.581/2014). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

The present study was a randomized double-blinded PLA -controlled experimental trial. The participants undertook four visits for baseline assessments (pre-training, week 1) where they underwent: (1) familiarization with the procedures, (2) incremental exercise tests to determine V_{peak}, (3) a constant load test (rectangular test) to determine the time limit (t_{lim}) at V_{peak}, and (4) 5-km running performance. The first three visits were performed under laboratory conditions, and the tests were

TABLE 1 | Participant's characteristics for both PLA group and PBMG at pre-training.

Variables	PLA (n = 15)	PBMG (n = 15)
Age (years)	27.3 ± 5.2	27.4 ± 3.7
Body mass (kg)	80.2 ± 10.3	79.2 ± 7.0
Height (m)	1.8 ± 0.1	1.8 ± 1.0
Body fat (%)	17.2 ± 5.4	17.7 ± 5.7
Body mass index ($kg \cdot m^{-2}$)	25.7 ± 2.9	25.3 ± 2.5

No statistical differences.

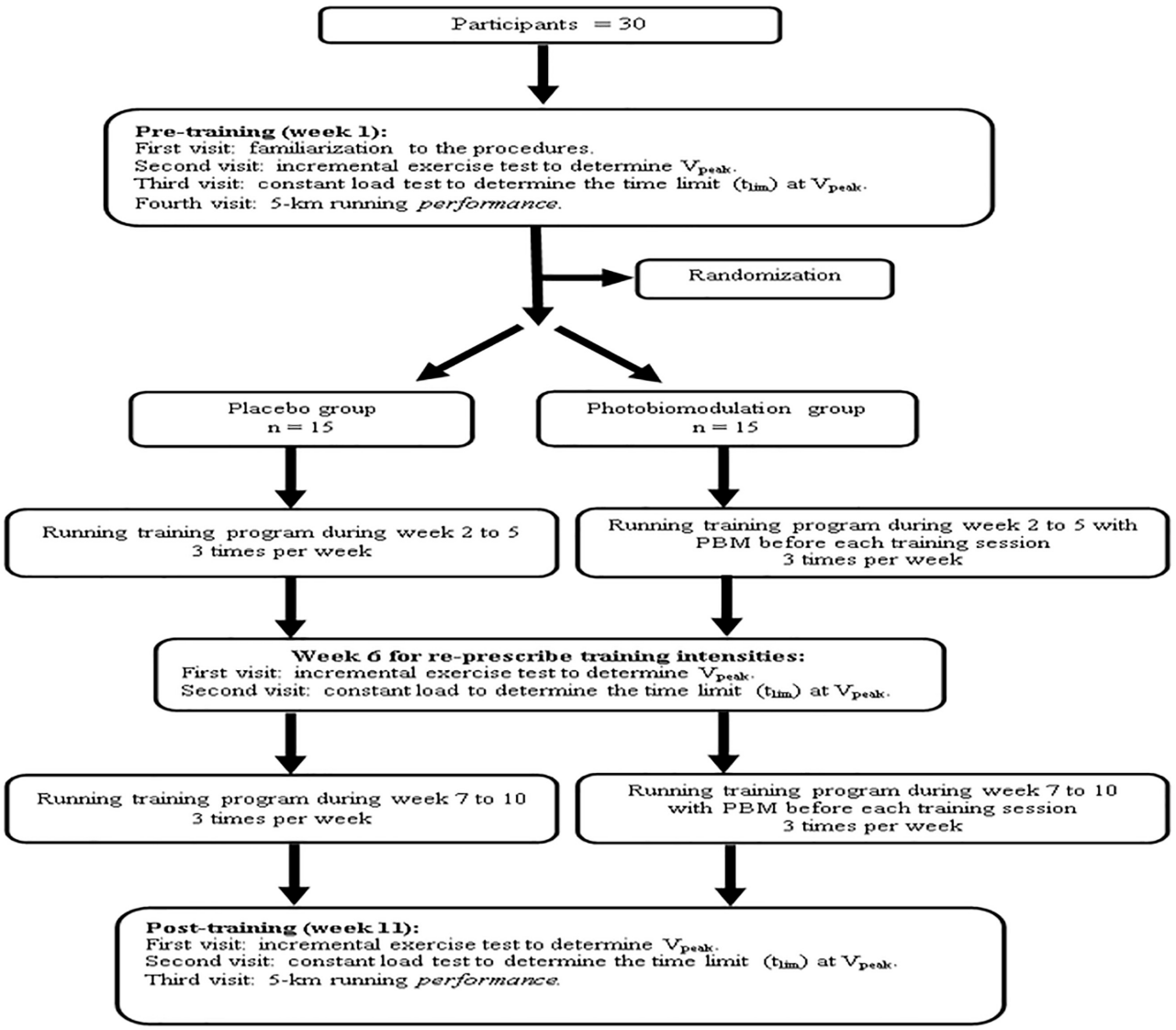

FIGURE 1 | Flow chart of the study design.

performed on a motorized treadmill (Super ATL; Inbrasport, Porto Alegre, Brazil). The 5-km running performance was performed on an outdoor track. All evaluations were performed within a maximum period of 7 days during which the participants did not perform training sessions. For the three maximal exercise tests, there was an intervening period of at least 48 h between tests to ensure the recovery of the participants between procedures. After the baseline assessments, the participants were randomly distributed in two experimental groups: the PLA group or the PBM group (PBMG). Both PLA and PBM groups performed the same running training program consisting of 8 weeks of endurance training, in which the only difference between the groups was that the PBMG received LED application before each training session. The V_{peak} test and its t_{lim} evaluation were repeated after 4 weeks of running training (i.e., from week 2 to 5) at week 6 to adjust training intensities during weeks 7–10 of training (i.e., the intensity prescription was based on V_{peak} and its t_{lim}). During week 6 participants did not perform training sessions. Furthermore, all the assessments were repeated in the week after the end of the running training program (at post-training, week 11). Thus, the participants completed 11 weeks of training that consisted of 8 weeks of endurance running training and the other 3 weeks for assessment (week 1, 6, and 11). **Figure 1** brings the study design.

Incremental Exercise Test to Determine Peak Running Velocity (V_{peak})

After a warm-up, comprised walking at 6 km · h^{-1} for 3 min, the incremental protocol started with a velocity of 8 km · h^{-1}

and increased by 1 km · h^{-1} between each successive 3-min stage until participants reached volitional exhaustion, with the gradient set at 1% (Machado et al., 2013; Peserico et al., 2015). This protocol was chosen because we previously demonstrated that this incremental rate and stage duration presented the highest correlations with endurance running performance and has been suggested as a tool for endurance running training prescription (Machado et al., 2013; Peserico et al., 2015). The V_{peak} of the incremental test was calculated as the velocity of the last complete stage added to the completed fraction of the incomplete stage (Kuipers et al., 2003), calculated according to the equation $V_{peak} = V_{complete} + t/T \times inc$ in which $V_{complete}$ is the running velocity of the last complete stage, t the time in seconds sustained during the incomplete stage, T the time in seconds required to complete a stage, and inc is the speed increment. During the test, the HR (Polar RS800sd; Polar, Finland) and rating of perceived exertion (RPE) (Borg, 1982) were monitored and the maximal HR (HR_{max}) and maximal RPE (RPE_{max}) were defined as the highest HR and RPE values, respectively, obtained during the test.

Constant Load Test to Determine the Time Limit (t_{lim}) at V_{peak}

After a 15-min warm-up at 60% of V_{peak}, the treadmill velocity was quickly increased (within approximately 6 s) to the individual V_{peak} (Billat et al., 1996) and the treadmill gradient was set at 1%. Participants were encouraged to invest maximal effort and the time of permanency in this intensity was considered the t_{lim} at V_{peak}. During the test HR and RPE were monitored and the HR_{max} and RPE_{max} were defined as the highest HR and RPE values, respectively, obtained during the test.

5-Km Running Performance

The 5-km time trial running performance was performed on a 400 m outdoor track and preceded by a self-determined warm-up of 10 min. Participants freely choose their pacing strategy during the performance. All participants were encouraged to give their best performance. The 5-km time for each participant were recorded and registered by the evaluator to determine the test duration (t_{5-km}), and this result was considered the running performance of the participant.

Muscle Soreness

For evaluated the perception of soreness we used a visual analog scale (VAS), which consisted of a 10 cm line labeled with "no soreness" on the left and "extremely sore" on the right. Previous studies have used a similar scale as a valid measure of muscle soreness (Baroni et al., 2010; Ferraresi et al., 2016a; Machado et al., 2017). This assessment was performed immediately pre-session (before the warm-up) in all training sessions. The pre-training session values of muscle soreness were considered DOMS from the preceding training sessions. The muscle soreness was elicited through a voluntary isometric contraction at 0 degrees of knee flexion and without load (Ferraresi et al., 2016b). Participants rated their perceived soreness related to the muscles involved in the isometric contraction (e.g., quadriceps, biceps femoris, and gastrocnemius) by placing a mark on the line that best corresponded to their perceived soreness and this assessment was quantified by measuring the distance in centimeters from the line on the left to the mark made by the participant.

Endurance Running Training Program

Both groups performed all training sessions on a 400 m outdoor track during the afternoon and evening due to the availability of the participants on Mondays, Wednesdays, and Fridays, which meant that the participants performed training sessions three times per week. If for any reason they missed a training session, they re-scheduled for another weekday (usually Tuesday or Thursday) in order to perform at least 90% of the training (22 of the 24 training sessions prescribed). Participants were recommended to keep the same time of day for their training and testing as strictly as possible to avoid circadian cycle influence.

Training sessions consisted of moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT). MICT and HIIT were both performed in the first (weeks 2–5) and last (weeks 7–10) training weeks. The MICT and HIIT were prescribed based on of V_{peak} and t_{lim} at V_{peak} determined during pre-training (week 1) and the exercise intensity was readjusted at week 6 (**Table 2**; Manoel et al., 2017). Training sessions were preceded by a 15 min warm-up, with 5 min of low self-selected intensity running, 5 min of stretching exercises, and 5 min of running at 60% of V_{peak}. After each session, participants had 10–15 min of cool-down. In total, both groups of participants performed 24 training sessions on non-consecutive days over a period of 8 weeks (weeks 2–5 and weeks 7–10). They completed 8 weeks of training with MICT and HIIT training every other day. All training sessions were monitored by session-RPE and training load was quantified by multiplying the whole RPE using the 10-point scale (CR-10) by its duration (Foster, 1998).

Photobiomodulation (PBM)

Photobiomodulation was performed by LED application with a double-blind control, in which neither the participant nor the principal researcher knew about who received LED application or not. Thus, during the application the participants from both groups remained standing wearing a headset with music and blindfolded to avoid identification of the experimental

TABLE 2 | MICT and HIIT used during training sessions for both PLA group and PBMG.

1st 4 weeks of training	
MICT	30 ± 2.5 min at 75 ± 4% of V_{peak}
HIIT	X[a] series at 100 ± 2% of V_{peak} with duration of 60% of t_{lim} and intervals de 60% do t_{lim}
2nd 4 weeks of training	
MICT	40 ± 2.5 min at 75 ± 4% of V_{peak}
HIIT	X[a] series a 100 ± 2% of V_{peak} with duration of 60% of t_{lim} and intervals of 60% do t_{lim}

[a] *The number of series of each participant was adjusted for a duration of 30 ± 2.5 min (in the 1st 4 weeks of training) and 40 ± 2.5 min (in the 2nd 4 weeks of training).*

TABLE 3 | Parameters of photobiomodulation.

Number of LED diodes: 104 (56 red diodes; 48 infrared diodes)
Wavelength: Mixed, 660 nm (red diodes) and 850 nm (infrared diodes)
Frequency: Continuous; 0–1500 Hz
Optical output (for each diode): 10 mW (660 nm) and 30 mW (850 nm)
LED spot size (each diode): 0.2 cm^2
LED cluster size: 46.3 cm^2
Power density (for each diode): 50 mW/cm^2 (660 nm) and 150 mW/cm^2 (850 nm)
Energy density (for each diode): 1.5 J/cm^2 (660 nm) and 4.5 J/cm^2 (850 nm)
Application time: 30 s at each point
Energy: 60 J at each application point (0.3 J from each 660 nm diode; 0.9 J from each 850 nm diode)
Number of irradiation points per leg: 5
Total energy delivered per leg: 300 J

group by audible and visual signals from the LED device. A second researcher controlled the groups, turning on the LED equipment (PBMG) or not (Placebo). The LED application was performed immediately before all training sessions (Vanin et al., 2016) and had a total duration of two and a half minutes (30 s per point, with application in both legs simultaneously), respecting the absence or presence of light emission for each group. For LED application, the method used was direct contact of the equipment with the site to be irradiated at an angle of 90° to the skin surface. This method has been previously used in other studies (Baroni et al., 2010; De Marchi et al., 2012).

The LED application was done on two regions of the quadriceps muscle, two regions of the biceps femoris, and one region of the gastrocnemius muscle, along the axis of muscle fibers distribution in both legs, and for 30 s each application point (De Marchi et al., 2012; Alves et al., 2014). LED was applied using an LED multidiode with cluster probe (THOR® DD2 control unit, THOR, London, United Kingdom) with 56 diodes of red light (660 nm) and 48 diodes of infrared light (850 nm). The LED device information and application parameters are presented in **Table 3**.

Statistical Analysis

Data are presented as means ± standard deviations (SD) and were analyzed using the Statistical Package for the Social Sciences 17.0 software (SPSS Inc., United States). Initially, the Shapiro-Wilk test was used to check the normality of the data distribution. The variables were analyzed using mixed ANOVA for repeated measures. In addition, the Mauchly's test of sphericity was applied and the Greenhouse-Geisser Epsilon correction was used when the sphericity criteria was not met. The analyses were completed with the Bonferroni *post hoc*. The main effects of group and the time in which the measurements were done and their interactions were also analyzed. The comparisons between groups at pre-training were made using the Student's t test for independent samples. Statistical significance was set at $P < 0.05$.

In addition to conventional statistical analysis, magnitude-based inference analysis was used for comparisons between groups (Batterham and Hopkins, 2006) using spreadsheet designed for sports science research[1]. The values are expressed as the standardized mean difference (Cohen's d ± confidence limits of 95%) (Cohen, 1988), which was calculated using pooled standard deviation as the denominator. The threshold values for ES were: <0.20 (trivial), 0.20–0.59 (small), 0.60–1.20 (moderate), >1.20 (large) (Hopkins et al., 2009). If the probabilities of the effect being substantially positive and negative were both >5%, the effect was reported as unclear, or, if not, the effect was clear. Thus, the changes were evaluated as follows: ≤1% most unlikely, >1–5% very unlikely, >5–25% unlikely, >25–75% possibly, >75–95% likely, >95–99% very likely, >99% most likely (Hopkins et al., 2009).

RESULTS

A total of 30 participants completed the study and there were no differences between groups at pre-training for all variables evaluated ($P > 0.05$). In addition, the mean training load was not different between groups for all MICT sessions[PLA = 304.7 ± 94.1 arbitrary unit (AU); PBMG = 317.9 ± 94.4 AU; $P = 0.705$] and for all HIIT sessions (PLA = 381.0 ± 94.7 AU; PBMG = 373.7 ± 93.7 AU; $P = 0.834$). In addition, the mean training loads calculated for each training week were not different between groups ($P > 0.05$).

The results from pre- and post-training for the variables obtained during the incremental test, constant load test, and 5-km running performance are presented in **Table 4**. The mixed ANOVA for repeated measures revealed a significant main effect of time on the V_{peak}, the HR_{max} from V_{peak} test, and from the t_{lim} test and t_{5-km}; however, a group effect and group-time interaction was not found for all variables ($P > 0.05$). The V_{peak} increased in both groups ($P < 0.001$) with a moderate ES for the comparison between pre and post-training (PLA = 0.82 and PBMG = 1.01), whereas the HR_{max} obtained in the incremental test decreased in both groups ($P < 0.001$). The t_{lim} did not change post-training for PLA and PBMG; however, the HR_{max} from the t_{lim} test decreased in the PBMG ($P < 0.001$). The 5-km running time (t_{5-km}) decreased in the PLA group and PBMG ($P < 0.001$) with a moderate ES for the comparison between pre and post-training (PLA = −0.82 and PBMG = −1.18) The magnitude based-inference analysis showed a *possibly positive* effect on V_{peak} and t_{5-km} and for PBMG compared to PLA group (**Table 4**). In addition, to illustrate a greater result in the PBMG for the t_{5-km}, **Figure 2** brings individual change of t_{5-km} from each group.

Table 5 brings the results from pre-session muscle soreness evaluated by VAS during 8 weeks of endurance running training (weeks 2–5 and weeks 7–10). It was not demonstrated a group effect, time effect and group-time interaction ($P > 0.05$) on the muscle soreness values. In addition, a moderate ES of 0.82 was found for the comparison between groups (1.2 ± 1.3 cm vs. PLA and 0.6 ± 0.6 cm for PBMG) in the third week of endurance running training (week 4). The magnitude based-inference analysis showed *unclear* effect on muscle soreness

[1]http://www.sportsci.org

TABLE 4 | Performance variables obtained from the V_{peak} test, t_{lim} test, and 5-km running performance for both groups at pre and post-training.

Variables	PLA (n = 15)			PBMG (n = 15)					Magnitude-based inference analysis		
	Pre-training	Post-training	Cohen's ES pre × post [CI 95%]	Pre-training	Post-training	Cohen's ES pre × post [CI 95%]	Time effect (F; P)	Interaction Time × group (F; P)	Cohen's ES [CI 95%]	% chances (positive/ trivial/negative)	Qualitative inference
V_{peak} (km · h^{-1})	13.4 ± 1.1	14.4 ± 1.0*	0.82 [0.65 – 1.00]	13.4 ± 1.2	14.6 ± 1.0*	1.01 [0.77 – 1.25]	178.39; <0.001	2.282; 0.142	0.21 [–0.08 – 0.50]	53/47/0	Possibly positive for PBMG
HR_{max} from V_{peak} test (bpm)	193 ± 9.3	187 ± 7.3*	–0.56 [–0.87 – –0.26]	196 ± 10.7	189 ± 9.8*	–0.56 [–0.68 – –0.44]	59.733; < 0.001	0.271; 0.606	–0.08 [–0.38 – 0.23]	4/76/20	Very Unlikely
RPE_{max} from V_{peak} test (6–20)	19.7 ± 0.6	19.9 ± 0.4	0.31 [–0.17 – 0.38]	19.7 ± 0.8	19.9 ± 0.3	0.31 [–0.20 – 0.82]	3.430; 0.075	0.070; 0.793	0.09 [–0.60 – 0.78]	37/43/20	Unclear
t_{lim} (min)	6.6 ± 0.7	6.9 ± 1.1	0.46 [–0.62 – 1.53]	6.6 ± 1.1	6.8 ± 1.2	0.23 [–0.47 – 0.92]	1.290; 0.266	0.013; 0.910	–0.06 [–1.18 – 1.06]	32/28/40	Unclear
HR_{max} from t_{lim} test (bpm)	188 ± 9.6	186 ± 8.0	–0.18 [-.048 – 0.11]	192 ± 10.6	187 ± 9.5*	–0.42 [–0.56 – –0.28]	17.983; <0.001	3.393; 0.076	–0.27 [–0.57 – 0.03]	0/32/67	Possibly positive
RPE_{max} form t_{lim} test (6–20)	19.9 ± 0.3	19.8 ± 0.4	–0.49 [–1.54 – 0.56]	19.9 ± 0.4	19.9 ± 0.5	0.00 [–0.59 - 0.59]	0.606; 0.443	0.606; 0.443	0.41 [–0.64 – 1.47]	66/22/12	Unclear
t_{5-km} (min)	27.0 ± 3.3	24.1 ± 2.5*	–0.82 [–1.04 – –0.61]	27.6 ± 3.0	23.9 ± 2.2*	–1.18 [–1.48 – –0.88]	135.46; <0.001	2.218; 0.148	–0.26 [–0.61 – 0.10]	1/37/63	Possibly positive for PBMG

*PLA, placebo group; PBMG, PBM group; ES, effect size; V_{peak}, peak running velocity; HR_{max}, maximal heart rate; RPE_{max}, maximal rating of perceived exertion; t_{lim}, time limit; t_{5-km}, 5-km time. *$P < 0.05$ compared with pre-training in the same group.*

values in all training weeks, except for the third week that presented a qualitative inference of *very unlikely* (**Table 5**).

DISCUSSION

The present study aimed to investigate the influence of endurance running training associated with PBM on endurance performance variables and muscle soreness in untrained men. The main finding was that although the magnitude-based inference analysis demonstrated a *possibly positive* effect on V_{peak} and t_{5-km} and for PBMG compared to PLA group and a moderate ES on attenuation in muscle soreness in the third week of endurance running training, no significant difference were found between PBMG and PLA interventions.

The data obtained from the incremental test demonstrated that V_{peak} and t_{5-km} improved in both groups (pre to post-training). Additionally, our findings highlighted that, despite the differences between groups not being statistically significant, PBM had a *possibly positive* effect on improving performance variables compared to PLA. Previous transversal studies reported better V_{peak} improvements with PBM conditions compared to that under the PLA conditions (Dellagrana et al., 2018; Mezzaroba et al., 2018). Another important physiological adaptation observed in our study was the decrease of the HR_{max} values obtained from the maximal tests in both groups; it was postulated that HR_{max} can be altered by 3–7% with an ES of –0.48 after aerobic training (Zavorsky, 2000).

Other human studies also found that PBM caused greater improvements in variables related to aerobic capacity after training than PLA or control group (Paolillo et al., 2011, 2013; Miranda et al., 2018), however, none of them investigated running performance on a time trial. Miranda et al. (2018) examined the effects of PBM applied at different moments (before and/or after each training session) and concluded that PBM applied before and after sessions can improve variables evaluated during progressive cardiopulmonary tests on a treadmill (e.g., VO_2 and time to exhaustion) when compared to the PLA.

Studies by Paolillo et al. (2011, 2013) investigated the effects of PBM application in post-menopausal women during training sessions and found positive effects of PBM on muscle power evaluated by isokinetic testing (Paolillo et al., 2011), maximal performance during the Bruce protocol and fast post-exercise recovery assessed by the time in which the HR and blood pressure returned to baseline values (Paolillo et al., 2013).

In contrast, Vieira et al. (2012) examined the effects of a cycle ergometer training program with PBM applied after each training session on isokinetic variables in healthy women. The authors found that only the low-level laser group had a significant decrease in the fatigue index of the knee extensor muscles. However, it is important to note that these studies (Paolillo et al., 2011, 2013; Vieira et al., 2012) did not perform evaluations or tests whose results could be extrapolated to running performance or assess aerobic capacity; thus, our study is the first to present endurance running-related variables. Furthermore, using animal models, Guaraldo et al. (2016) investigated the effects of

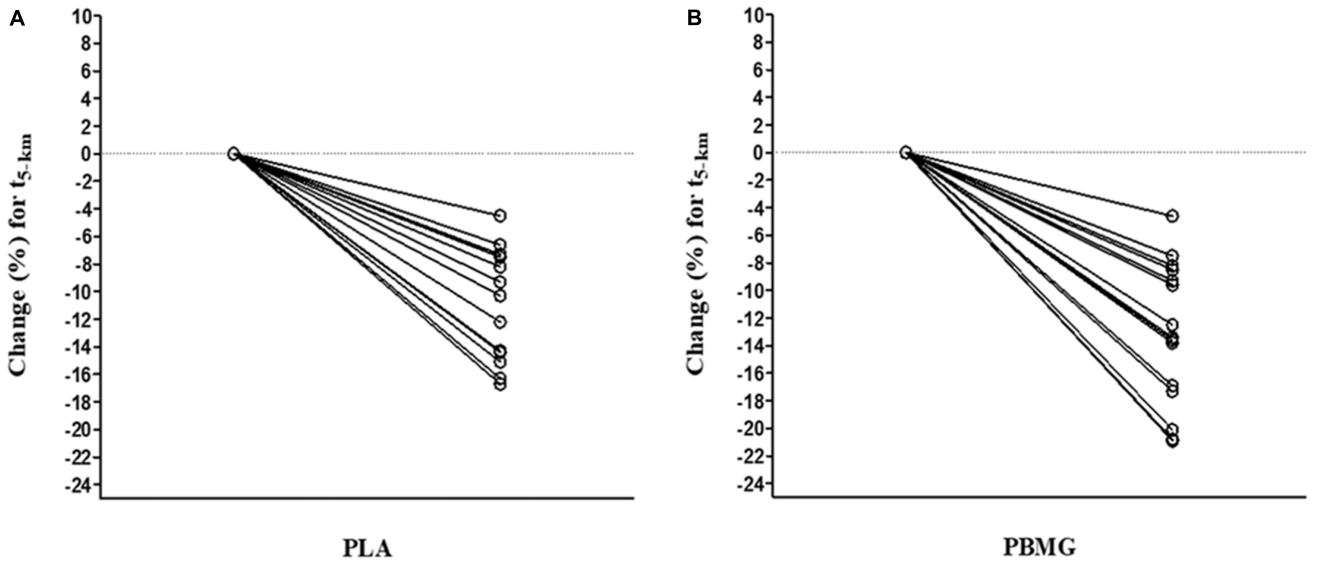

FIGURE 2 | Individual change of time to complete 5 km (% change of t_{5-km}) in PLA group and PBMG. **(A)** Individual change in the PLA group. **(B)** Individual change in the PBM group (n = 15 each group).

6 weeks of swimming aerobic training in conjunction with low level laser application before all training sessions, and reported that the training group which received low level laser presented greater improvements in aerobic performance, represented by the VO_{2max} and V_{peak} variables, compared to the other control groups.

Based on our results and previous data that showed the positive effects of PBM application on aerobic parameters (Dellagrana et al., 2018; Lanferdini et al., 2018a; Miranda et al., 2018) we suggest that PBM can be an ergogenic aid with positive effects on oxidative metabolism. There are several possible physiological mechanisms to explain these positive effects (Leal Junior et al., 2015); for example, the increased blood flow and the increased activity of oxidative enzymes such as cytochrome c oxidase inside the mitochondria leading to increased ATP synthesis (Albuquerque-Pontes et al., 2015; Ferraresi et al., 2015).

TABLE 5 | Muscle soreness responses evaluated by visual analogic scale (VAS) during weeks of running training.

Training weeks	PLA (n = 15) (cm)	PBMG (n = 15) (cm)	Cohen's ES [CI 95%]	% chances (positive/ trivial/ negative)	Qualitative inference
2	1.1 ± 1.4	0.9 ± 0.8	0.22 [−0.65 – 1.09]	52/32/16	Unclear
3	1.2 ± 1.1	1.0 ± 0.8	0.26 [−0.54 – 1.05]	56/32/12	Unclear
4	1.2 ± 1.3	0.6 ± 0.6	0.82 [−0.43 – 2.07]	85/10/5	Very Unlikely
5	0.8 ± 1.0	1.0 ± 0.8	−0.16 [−0.84 – 0.53]	14/41/45	Unclear
7	1.0 ± 0.9	1.2 ± 1.2	−0.15 [−0.77 – 0.47]	12/45/43	Unclear
8	0.8 ± 0.9	1.0 ± 1.0	−0.14 [−0.71 – 0.44]	11/48/41	Unclear
9	0.9 ± 0.7	0.8 ± 0.7	0.08 [−0.48 – 0.65]	33/51/15	Unclear
10	0.9 ± 1.0	0.7 ± 0.9	0.20 [−0.40 – 0.79]	50/42/9	Unclear

PLA, placebo group; PBMG, PBM group. No statistical differences.

Concerning the muscle soreness results (i.e., DOMS), although the comparisons did not demonstrate statistical differences between groups, a moderate ES of 0.82 was observed for the comparison between groups in the third week of endurance running training (**Table 5**). Previous studies also reported that PBM applied before maximal exercise of knee eccentric contractions (Antonialli et al., 2014) and after running time trials (Hausswirth et al., 2011; Machado et al., 2017) had a greater ability to reduce DOMS compared to PLA conditions. In contrast, using the low-level laser application before a maximal lower limb resistance exercise, Baroni et al. (2010) did not demonstrate significant differences between the DOMS responses in the PLA group and the low level laser group. Only Ferraresi et al. (2016a) evaluated DOMS during a 12-week strength training program by applying the visual analog scale 24 h after the first, 13th, 25th, and 36th training sessions; lower visual analog scale scores were reported by the participants with low level laser application after all sessions compared to those under PLA conditions.

Despite the important findings, the present study had some limitations; for example, the lack of another control group with only the PBM application and without endurance running training. Furthermore, it is important to note that because different PBM parameters (e.g., wavelength, time to apply PBM, dosage) influence the responses of different variables (Lanferdini et al., 2018b; Miranda et al., 2018), we suggest that the dosage and the time of application used could influence the magnitude of our results. For example, Ferraresi et al. (2016a) in a recent review on PBM in human muscle tissue, reported some evidence in favor of applying PBM before exercise in association with training programs, as used in our study, to increase performance, limit muscle damage and prevent pain from 1 h until 72–96 h after exercise. However, concerning the time that elapses between

application of the PBM and the exercise performance, it was demonstrated that PBM applied 3–5 min before a bout of exercise may not actually be the best time point and that a longer duration before exercise may be more favorable (Ferraresi et al., 2016a). However, it is important to emphasize that this assumption is not completely clear in the literature and that further investigations could answer this question (Ferraresi et al., 2016a).

Therefore, we concluded that although the magnitude-based inference analysis demonstrated a *possibly positive* effect on V_{peak} and t_{5-km} and for PBMG compared to PLA group and a moderate ES on attenuation in muscle soreness in the third week of endurance running training, non-significant differences were found between the PBMG and PLA interventions.

PRACTICAL APPLICATION

In terms of practical application, these results demonstrated that, despite being non-significant, there is the possibility for using PBM as strategy for practitioners and recreational runners during endurance running training to optimize their adaptations. In sports performance small improvements can determine the winner or loser. However, further research is required to examine the chronic effects of endurance running training with different PBM doses and analyze other biomarkers related to muscle soreness, inflammation, and oxidative metabolism.

AUTHOR CONTRIBUTIONS

CP and FM designed the work and acquired the data. CP, AZ, and FM contributed to the analysis and interpretation of the data. CP and FM drafted the work. AZ revised it critically. All authors approved the final version of the manuscript.

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A Meta-Analysis of the Effects of Foam Rolling on Performance and Recovery

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Foam rolling is thought to improve muscular performance and flexibility as well as to alleviate muscle fatigue and soreness. For this reason, foam rolling has become a popular intervention in all kinds of sport settings used to increase the efficiency of training or competition preparation as well as to speed post-exercise recovery. The objective of this meta-analysis was to compare the effects of foam rolling applied *before* (pre-rolling as a warm-up activity) and *after* (post-rolling as a recovery strategy) exercise on sprint, jump, and strength performance as well as on flexibility and muscle pain outcomes and to identify whether self-massage with a foam roller or a roller massager is more effective. A comprehensive and structured literature search was performed using the PubMed, Google Scholar, PEDro, and Cochrane Library search engines. Twenty-one studies were located that met the inclusion criteria. Fourteen studies used pre-rolling, while seven studies used post-rolling. Pre-rolling resulted in a small improvement in sprint performance (+0.7%, $g = 0.28$) and flexibility (+4.0%, $g = 0.34$), whereas the effect on jump (−1.9%, $g = 0.09$) and strength performance (+1.8%, $g = 0.12$) was negligible. Post-rolling slightly attenuated exercise-induced decreases in sprint (+3.1%, $g = 0.34$) and strength performance (+3.9 %, $g = 0.21$). It also reduced muscle pain perception (+6.0%, $g = 0.47$), whereas its effect on jump performance (−0.2%, $g = 0.06$) was trivial. Of the twenty-one studies, fourteen used foam rollers, while the other seven used roller massage bars/sticks. A tendency was found for foam rollers to offer larger effects on the recovery of strength performance (+5.6%, $g = 0.27$ vs. −0.1%, $g = -0.01$) than roller massagers. The differences in the effects between foam rolling devices in terms of pre-rolling did not seem to be of practical relevance (overall performance: +2.7 %, $g = 0.11$ vs. +0.4%, $g = 0.21$; flexibility: +5.0%, $g = 0.32$ vs. +1.6%, $g = 0.39$). Overall, it was determined that the effects of foam rolling on performance and recovery are rather minor and partly negligible, but can be relevant in some cases (e.g., to increase sprint performance and flexibility or to reduce muscle pain sensation). Evidence seems to justify the widespread use of foam rolling as a warm-up activity rather than a recovery tool.

Keywords: rolling massage, sprint, jump, strength, flexibility, muscle pain

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INTRODUCTION

In recent years, foam rolling has become a common practice in all kinds of sport settings and is highly regarded within the strength and conditioning field for increasing the efficiency of training or competition preparation and for accelerating post-exercise recovery (Healey et al., 2014; Jones et al., 2015; Monteiro and Neto, 2016). Foam rolling (FR) is a form of self-massage in which the targeted musculature is rolled and compressed utilizing a FR device (Peacock et al., 2014). Common FR tools include the foam roller and various types of roller massage bars/sticks, which come in several sizes and foam densities.

With foam rollers, athletes use their bodyweight to apply pressure to the soft tissues during the rolling motion, while roller massagers are applied with the upper extremities to the target muscles (Cheatham et al., 2015). The motions place both direct and sweeping pressure on the soft tissue, stretching it and generating friction between it and the FR device. Consequently, FR can be considered a form of self-induced massage because the pressure that the roller exerts on the muscles resembles the pressure exerted on the muscles through manual manipulation by the user himself (Pearcey et al., 2015). Some reasons why self-massage through FR has become a popular intervention technique used by both elite athletes and recreationally active individuals may be its affordable, easy, and time-efficient applicability as well as its close relationship to massage, which in turn is believed to benefit athletes by enhancing performance and recovery (Weerapong et al., 2005).

However, despite the popularity of FR, no consensus exists on its benefits (Cheatham et al., 2015; Pearcey et al., 2015). This may be partly due to the fact that few studies have examined the underlying physiological mechanisms of FR. Nevertheless, the potential effects of FR have been attributed to mechanical, neurological, physiological, and psychophysiological parameters (Aboodarda et al., 2015; Cavanaugh et al., 2017; Monteiro et al., 2018; Phillips et al., 2018). The mechanical mechanisms are comprised of a number of sub-mechanisms, such as reduction in tissue adhesion, altered tissue stiffness, and thixotropic responses (Aboodarda et al., 2015; Kelly and Beardsley, 2016). Within neurological models, it is theorized that FR may potentiate analgesic effects and muscular recovery by mediating pain-modulatory systems (e.g., nociceptor and mechanoreceptor sensitivity and/or diffuse noxious inhibitory control) (Cavanaugh et al., 2017; Jo et al., 2018). The proposed physiological mechanisms are increased blood flow and parasympathetic circulation, as well as inflammatory responses and associated trigger-point break down (Aboodarda et al., 2015; Kelly and Beardsley, 2016). Psychophysiological responses may include improved perceptions of well-being and recovery due to the increase of plasma endorphins, decreased arousal level, an activation of the parasympathetic response and/or placebo effect (Weerapong et al., 2005; Phillips et al., 2018).

Due to the potential underlying physiological mechanisms, it is believed that FR can improve both acute athletic performance as well as recovery from an intensive bout of physical activity (Cheatham et al., 2015). Therefore, studies on the effects of FR have either determined whether massage-like mechanical pressure with a foam roller or roller massager *prior* to activity affects muscle performance (i.e., pre-rolling as a warm-up activity), or whether FR *after* an intense bout of exercise enhances muscle recovery (i.e., post-rolling as a recovery tool). Unfortunately, the literature on FR that does exist is equivocal and insufficient, which is why the widespread use of FR is to date not fully supported by the available empirical data. In addition, there is currently no meta-analysis that has evaluated the literature and calculated the pooled effects of FR. This creates a gap in the translation from research to practice for strength and conditioning coaches who use FR tools and recommend these products to their athletes (Cheatham et al., 2015). Accordingly, the aims of the study were to conduct a meta-analytical review of the effects of pre-rolling and post-rolling on performance, flexibility, and muscle pain outcomes in healthy and physically active individuals and to identify whether self-massage with a foam roller or a roller massager is more effective.

METHODS

Search Strategy

A comprehensive and structured search of articles were performed using the PubMed, Google Scholar, PEDro, and Cochrane Library search engines. Different sets of seven key terms ("self-massage," "foam rolling," "roller massage," "roller massager," "self-myofascial release," "performance," "recovery") were combined by Boolean logic ("AND," "OR"), and the results were limited to human subjects that were healthy and physically active as well as to articles written in English. Each database was searched from the earliest available article up to December 2017. We also searched the reference lists of all incoming articles and extracted the appropriate publications. From the 954 abstracts reviewed, 110 potentially suitable articles were identified (**Figure 1**).

Selection Criteria

The selection of articles for inclusion in this meta-analysis was based on the following criteria. First, only publications that appeared in an international, peer-reviewed scientific journal were selected. Second, a FR intervention had to have been done as part of the analysis, regardless of which type of FR device was used for the intervention. Third, the FR intervention had to have been used either as a warm-up or a recovery routine. Fourth, before and after the FR intervention, measurements of performance, flexibility, and/or muscle pain outcomes had to have been conducted. Fifth, there had to have been a control condition, where athletes were subdivided either as their own controls or randomly into an intervention and control group. The first author was responsible for the study selection. After the selection process, all studies were discussed among three authors. In case of disagreement about the inclusion of a study, a voting process was used to determine if a study should be included or not. **Figure 1** provides a flow chart of the literature search.

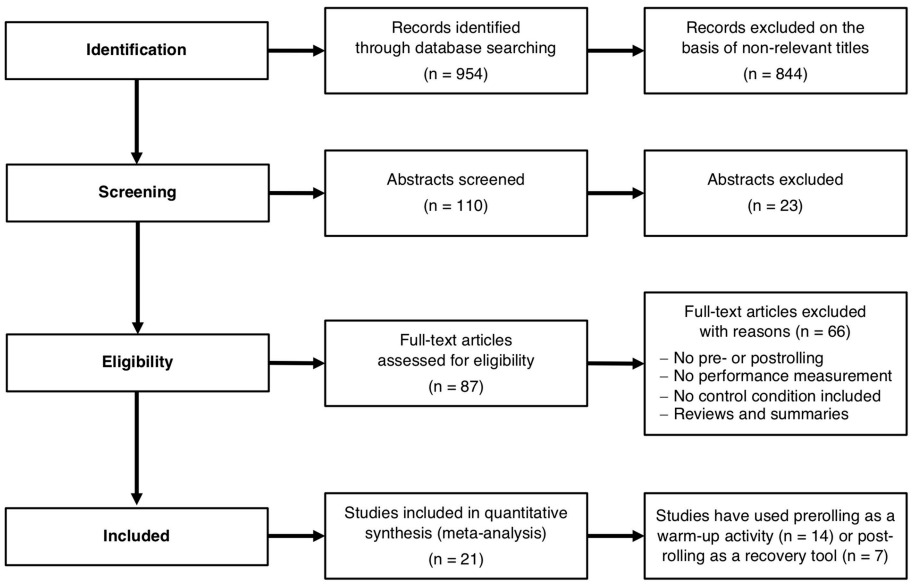

FIGURE 1 | Overview of the selection process for the studies included in this meta-analysis. *N* indicates the number of studies.

Classification and Quality Assessment of the Studies

The inclusion criteria were met by 21 studies, 14 of which used pre-rolling as an exercise warm-up routine, while seven used post-rolling to enhance recovery mechanisms. For further analysis, the studies were categorized according to the type of FR device used (i.e., foam roller or roller massager). Several studies were included more than once in the analysis. This was the case, for example, when several follow-up examinations were carried out (e.g., after 24 and 48 h) or several types of performance indicators were measured. The Cochrane risk of bias tool (Higgins et al., 2011) was used to assess the quality of each included study.

Statistical Analysis and Assessment of Effect Sizes

A standardized form was used to extract all relevant data and important methodological details from the studies. For each study, relative changes in performance, flexibility, and muscle pain were calculated for the treatment condition and the control condition. By subtracting the two values, the net effect of the treatment on changes in performance, flexibility, and muscle pain was calculated. Effect sizes (ES, Hedges' *g* values) were estimated according to the following formula:

$$g = c_P \frac{\left(M_{post,\ foam\ rolling} - M_{pre,\ foam\ rolling}\right)\ \left(M_{post,\ control} - M_{pre,\ control}\right)}{SD_{pre}}$$

where c_p is a bias factor recommended for small sample sizes (Morris, 2008), $M_{pre,\ foam\ rolling}$, $M_{post,\ foam\ rolling}$, $M_{pre,\ control}$, and $M_{post,\ control}$ are the respective mean values of performance, flexibility, and muscle pain, and SD_{pre} is the pooled pre-test standard deviation. This method was chosen because it has been suggested for the ES calculation of controlled pre-test-post-test study designs in meta-analyses (Higgins et al., 2011). Negative effects on performance, flexibility, and muscle pain are marked with a minus sign ES deviations and 95% confidence intervals were calculated as described by Borenstein et al. (2011). In addition, the ES was converted to percentiles as described by Coe (2002). For example, an ES of 0.5 means that the score of the average subject in the FR group is 0.5 standard deviations above the average subject in the control group, and hence exceeds the score of 69%. The value of 69% indicates that the average subject in the FR group would score higher than 69% of the control group that was initially equivalent.

If more than one parameter of performance was measured, a combined effect was calculated by averaging the relative change and the ES, and calculating the combined ES variance (Borenstein et al., 2011). In this context, a correlation coefficient of 0.9 was used based on the values reported in studies by Harbo et al. (2012) and Nuzzo et al. (2008).

The total results for the analyzed conditions were determined by the calculation of inverse-variance-weighted *g*-values (Borenstein et al., 2011). For nine of the twenty-one studies, more than one result was included in the analysis because several follow-up examinations were performed (e.g., after 24 and 48 h). In these cases, the respective results were combined as described above, assuming correlation coefficients of 0.9. To combine the different types of sprint, jump,

and strength performances, a correlation coefficient of 0.6 was used.

The data of each individual study as well as weighted-average values are presented in forest plots. The magnitude of g was categorized according to Cohen (1992) (i.e., 0.00–0.19 = negligible effect, 0.20–0.49 = small effect, 0.50–0.79 = moderate effect, ≥0.80 = large effect). The values are given with 95% confidence intervals to express the uncertainty of the true effect. ES can be interpreted as evidence of the benefit of pre-rolling or post-rolling when the average and 95% confidence intervals are above zero.

RESULTS

Included Studies

Twenty-one studies with a total number of 454 subjects met the inclusion criteria, fourteen of which used pre-rolling as an exercise warm-up strategy (n = 306), while seven used post-rolling to enhance recovery (n = 148). Of the twenty-one studies, fourteen used foam rollers, while the other seven used roller massage bars/sticks. The characteristics of the included studies are summarized in **Supplementary Table 1** (studies using pre-rolling) and **Supplementary Table 2** (studies using post-rolling). The calculated ES for the effects of FR on performance, flexibility, and muscle pain outcomes are shown in **Figures 2–9**.

The use of the Cochrane risk of bias tool (Higgins et al., 2011) showed a comparable bias level for most of the included studies. Regarding selection bias, almost all studies mentioned a random assignment of their subjects into either a FR or control group. Accordingly, the risk of selection bias was considered low. However, in the research article by Sullivan et al. (2013), it was not explicitly stated how the participants were assigned to the different groups. Here, the selection bias remained unclear. The blinding of the subjects was not possible due to the nature of the FR technique. Consequently, the risk of a placebo bias was comparatively high. Mikesky et al. (2002) imposed blinding on researchers and participants during testing. Griefahn et al. (2017) stated that only the examiners were blinded during outcome assessments, whereas Cheatham et al. (2017) imposed blinding only on subjects. None of the other research articles provided any information on blinding. Regarding attrition bias, only one study reported on drop-outs (n = 2; Bushell et al., 2015).

Furthermore, to minimize possible learning effects, twelve studies (Mikesky et al., 2002; MacDonald et al., 2013; Healey et al., 2014; Jones et al., 2015; Pearcey et al., 2015; Zorko et al., 2016; Cavanaugh et al., 2017; Cheatham et al., 2017; D'Amico and Gillis, 2017; Grabow et al., 2017; Casanova et al., 2018; Phillips et al., 2018) provided participants an organized familiarization with performance tests prior to the first testing session; in nine studies (MacDonald et al., 2013; Peacock et al., 2014; Jones et al., 2015; Pearcey et al., 2015; Cheatham et al., 2017; D'Amico and Gillis, 2017; Grabow et al., 2017; Casanova et al., 2018; Phillips et al., 2018), the participants were instructed to avoid strenuous exercise before and/or during the experimental period; in seven studies (MacDonald et al., 2013; Pearcey et al., 2015; Cavanaugh et al., 2017; D'Amico and Gillis, 2017; Grabow et al., 2017; Rey et al., 2017; Phillips et al., 2018), diet control was mentioned and/or the subjects were asked to maintain their normal dietary intake and to refrain from nutritional supplements and alcohol intake during the experimental period; and in six studies (Mikesky et al., 2002; Macdonald et al., 2014; Zorko et al., 2016; Cavanaugh et al., 2017; Cheatham et al., 2017; Rey et al., 2017), it was explicitly stated that each participant was always examined at approximately the same time of day.

Overall, based on the quality assessment of the studies included in this meta-analysis, none of the studies was considered to have a high risk of bias, except for the high risk of placebo bias that can be inevitable for this kind of studies.

Pre-rolling

Pre-rolling resulted in a small improvement in sprint performance (+0.7%, g = 0.28) and flexibility (+4.0%, g = 0.34), whereas the effect on jump (−1.9%, g = 0.09) and strength performance (+1.8%, g = 0.12) was negligible. The weighted-average overall performance change due to pre-rolling was +1.5% (g = 0.20). Of the fourteen studies investigating the effects of pre-rolling on performance and flexibility, 10 (MacDonald et al., 2013; Healey et al., 2014; Peacock et al., 2014; Bushell et al., 2015; Jones et al., 2015; Murray et al., 2016; Cheatham et al., 2017; Griefahn et al., 2017; Sagiroglu et al., 2017; Phillips et al., 2018) used a cylindrical foam roller (overall performance: +2.7%, g = 0.11; flexibility: +5.0%, g = 0.32), while the remaining four studies (Mikesky et al., 2002; Sullivan et al., 2013; Cavanaugh et al., 2017; Grabow et al., 2017) used a type of roller massage bar/stick (overall performance: +0.4%, g = 0.21; flexibility: +1.6%, g = 0.39).

Post-rolling

Post-rolling slightly attenuated exercise-induced decreases in sprint (+3.1%, g = 0.34) and strength performance (+3.9%, g = 0.21). It also reduced muscle pain perception (+6.0%, g = 0.47), whereas the effect on jump performance (−0.2%, g = 0.06) was trivial. The weighted-average overall performance change in response to post-rolling was +2.0% (g = 0.19). The effects of post-rolling using a cylindrical foam roller (strength performance: +5.6%, g = 0.27; muscle pain: +6.0%, g = 0.55) were examined by four studies (Macdonald et al., 2014; Pearcey et al., 2015; Zorko et al., 2016; Fleckenstein et al., 2017), while the remaining three studies (D'Amico and Gillis, 2017; Rey et al., 2017; Casanova et al., 2018) used a type of roller massage bar/stick (strength performance: −0.1%, g = −0.01; muscle pain: +5.8%, g = 0.20).

DISCUSSION

There is a growing body of literature examining the use of FR as a warm-up activity (i.e., pre-rolling) or as a recovery strategy (i.e., post-rolling); however, the effectiveness of FR is still in question in both scenarios. The variation in methodological design, combined with the differences in FR intervention, exercise modality, and training status of the populations investigated, has perhaps contributed to the apparently inconsistent findings. This study used a meta-analytical approach to (1). Explore whether the use of pre-rolling and post-rolling are effective tools to improve

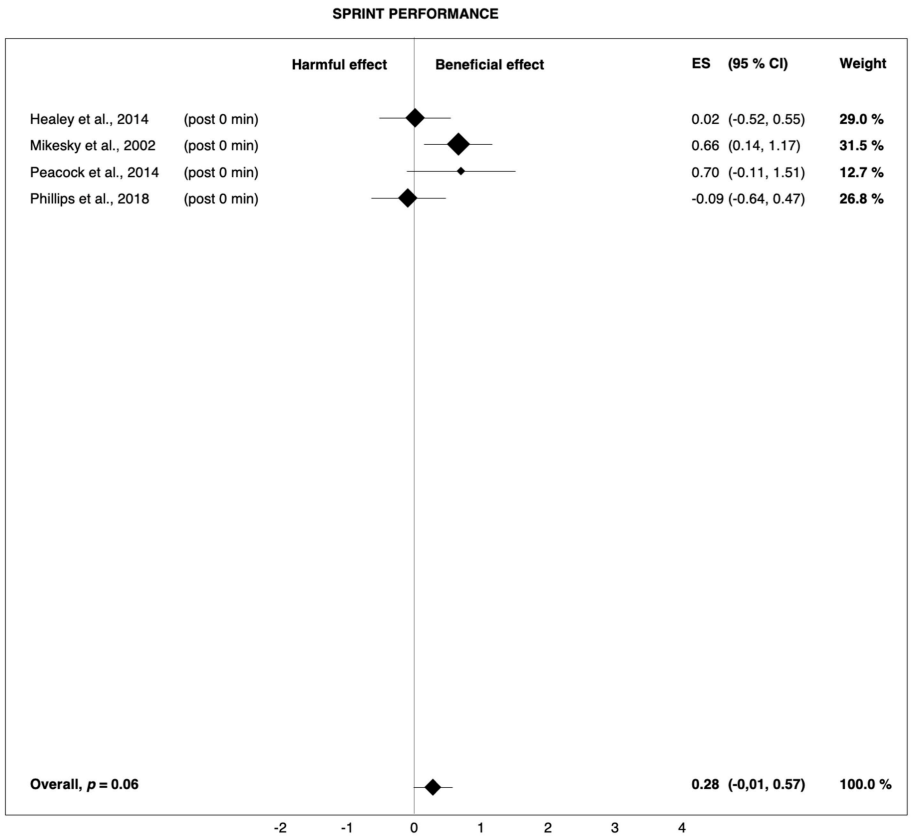

FIGURE 2 | Forest plot summarizing the effects of pre-rolling on sprint performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

sprint, jump, and strength performance as well as flexibility and muscle pain outcomes and (2). To identify whether self-massage with a foam roller or a roller massager is more effective. The results indicate that pre-rolling causes a small acute improvement in sprint performance and flexibility, while its effect on jump and strength performance was negligible. Second, when foam rolling is used as a recovery tool, participants experience slightly reduced decrements in sprint and strength performance and a small reduction in the severity of muscle pain. Third, a tendency was found for foam rollers to offer larger recovery effects than roller massagers, while the differences in the effects between FR devices in terms of pre-rolling did not seem to be of practical relevance.

Pre-rolling

Relevant effect sizes for average improvements in performance due to pre-rolling were found only for sprinting. The total Hedges' *g* of 0.28 (**Figure 2**) indicates that with the use of pre-rolling, 58% of the population is likely to experience increased sprint performance (Coe, 2002). However, the average percentage improvement in sprint performance was only 0.7%. In this context, Hopkins et al. (1999) defined the smallest worthwhile performance enhancement (i.e., in the case of the present study, the minimum improvement making pre-rolling worthwhile) as the value increasing the chance of victory for an athlete by 10%. Based on this definition, they concluded that an enhancement as small as 0.3–0.4 of the within-athlete standard deviation known as the coefficient of variation (CV) is important for at least the best athletes. For sprinting, Malcata and Hopkins (2014) as well as Tanner and Gore (2013) reported CVs of ∼0.8%. The smallest important change in sprint performance thus corresponds to ∼0.3%. This shows that although the effect size was rather small from a purely statistical point of view, when within-athlete variability is taken into account, the average improvement in sprint performance induced by pre-rolling is within a range that is relevant for elite athletes.

However, for recreational athletes, a change in sprint performance as small as ∼0.3% may be barely noticeable due to a likely greater within-athlete variability. In this case, the minimum worthwhile enhancement in sprint performance would be greater than the change in sprint performance induced by pre-rolling. Consequently, the effects of pre-rolling on sprint performance seem to be more relevant for elite athletes, while it is possible that recreationally active individuals may not benefit substantially from pre-rolling. Furthermore, the small average overall effect size for sprint performance is based on only four studies and is mainly due to the studies conducted by Mikesky et al. (2002)

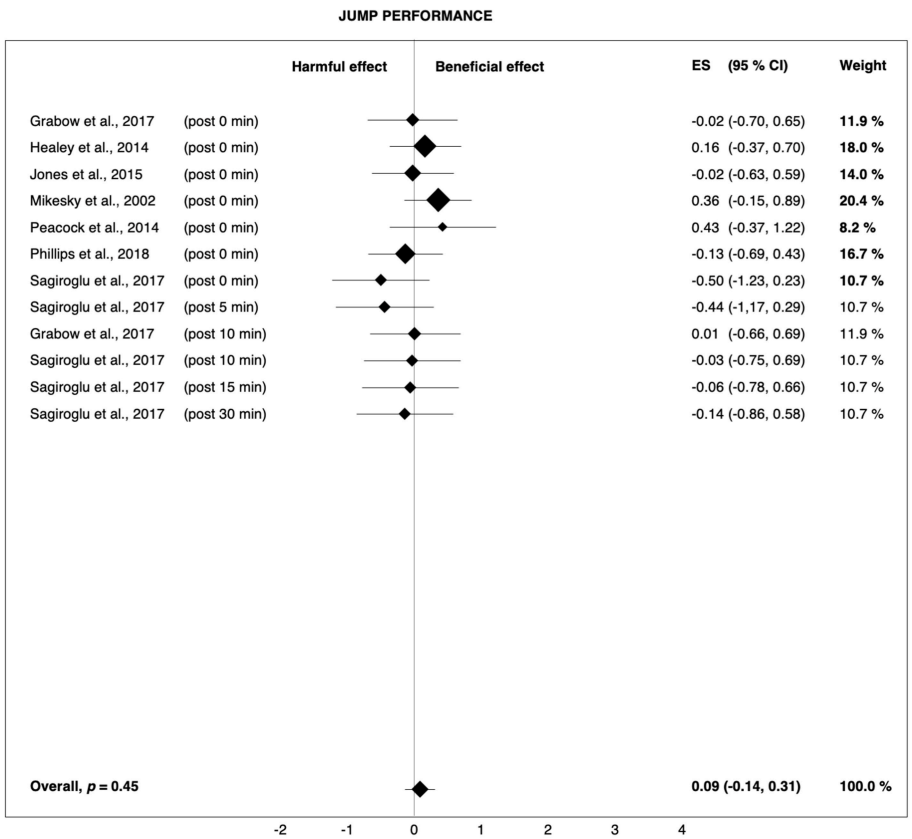

FIGURE 3 | Forest plot summarizing the effects of pre-rolling on jump performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

and Peacock et al. (2014) showing effect sizes of 0.66 and 0.70, respectively. Therefore, it can be speculated that sprint performance does not, per se, benefit more from pre-rolling than the other performance components and that the slightly larger effect sizes found for sprinting are, rather, due to methodological aspects and/or aberrations in the data. Consequently, sprint performance results should be interpreted with caution, as the number of available studies was limited and only two of them showed a clear positive effect.

Several potential physiological effects of FR could explain the trend of improved sprint performance following pre-rolling. One possibility is that FR immediately prior to sprinting breaks up what are known as barrier trigger-points (Bonci and Oswald, 1993). These are identified as inflexible bands of muscle containing knots resulting from muscle spasm. Barrier trigger-points are typically painless and can result in muscle weakness, muscle fatigue, and muscle stiffness (Mikesky et al., 2002). All of these factors could obviously have an impact on sprinting. FR may break up these trigger-points. Decreasing muscle spasms would not only decrease the amount of internal resistance to muscle movement, but also enable the previously spasmodic tissue to contribute to the athletic activity being performed (Mikesky et al., 2002). However, this explanation remains highly speculative, and there is no concrete evidence proving that the release of trigger-points makes FR effective.

Alternative explanations for acute benefits in performance could be a potential warm-up and/or placebo effect. Self-massage with a foam roller necessitates supporting one's partial body weight with the upper body, similar to with planking exercises. These exercises primarily involve isometrically holding the body in a prone position and are typically used to strengthen the core. Isometric exercises such as planking are in some ways similar to FR because the body position is maintained in an analogous manner, requiring similar isometric actions to support one's body weight. Planking in turn would have a warm-up effect through possible increased skin and muscle temperature, increased blood flow, and enhanced flexibility/mobility (Healey et al., 2014). Moreover, the observed effect of FR might have been confounded by a potential psychosomatic disorder, meaning the subjects may have performed better following FR treatment simply because they believed it would improve their performance (Jo et al., 2018).

It should also be noted that sprint performance mainly depends on muscle strength and neuromuscular coordination. However, similar to the findings of Cheatham et al. (2015), the effects of pre-rolling on jump ($g = 0.09$) and strength performance ($g = 0.12$) were negligible (**Figures 3**, **4**). Thus,

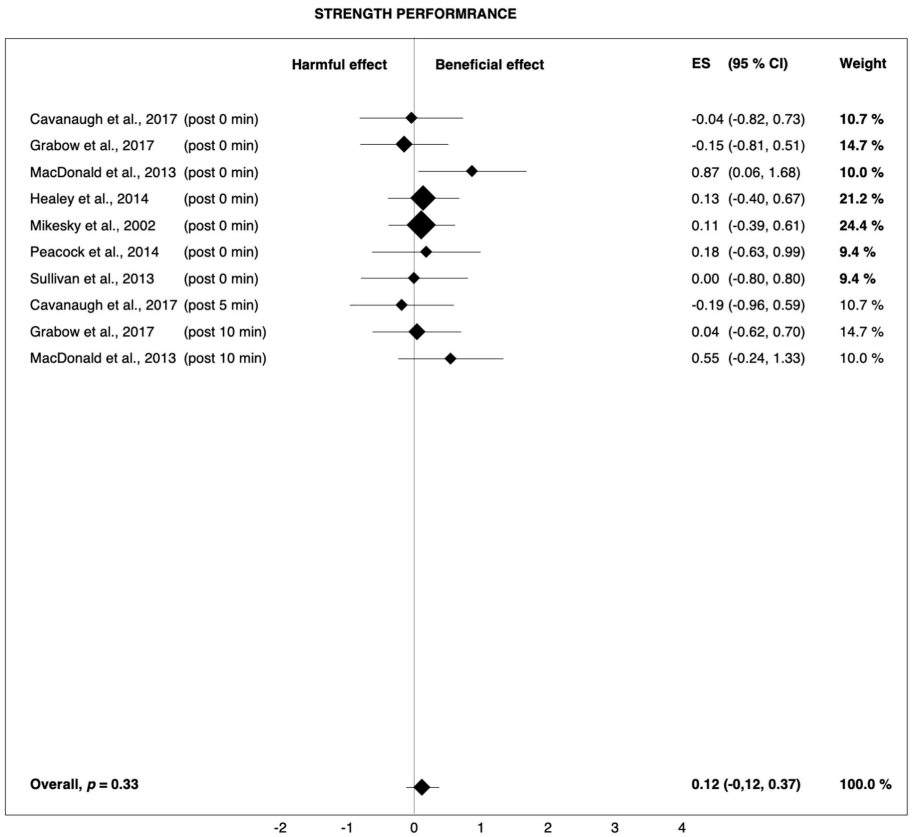

FIGURE 4 | Forest plot summarizing the effects of pre-rolling on strength performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

it cannot be definitively determined if the observed effects on sprint performance were really due to FR. For example, it is possible that the trend toward improved sprint performance was the result of a placebo effect, as FR can hardly be blinded. The reason the other measures of physical performance did not show trends toward improvements is unclear. Mikesky et al. (2002) suggested that the measures of jump capacity and strength are rather one-dimensional when compared with the complexity and coordination required to sprint. As such, large improvements in more isolated tasks are not as remarkable, while the combined effects on more complex, repetitive tasks become more evident. Although it is a coordinated task, jumping is so brief in duration, at least compared to sprinting, that any combined improvements are not afforded a chance to be revealed.

The largest average effect of pre-rolling was related to flexibility. The overall Hedges' *g* of 0.34 (**Figure 5**) indicates that 62% of the population will experience short-term improvements in flexibility when using pre-rolling as a pre-exercise warm-up (Coe, 2002). Cheatham et al. (2015) assumed that the effects of FR on flexibility would be attributed to the altered viscoelastic and thixotropic properties of the fascia (i.e., remobilizing the fascia back to a gel-like state), as well as increases in intramuscular temperature and blood flow due to the friction created by the foam roller and the mechanical breakdown of scar tissue. However, this is merely speculation by the authors and is not based on direct scientific observations. In addition, hypotheses related to the mechanisms of pressure-associated changes in myofascial properties have been questioned. The pressure that is required to deform firm fascial tissue is greater than the physical range that is usually achieved by FR (Schleip, 2003). Therefore, a change in the thixotropic property of the fascia surrounding the muscle may be more likely (Phillips et al., 2018). This change is possible because the fascia is composed of colloidal substances that can become more gelatinous when they encounter heat and mechanical stress (de Souza et al., 2019). However, in colloidal substances, the thixotropic effect only lasts as long as the pressure or heat is applied, and within minutes, the substance returns to its original gel state (Schleip, 2003). Therefore, it is unlikely that FR would have a sustained effect on flexibility by changing the thixotropic property of the fascia.

Apart from this, FR may increase flexibility due to a process known as autogenic inhibition. As the FR device applies pressure to the muscle tissue, it is believed that mechanoreceptors called Golgi tendon organs (GTO) send a message to the central nervous system that substantial tension is being placed on the muscle, causing the central nervous system to relax that muscle to

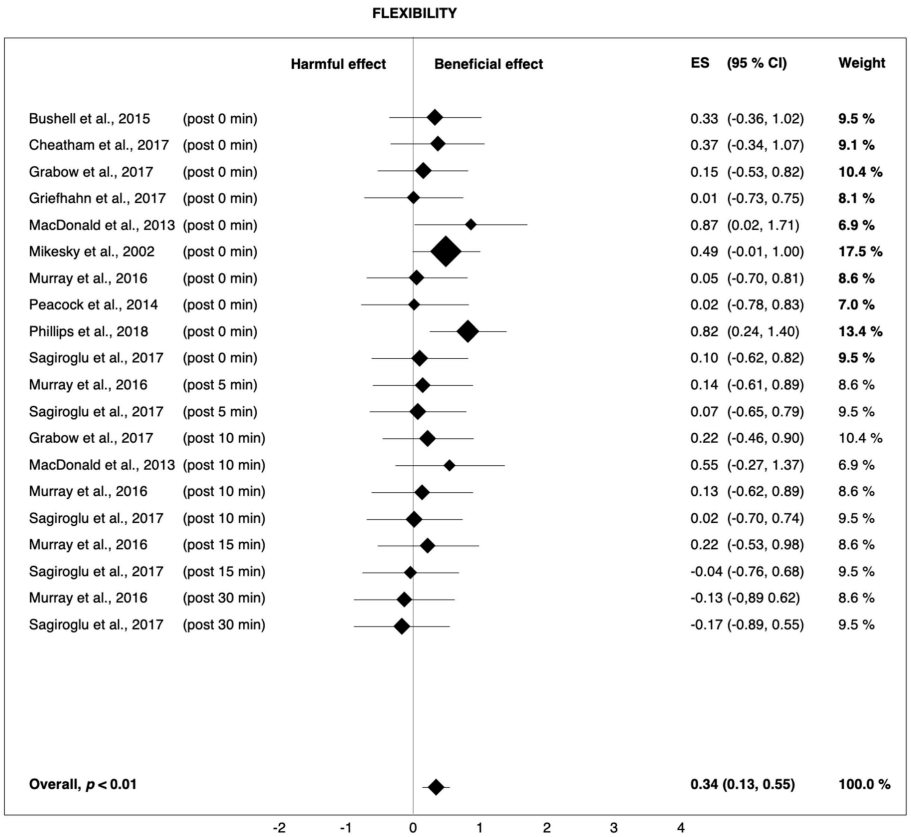

FIGURE 5 | Forest plot summarizing the effects of pre-rolling on flexibility. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

prevent it from tearing (Larson, 2014). However, Edin and Vallbo (1990) found that GTOs were insensitive to the tension produced on the tendon through stretching. If stretch-induced GTO inhibition exists, it is more likely to occur with large-amplitude stretches and not from the small tensile forces that are exerted during FR. Furthermore, any possible GTO inhibition subsides almost immediately after the cessation of tension in the tendon (Behm, 2018). Therefore, it seems unlikely that this mechanism would contribute to increased flexibility following FR. The most plausible explanation for short-term improvements in flexibility could be the effect of FR on the central pain-modulatory systems. For example, constant and vigorous pressure exerted on the soft tissues may overload the skin receptors, thus inhibiting or minimizing pain sensation and increasing stretch tolerance (Kelly and Beardsley, 2016; de Souza et al., 2019). This hypothesis is supported by the findings of Aboodarda et al. (2015) and Cavanaugh et al. (2017) who have shown that FR can improve pain perception.

Apart from the study by Sagiroglu et al. (2017), which has shown that pre-rolling has a harmful effect on jump performance, the research suggests that pre-rolling may offer small short-term benefits in promoting flexibility without negatively affecting muscle performance. This is an important finding to consider when putting together a menu of warm-up activities, since training and competition preparation should always aim to enhance performance. Nevertheless, additional research is necessary to identify different FR protocols that are relevant to different sports and to develop guidelines that ensure FR routines do not impair performance. For example, despite causing an acute increase in range of motion in the joints, prolonged static stretching of more than 60 s per muscle is likely to result in significant performance impairment (Kay and Blazevich, 2012; Behm et al., 2015; Reid et al., 2018). Therefore, one might assume that prolonged static stretching is not recommended during pre-event warm-up activities, especially when performance is required immediately after stretching. However, in studies that conducted performance tests >10 min after static stretching, performance changes were typically statistically trivial unless extreme stretch protocols were used (Behm et al., 2015). Moreover, Blazevich et al. (2018) and Reid et al. (2018) reported that potential performance decrements caused by static stretching are insignificant with shorter stretching durations (i.e., <60 s) and appear to be resolved after a complete, progressive pre-exercise warm-up routine. Therefore, strong evidence supports the deleterious effects of static stretching prior to performance (Behm and Chaouachi, 2011), but when used

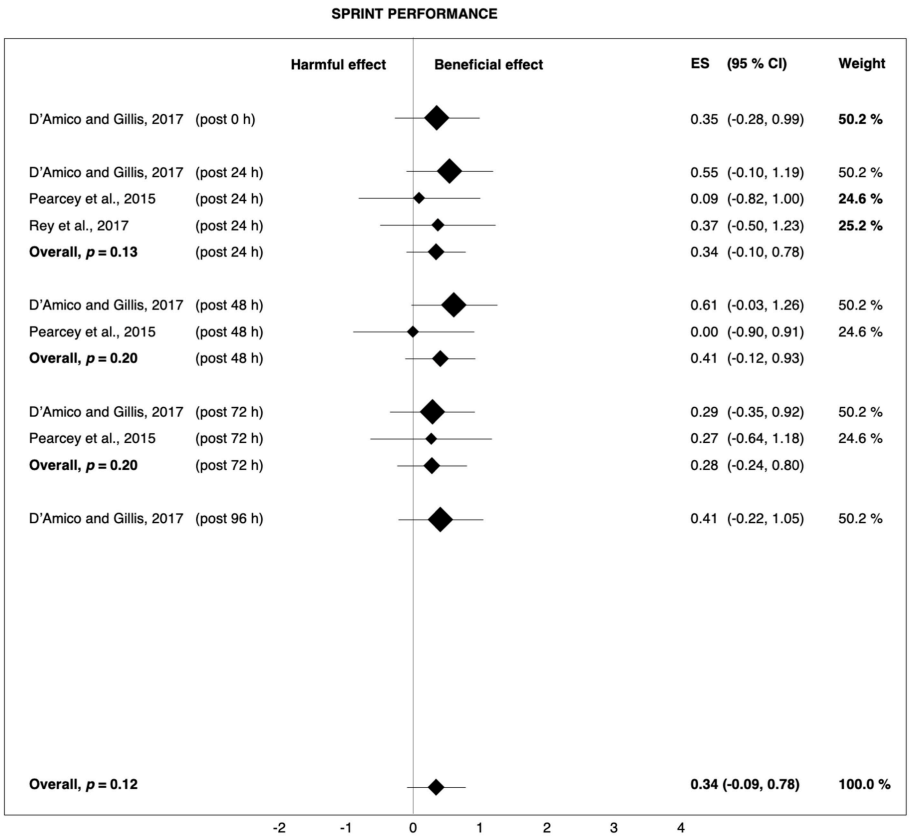

FIGURE 6 | Forest plot summarizing the effects of post-rolling on sprint performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

properly, static stretching can promote flexibility and injury prevention without negatively affecting muscle performance (Behm et al., 2015; Reid et al., 2018).

Post-rolling

The current review demonstrates that post-rolling recovers exercise-induced decreases in sprint and strength performance more quickly than passive recovery. The overall Hedges' *g* of 0.34 and 0.21 for sprint and strength (**Figures 6**, **8**) indicate that 62 and 58%, respectively, of the population will experience the accelerated recovery of sprint and strength performance when using post-rolling (Coe, 2002). The average sprint and strength performance improvements were 3.1 and 3.9%, respectively, reflecting a range that is clearly higher than the smallest worthwhile change defined by Hopkins et al. (1999).

Prolonged impairments in muscular function have been attributed to a multifaceted process from central factors involving the central nervous system and nervous pathways to peripheral factors occurring within the muscle itself. However, it is assumed that subsequent fatigue after intensive exercise would account for about 80% of the impairments originating from a peripheral factor (Wiewelhove et al., 2017). Peripheral fatigue includes physical signs, such as the ultrastructural damage of connective tissue and muscle tissue, as well as an increase in muscle soreness. Nevertheless, previous research has indicated that ultrastructural damage does not always occur following intensive exercise that leads to muscle soreness (Yu et al., 2002). Therefore, it seems reasonable that reduced voluntary muscle activation (e.g., central fatigue due to inhibition caused by muscle soreness, swelling, and stiffness) also contributes to a reduction in muscular function (Byrne et al., 2004). Considering this, the recovery of dynamic performance measures with the use of post-rolling is due to either the facilitated process of soft-tissue restoration, the accelerated restoration of central factors, or both.

Although Pearcey et al. (2015) did not directly investigate the physiological mechanisms of FR, they speculated that post-rolling might enhance post-exercise recovery of dynamic performance measures via systemic biomechanical effects. These include: increased levels of circulating neutrophil; smaller increases in post-exercise plasma creatine kinase; activated mechano-sensory sensors that signal transcription of COX7B and ND1, indicating that new mitochondria are being formed, which presumably accelerate the healing of the muscle; and less active heat-shock proteins and immune cytokines, thus reflecting less cellular stress and inflammation. Furthermore, the reduced perception of pain may positively affect the short-term recovery

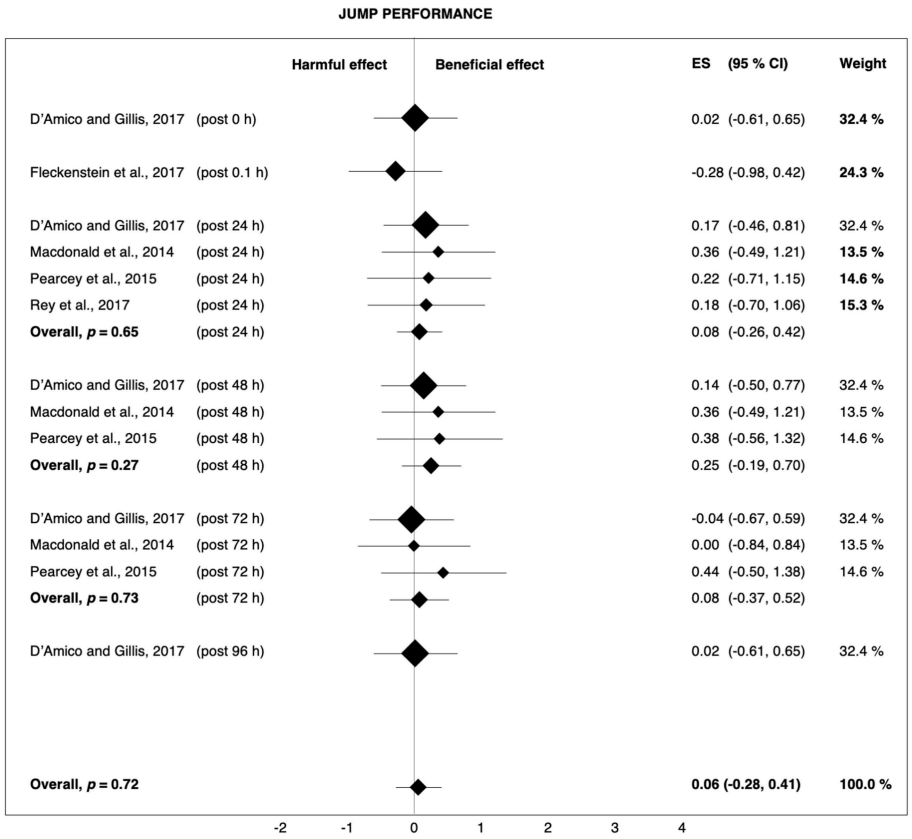

FIGURE 7 | Forest plot summarizing the effects of post-rolling on jump performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

process of muscular function (Zorko et al., 2016), and improved perception of muscle soreness may be critical for the restoration of exercise performance, since muscular function is impaired in the presence of muscle pain (Graven-Nielsen et al., 2002). These mechanisms will be described in more detail below.

However, the effects of post-rolling on performance should again be interpreted with caution, as the overall effects on sprint ($p = 0.12$) and strength performance ($p = 0.28$) were not significant and the number of available studies was limited. Merely one study found a clear benefit of post-rolling for sprint performance (D'Amico and Gillis, 2017), while only two studies showed post-rolling had a clear positive effect on strength performance (Zorko et al., 2016; Fleckenstein et al., 2017). In addition, the effects of post-rolling on jump capacity were negligible (**Figure 7**), although research has demonstrated a clear relationship between sprint, jump, and strength performance in athletes (Comfort et al., 2014). Therefore, it remains questionable whether the average post-rolling-induced enhancements of performance recovery were really due to a true physiological effect of FR or whether the placebo effect or methodological aspects contaminated these results.

The largest average effects of FR in general and post-rolling in particular were found for the alleviation of perceived muscle pain. The total Hedges' g of 0.47 (**Figure 9**) indicates that with the use of post-rolling, 66% of the population is likely to experience reduced muscle pain (Coe, 2002). In terms of athletic performance, muscle soreness, as previously described, can have negative consequences. It may result in altered muscle functions. These alterations may substantially reduce the performance or optimal training intensity of athletes (Pearcey et al., 2015). For example, Byrne et al. (2004) reported the negative effects of perceived muscle pain on sprint, jump, and strength performance, all of which are important during many athletic events.

Several theories have been proposed to explain the underlying mechanisms of exercise-induced muscle soreness. Some authors suggest that perceived muscle pain arises from disruption to the muscle fiber and surrounding connective tissue, while others suggest that it is associated with the inflammatory response, and other suggest that it is a combination of both (Hill et al., 2014). However, since muscle enzyme efflux and myofibrillar damage are not correlated with the actual sensation of muscle soreness, it has been postulated that exercise-induced muscle soreness may be more related to connective tissue damage and the

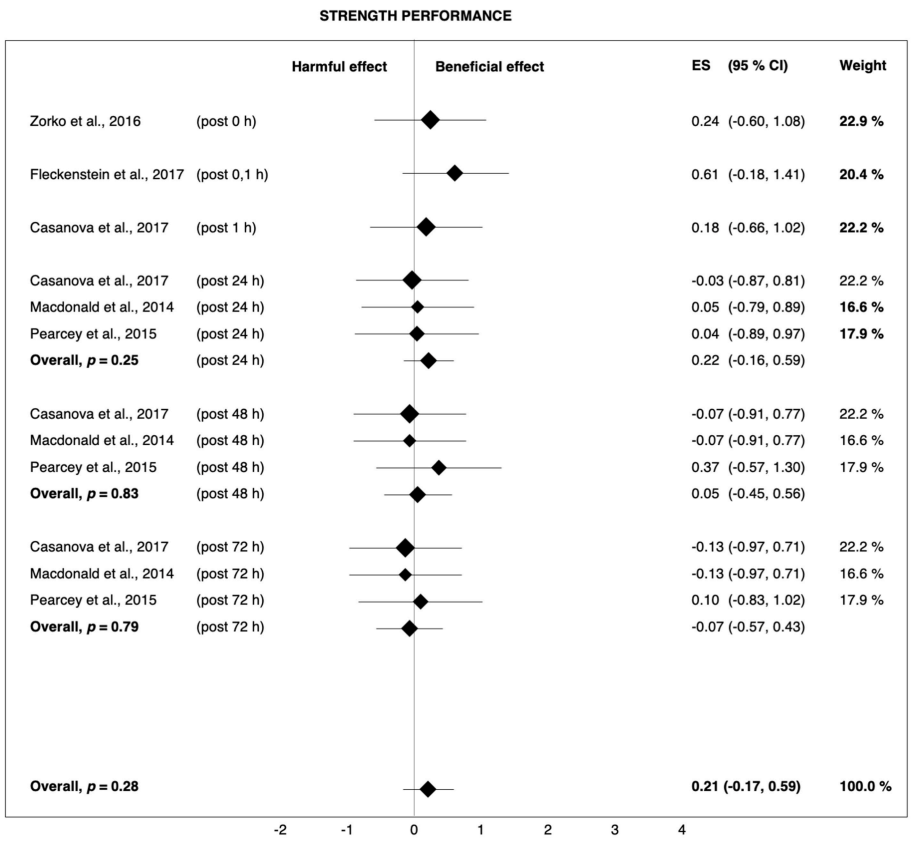

FIGURE 8 | Forest plot summarizing the effects of post-rolling on strength performance. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

inflammatory response rather than the actual muscle cell damage incurred (Macdonald et al., 2014). For example, damaged connective tissue stimulates mechanically sensitive receptors, giving rise to pain when stretched or pressed, while the inflammatory response, which follows tissue damage, creates an increase in tissue osmotic pressure that sensitizes the nociceptors, also resulting in the sensation of pain (Hill et al., 2014; Macdonald et al., 2014).

In this regard, FR-like treatments in animal models have been shown to induce an anti-nociceptive response by mediating an endogenous release of oxytocin into the plasma and in the central grey matter located around the cerebral aqueduct in the midbrain (Agren et al., 1995; Lund et al., 2002; Jay et al., 2014). Thus, one plausible mechanism to explain the reduction in muscle soreness following FR is the activation of descending inhibitory pathways, using the central gray matter-opioid system and oxytocin (Jay et al., 2014). Moreover, it has been proposed that FR-like mechanical stress may remove trigger points from the muscle tissue, leading to improved pain perception. Myofascial trigger points are a common source of musculoskeletal pain. It is thought that application of massage-like mechanical pressure on trigger points can prevent the unnecessary firing of muscle spindles afferent discharges from the trigger point, can reduce trigger point-induced muscle spasms, and ultimately decrease pain (Aboodarda et al., 2015). However, this explanation remains highly speculative because there is no concrete evidence for the effectiveness of brief rolling massages for trigger point therapy.

As proposed by Aboodarda et al. (2015) and Cavanaugh et al. (2017), the most plausible explanation for the mediation of perceived muscle pain following FR could be the effect of rolling massages on the central pain-modulatory systems. Their findings suggest that FR performed on muscles that contain a hypersensitive tender spot and FR performed on the contralateral muscle group can both provide an acute increase in the pain threshold. Since the increase in the pain threshold has a transient and non-localized effect, they suggest that massage-like mechanical pressure can provide analgesic effects through the ascending pain inhibitory system (gate theory of pain) and the descending anti-nociceptive pathway (diffuse noxious inhibitory control), respectively. Although the physiological mechanisms underlying the analgesic effect of FR have yet to be demonstrated empirically, a reduction in the sensation of muscle soreness is beneficial to athletes and may improve their readiness to participate in physical activity (Hill et al., 2014).

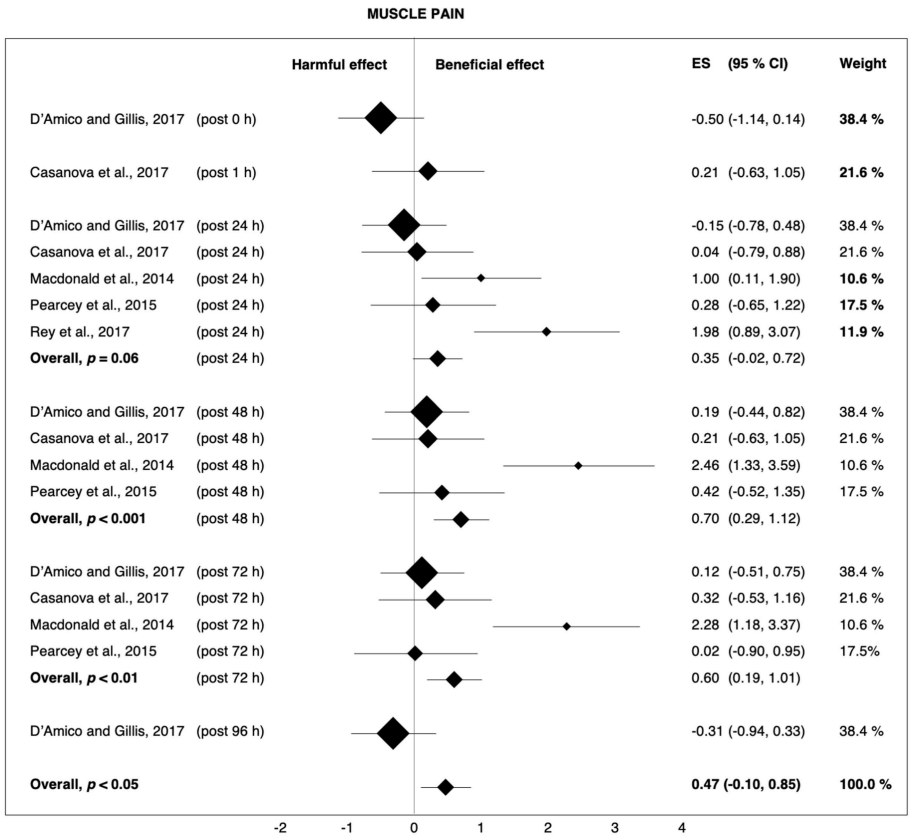

FIGURE 9 | Forest plot summarizing the effects of post-rolling on muscle pain. For each study, the timing of the post-test is included in parentheses. The studies are sorted by increasing the duration between the foam rolling intervention and the post-test. The rectangles represent the weighted effect size (ES) and the lines are the 95% confidence intervals (CI). The size of the rectangles indicates the weight of the study.

Foam Rollers vs. Roller Massagers

Although pre-rolling effects were greater with the use of roller massagers, larger average percentage changes were seen with the use of foam rollers. Due to this contradictory finding, it is difficult to conclude whether pre-rolling with foam rollers or with roller massagers is superior. On the other hand, post-rolling showed both larger effects and greater percentage changes when it was administered with foam rollers, while the benefits of pre-rolling with roller massagers were less significant. Consequently, post-rolling seems to be more effective if foam rollers are used. However, as the number of high-quality and well-designed studies on FR is limited, the conclusions drawn above should be treated cautiously. Further studies would be necessary to confirm that different FR devices lead to different effects.

Limitations

The results from the present meta-analysis provide evidence that the effects of FR on performance and recovery are rather minor and partly negligible, but can be relevant in some cases (e.g., to increase flexibility or to reduce muscle pain sensation). However, any meta-analysis is limited by the data available and there are several limitations for this analysis. First, most of the included studies contain small sample sizes, which result in reduced statistical power. Second, none of the included studies were able to blind their patients to the treatment due to the nature of the FR technique. As such, the placebo effect cannot be eliminated. Third, the methodology varied widely between the studies. For example, the majority of the published studies have not standardized and/or measured the applied pressure of the rolling action, while only a few studies have used a kind of pressure roller apparatus to maintain a constant rolling intensity. Consequently, there may be large ranges in the pressure exerted through FR. Furthermore, the FR procedures differed in terms of the duration of the application, the FR device used, and the muscles targeted. Fourth, with respect to the subjects' training status, the studies recruited both elite athletes and recreationally active or untrained individuals. Overall, these differences may explain some of the inconsistent findings within the current literature and future research should account for this inconsistency by at least directly measuring as well as reporting and standardizing the degree of pressure induced through FR. Finally, the statistical analysis is based only on articles that were published before January 2018. However, the vast majority of articles published since January 2018 confirm the pooled effects of FR, which are calculated in this meta-analysis. For example, Cheatham and Stull (2018), de Souza et al. (2019), Hall and

Chadwick Smith (2018), Killen et al. (2018), Macgregor et al. (2018), Madoni et al. (2018), Monteiro et al. (2018, 2019a,b), and Smith et al. (2018, 2019) were able to confirm that FR can improve flexibility, perceived muscle pain perception, and/or the recovery of strength performance, while Madoni et al. (2018) found that FR had no effect on jump performance.

CONCLUSION

In conclusion, this meta-analysis illustrates that pre-rolling seems to be an effective strategy for short-term improvements in flexibility without decreasing muscle performance. The review has also shown that the improvement of sprint performance to be expected from the use of pre-rolling, as well as the recovery rate of the performance measures of speed and strength with post-rolling, are significant enough to be relevant for at least elite athletes. The underlying mechanisms, however, remain elusive and the effects are in part contradictory. While the effects of FR on muscle function were less clear, the positive effects of alleviating muscle soreness with a larger body of evidence endorse the utilization of post-rolling. As psychological aspects play an important role in most sports, the fact that an athlete feels less pain after pre-rolling might be sufficient to justify its use despite the absence of measurable physiological benefits (Poppendieck et al., 2016). In addition, the almost complete absence of side effects might favor recovery-supporting FR intervention.

However, since the physiological mechanisms of the potential benefits of FR are not fully understood, care should be taken by athletes and coaches, particularly when considering that potential harmful side effects are also suggested when FR is applied (Freiwald et al., 2016). Further, it must be noted that in the available studies, different FR intervention protocols (e.g., different FR devices) were combined with different types of fatigue-inducing exercises and outcome measures, making it difficult to compare the results. In addition, there are not enough high-quality and well-designed studies on FR to draw any definite conclusions. Finally, due to the heterogeneity of the methodological designs among the included studies, there is no consensus on the optimal FR intervention (i.e., in terms of treatment time, pressure, and cadence, etc.). The existing literature thus provides some evidence to support the utilization of FR interventions in sports practice. However, the limited evidence should be considered prior to integrating foam rolling as a warm-up activity and/or a recovery tool.

AUTHOR CONTRIBUTIONS

TW and AD searched and reviewed studies, extracted and analyzed the data, and drafted and proofed the manuscript. CS contributed to data collection, statistical analyses, and reviewed the manuscript. LH reviewed and edited the manuscript. TM, MK, MP, and AF directed the project and contributed to discussion as well as reviewed and edited the manuscript.

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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.2019.00376/full#supplementary-material

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Can Off-Training Physical Behaviors Influence Recovery in Athletes? A Scoping Review

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Recently, the attention on recovery in sport increased enormously although there is lack of scientific evidence on the role of lifestyle in terms of movement [i.e., physical behaviors (PBs)], apart from sleep. Few studies assessed physical activity (PA) and sedentary behavior (SB) in athletes. The aims of this scoping review were to answer to the following scientific questions: (1) How active/inactive are competitive athletes out of training? (2) Do off-training PBs affect recovery, performance, and health? (3) What strategies can be implemented to improve recovery using off-training PBs, apart from sleep? From 1,116 potentially relevant articles, nine were eligible for inclusion in this review. The main issues identified were related to the heterogeneity concerning the types of sports, age category, gender, competitive level, sample size, and instruments/devices adopted, the paucity of studies investigating the effects of PBs while awake on recovery, and the lack of experimental designs manipulating PBs while awake to accelerate recovery. Furthermore, PA and SB domains were rarely investigated, while no research articles focused on the combined effect of 24-h PBs. Eight out of nine studies measured PA, seven SB, and two included sleep. Three studies included training practice into PA measurement by the means of accelerometry. Overall, almost the totality of the athletes achieved recommended PA levels although they sustained prolonged SB. In conclusion, more descriptive researches are needed in different athletic populations and settings. Furthermore, experimental designs aimed at investigating the effects of PBs manipulation on recovery and the putative mechanisms are encouraged.

Keywords: non-exercise activity, physical activity measurement, sitting interruptions, screen time behavior, athletes' health and life, accelerometry, physical activity questionnaires, sedentary behavior

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The attention on recovery in sport has increased enormously in the last years and, nowadays, it is almost as important as training for performance and sport success. A plethora of recovery strategies has been developed in the last years including active and passive recovery, cold-water immersion, compression garments, massage and many others, but some of them lack scientific evidence to support their use (McGuigan, 2017). Surprisingly, although a healthy lifestyle is considered a key factor for athletic recovery, practitioners, and sport scientists often overlook this aspect except for sleep, which has attracted a widespread attention in the last years (Costa et al., 2018; Nedelec et al., 2018; Vitale et al., 2019a). On the other hand, the amount of evidence on the role of an active

lifestyle for health is enormous and various global and national guidelines and updates have been developed during years on the type and amount of physical activity (PA) recommended to improve and maintain health and to prevent and manage chronic diseases, in different populations (Ministry of the Education and Culture Finland, 2016; Graf et al., 2017; Pfeifer and Geidl, 2017; Mottola et al., 2018; Piercy et al., 2018). Physical activity positively affects cardiorespiratory fitness, muscular strength and endurance, blood lipids and glucose levels, body composition, balance and coordination, and mental health and well-being. On the other hand, sedentary behavior (SB) and the lack of PA affect health and well-being, negatively acting at all the above-mentioned levels. According to the recent and more comprehensive definition of PA proposed by the DEDIPAC-KH research team, it consists of any bodily movement produced by skeletal muscles that results in energy expenditure and this may be unstructured and everyday life activity, exercise that includes prearranged, deliberate, and repetitive activity and grassroots sports and competitive sports (Condello et al., 2016). Unstructured and everyday life activity is also defined spontaneous physical activity (SPA), namely every daily PA excluding any volitional exercise (Garland et al., 2011). SB is defined as any waking behavior characterized by an energy expenditure $\leq$1.5 metabolic equivalents (METs), while in a sitting, reclining, or lying posture. Consequently, physical inactivity is considered the lack of meeting the recommended levels of PA and not a synonymous of SB (Tremblay et al., 2017a). Collectively these terms, together with sleep, are named 24-h movement behaviors (McGregor et al., 2018) or physical behaviors (PBs) (Freedson, 2018). The World Health Organization (WHO) recommends adults to perform at least 150 min/week of moderate-intensity physical activity (MPA) or 75 min/week of vigorous physical activity (VPA), or any combination of both intensities that meets the recommended amount. Notably, it recognizes the importance of sitting less, though no quantitative key guideline for sitting time or how to break up sitting duration are proposed (Piercy et al., 2018). Furthermore, 24-h movement guidelines have been recently published for children in different countries (New Zealand Ministry of Health, 0000; Tremblay et al., 2016, 2017b; Okely et al., 2017) and it is very probable that the same format will be adopted for future recommendations (Chaput et al., 2014). The aforementioned recommendations are considered the minimum amount of PA to obtain noticeable benefits for health. Additional health benefits are gained by doing physical activity beyond the equivalent of 300 min/week of MPA (Piercy et al., 2018). On the other hand, athletes are active "*de facto*" because they exercise regularly and athletes from most sports disciplines exceed those recommendations. Indeed, the mortality risk has been reported to be lower in elite athletes compared to non-athletes, particularly in endurance athletes (Garatachea et al., 2014; Kettunen et al., 2015; Lemez and Baker, 2015). However, the amount of time spent in training represents a reduced part of the full day and at present the majority of studies focus their attention only on the effects of sleep on recovery and performance, while PA and SB, during almost two-thirds of the day, have been investigated in a restricted number of studies. It is well-known that PBs while awake affect both bodily systems (e.g., vascular system, endocrine system, and immune system) and metabolic pathways (i.e., glucose and lipids), as well as systemic and local inflammation, mood, fatigue, and cognition (Pedersen and Saltin, 2015). However, there is a lack of evidence in scientific literature about the repercussions of off-training PBs in competitive athletes on recovery, training adaptations and performance, as well as on short and long-term career. Given the above considerations and the recent progresses in accelerometry micro-technology (Freedson, 2018), an overview of the studies describing off-training PBs in competitive athletes throughout the days, not only during sleep is warranted. For this purpose, considering the heterogeneity and the complex nature of the topic, and considering that it has not yet been comprehensive revised, a scoping review is appropriate. The aim of a scoping review is to identify knowledge gaps, scope a body of literature, clarify concepts or to investigate research conduct in emerging body of evidence and make recommendations for future researches. It answers to broader questions beyond those related to the effectiveness of an intervention or treatment (Peters et al., 2015). Then, the aim of this scoping review article is to provide an overview of the body of literature describing PBs while awake in competitive athletes and their effects on recovery, training adaptation, and performance, as well as on related factors (e.g., lactate clearance). This article aims to answer to the following scientific questions: (1) How active/inactive are competitive athletes out of training and do they differ on the basis of competitive level, age categories, and particular settings (e.g., home-based training, training camp, traveling, and tournament participation)? (2) Do off-training PBs affect recovery, performance, health, physical fitness, and career in competitive athletes and by means of which mechanisms? (3) What strategies can be implemented in competitive athletes to improve recovery using off-training PBs, apart from sleep? For the purpose of clarification, a competitive athlete is considered in this scoping review article a highly trained individual who engages in regular organized physical training within a particular sporting discipline and competes at county or national or international level (Sharma, 2003). This scoping review introduce the 3ST (Sleep, Sedentary behavior, Spontaneous physical activity, and Training) project which proposes to (1) provide a complete and detailed description of PBs of competitive athletes using an observational design; (2) explore associations between competitive athletes' PBs and physiological/psychological markers of health, performance, and recovery, as well as describe the mechanisms that regulate such associations and; (3) test the effectiveness of interventions (e.g., education and therapies) to improve competitive athletes' lifestyles and habits, aiming at optimizing training adaptation, recovery, and performance. Information gathered for this scoping review and the potential future results of the 3ST project could be useful for sports scientists, practitioners, and, ultimately, athletes, as it may help in the understanding of the conceptual and methodological gaps in the current health-enhancing and training recovery literature. Additionally, this review and the associated 3ST project

may inspire the design of high-quality studies in the field across different athletic populations and the various forms of training-related demands.

METHODS

Search Strategy

A three-step search strategy was utilized in this review up to February 8, 2019. An initial limited search of MEDLINE was undertaken using the terms: physical activity, sedentary behavior, athletes, and recovery, followed by an analysis of the text word contained in title and abstract and of the index terms used to describe the articles. A second search of MEDLINE, ISI Web of Science, and Scopus was undertaken using all the identified keywords and index terms in the following four categories: population, assessment, comparison/subgroup, outcome/phenomenon of interest (**Table 1**). Search syntax is available in **Supplementary Material**. Thirdly, the reference list of all the identified manuscripts were searched for additional studies. Considering the broad international scope of the current review, no restrictions about language, type of study, and journal categories were applied. Furthermore, no age and gender criteria were imposed according to the objective of investigating the role of different group and settings on PBs. Based on the relevant recent definition of SB (Sedentary Behaviour Research Network., 2012) date criteria was limited to the articles published during and after 2012.

Inclusion Criteria

Included studies were those that incorporated competitive athletes without restriction concerning age, gender, and competitive level. Since the aim of this scoping review is to map the knowledge concerning PBs in athletes, studies including those behavior as primary or secondary outcomes were included. Studies concerning sleep were considered only when sleep measurement and assessment were accompanied by some other PBs while awake.

TABLE 1 | Identified terms for the search strategy according to the categories of interest.

Population	Assessment	Comparison/ subgroup	Outcome/ phenomenon of interest
Player*	Self-reported time	Highly trained	Sitting
Athlete*	Questionnaire*	Injur*	Physical activit*
Amateur*	Acceleromet*	Recreational	Sedentar*
Professional*		Young adult*	Recovery
Elite		Master*	Performance
		Young athlete*	
		Adolescent*	
		Youth	
		Non-athlet*	

*The identified keywords were searched according to the four categories in the table to provide a better structure to the search strategy. *indicates end-truncation in search strategy.*

Exclusion Criteria

Studies including only recreational sport participants were not included. Furthermore, no studies were considered in which it was not possible to infer if participants were competitive or not.

Data Extraction

From potentially relevant articles, generic information (e.g., author name, journal name, and year) and abstract were saved for the analysis. Two independent researchers independently processed all data, with one extracting information and the other verifying. Quantitative data (e.g., sample size, period of assessment) and qualitative data (e.g., phenomena of interest, setting, authors conclusions) were extracted.

Eligible Articles

From 1,116 articles, 1107 were excluded (**Figure 1**), leaving 9 eligible for data extraction.

RESULTS

General Information

Items considered for data extraction and the identified categories are summarized in **Figure 2**. All the nine eligible articles were written in English and published in 9 different journals, from 5 countries, between 2014 and 2019 (**Figure 3**). One study compared PA and SB between athletes and non-athletes (Clemente et al., 2016), and two studies correlated PBs with some physiological/psychological markers, in competitive athletes (Júdice et al., 2014; Sufrinko et al., 2018). Overall, there is substantial heterogeneity in the selected studies concerning the types of sports, age category, gender, competitive level, sample size, and instruments/devices adopted to assess PBs (**Table 2**). Júdice et al. (2014) performed the study in a crucial time of the competitive season (e.g., the last days before engaging in an international competition), Weiler et al. (2015) during a week of the competitive season, Clemente et al. (2016) assessed university athletes the majority of which lived in rented flats or in the campus hostels with a small home-to-university distance, but they did not give information about the competitive period, as well as McCracken and Dogra (2018); Sperlich et al. (2017) performed the study during a preparation training camp in the pre-season, while Exel et al. (2018, 2019) during a typical week of the competitive season. Finally, Sufrinko et al. (2018) performed the study during recovery after concussion. Not all the studies reported the monitoring device sampling frequency, as well as the epoch length at which data were collected and analyzed/collapsed (**Table 3**).

Off-Training Physical Behaviors While Awake in Competitive Athletes

All the included studies, except for the one from Júdice et al. (2014) assessed PA. One study (McCracken and Dogra, 2018) assessed PA in master athletes using the short form of the International Physical Activity Questionnaire (short-IPAQ), which assesses MPA, VPA, walking, and sitting time. Six out of seven accelerometry studies categorized PA according to its intensity in LPA (1.5–3 METs), MPA (3–6 METs), and VPA

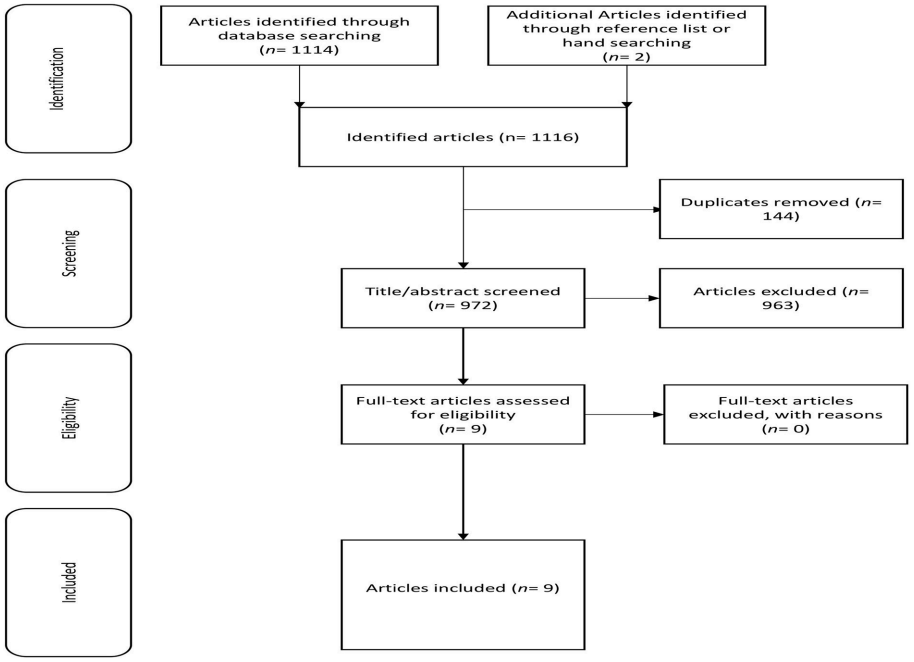

FIGURE 1 | Flow chart illustrating the different phases of the search and study selection.

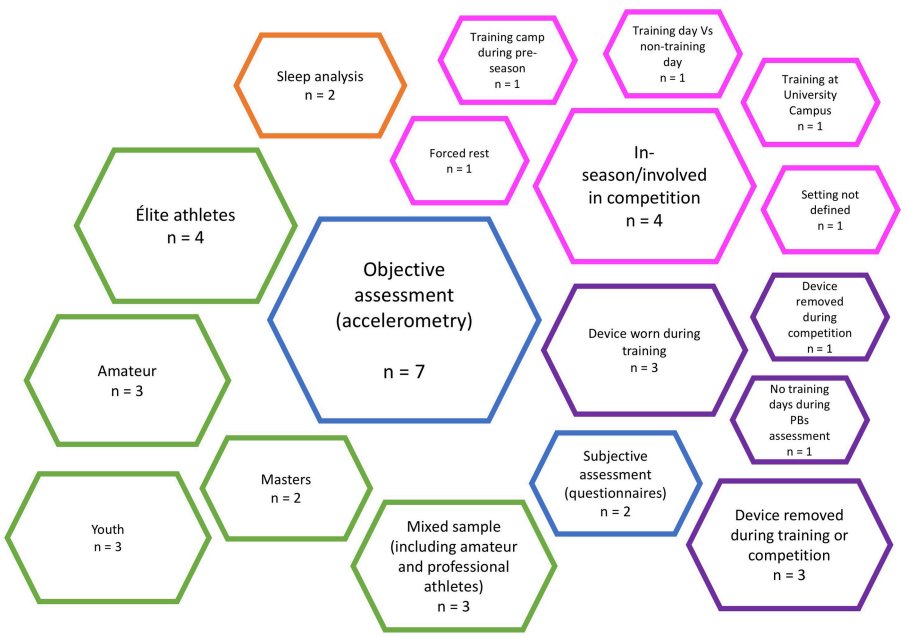

FIGURE 2 | Map of the identified qualitative items by number of studies. Blue, method of assessment; purple, inclusion/exclusion of training/competition during PBs assessment; orange, sleep analysis; magenta, setting, phase of the season; green, sample.

(>6 METs), although not all the studies reported the cut-points used to calculate the intensity (**Table 3**). On the other hand, Sufrinko et al. (2018) only reported total daily vector magnitude (VM) counts, average VM counts per minute, and maximum VM counts per minute, making it difficult to categorize PA levels and to compare with other studies. Nevertheless, even among studies using the same threshold-based analysis for classifying PA by its intensity, the comparison remains difficult, due to the lack of some information on the technical specifications and the differences in instrumentation, setting, and data processing

FIGURE 3 | Eligible articles published per year and country.

(**Table 3**). Overall, the recommendation on PA levels for health was met on average by all the samples of the considered studies that reported PA levels, apart from children in days without sport practice (Ala-Kitula et al., 2019). Clemente et al. (2016) and McCracken and Dogra (2018) compared university and master competitive athletes with non-athletes and recreational athletes, respectively. Despite some statistical differences with small effect size (LPA for men and SB, LPA, and VPA for women), the results of Clemente et al. (2016) suggest proximity between PA levels of athletes and non-athletes. On the other hand, male master athletes in the study from McCracken and Dogra (2018) showed significantly higher levels of VPA compared to male (age-matched) recreational athletes. Another study on male master runners and footballers (runners were recreational participants) also indicated that master athletes meet global recommendations on PA (including training time) (Exel et al., 2019). A study on young footballers compared days with soccer practice with days without soccer practice showing that in the days without training children fail to reach the national recommendations on MVPA (Ala-Kitula et al., 2019).

All the studies that assessed SB reported worrisome levels of sedentariness, regardless of using subjective or objective methods. No difference emerged when competitive athletes were compared to non-athletes (Clemente et al., 2016) or recreational masters (McCracken and Dogra, 2018). However, when considering the domains of SB, some differences were found between master and recreational athletes: male master athletes spent less time watching TV than the recreational ones and less time in hobbies that imply sitting, as well as at work and during leisure time, compared to both male recreational athletes and female master athletes. On the other hand, female master athletes spent more time sitting at work and during leisure time than female recreational athletes, although this result may be due to the higher rate of workers among master than recreational athletes. Interestingly, Exel et al. (2018) found that young athletes showed different patterns of activity (i.e., more or less active), in spite of the time spent in environments which can favor SB, such as school and home. Adult recreational runners and master footballers competing at local level showed no difference in terms of SB, although they differed in LPA, MPA, and VPA but not in MVPA cumulated in bouts of at least 10 min (Exel et al., 2019). Overall, these studies suggest that athletes have a similar SB to the general population but the majority meet PA recommendations, although results about children are matter of concern.

Association Between PBs While Awake and Markers of Recovery, Performance, Health, and Physical Fitness, in Competitive Athletes

Júdice et al. (2014) reported that sitting time was positively associated with total and trunk adiposity while weight class sports showed higher values of sitting time, in spite of a lower amount of training, and a stronger association between sitting time and adiposity. Sufrinko et al. (2018) showed that both PA and sleep changed throughout the course of the recovery from sport-related concussion. In particular, PA intensity (i.e., maximum VM counts per minute) increased and total time in bed decreased throughout concussion recovery, without any difference in total sleep time and sleep efficiency. Furthermore, both PA and sleep were associated with clinical outcomes of recovery. Both PA and sleep were associated with clinical outcomes of recovery: visual memory scores at follow up (sub-acute phase) were linearly and positively associated with PA intensity during the first week after recovery (acute phase), while very high and very low average PA counts during the acute phase were associated with worse motor speed scores at follow up (inverted "U" relationship). In addition, PA during the first week postinjury was also associated with worse vestibular/oculomotor scores at follow up. Finally, Ala-Kitula et al. (2019) investigated the relationship between SPA preceding training and PA during training: SPA before the practices had significant positive correlations with different activity levels during practices on weekdays and on weekend days. The highest correlation was found between preceding LPA and MPA during training on the same days, on weekdays and on weekend days.

Intervention Studies on Physical Behaviors While Awake in Competitive Athletes

No studies were found that manipulated PBs while awake with the aim of improving health, recovery, performance, training adaptations, or any other related outcomes in competitive athletes.

TABLE 2 | General characteristics and main results of the selected articles.

Authors	Disciplines	Gender	Competitive level	Sample size (num)	Age (years, Mean ± sd)	Assessed behaviors	Main results
Júdice et al., 2014	Various disciplines	Males	Elite	82	21.8 ± 4.8	Sitting time	Sitting time predicts total fat and trunk fat mass independent of age, weekly training time, and residual mass but not abdominal fat. Weight-class sports is the category most responsible of this association, compared to non-weight sensitive sports and gravitational sports.
Weiler et al., 2015	Soccer	Males	Elite	25	26.8 ± 4.4	SB, LPA, MPA, VPA	The majority (79%) of post-training time of elite soccer players from an English Premier League football club is spent in sedentary activities.
Clemente et al., 2016	Various disciplines	Males and females	Amateurs and professionals	33	NA	SB, LPA, MPA, VPA, steps	Despite some statistical differences with minimal effect size (LPA for men and SB, LPA, and VPA for women), the results of this study suggested proximity between PA levels of athletes and non-athletes, mainly in the case of SB.
Sperlich et al., 2017	Rowing	Males	Elite (U23 men's national team)	11	20.0 ± 2.0	SB, LPA, MPA, VPA, sleep	Rowers display a considerable amount of time spent in sedentary pursuits (about 11.5 h/day).
McCracken and Dogra, 2018	Various disciplines	Males and females	Local to international masters	79	63.6 ± 7.2	Sitting time, MPA, VPA, PA and SB domains	Male recreational athletes spend more time in SB and less time in VPA compared to master athletes, while female recreational athlete spend less time in SB in comparison to master athletes. Although older athletes accumulate high volumes of SB, they also accumulate the suggested 60–75 min of moderate-vigorous intensity PA per day to negate the detrimental effects of sitting.
Exel et al., 2018	Various disciplines	NA	Elite (youth)	8	15.7 ± 2.0	SB, LPA, MPA, VPA, MVPA, standing, sitting, lying, sedentary breaks over 30 min	Young athletes showed different patterns of PA and SB. Most weekdays waking hours are spent in places that promote sedentarism (school and home). Some athletes still manage to balance healthy PA and SB levels and may serve as a reference.
Sufrinko et al., 2018	Various disciplines	Males and females	NA	19	15.5 ± 1.9	Bed time, sleep time, sleep efficiency, variation in total sleep time, total PA, mean PA, PA intensity	PA increases during recovery from concussion while total time in bed decreases, although total amount of sleep and sleep efficiency did not change. Both PA and sleep are associated with neurocognitive and vestibular/oculomotor outcomes.
Exel et al., 2019	Footballers and runners	Males	Footballers: local masters; Runners: recreational masters	29	43.9 ± 3.9	SB, LPA, MPA, VPA, MVPA in 10 min bouts,	Different sports determine different distributions of PA levels in adults. Amateur runners tend to higher amounts of VPA, while footballers perform higher amounts of LPA and MPA. There are no differences in terms of SB.
Ala-Kitula et al., 2019	Soccer players	Males	National	18	12. 6 ± 0.3	LPA, MPA, VPA, MVPA, SB lasting at least 30 min	The amount of MVPA attained on practice days is not achieved on days without practice. On weekdays without practice the MVPA recommendations are not met. Previous PA of the same day before soccer practice has positive correlation with PA during soccer practice at several different activity levels.

SB, sedentary behavior; PA, physical activity; LPA, light-intensity physical activity; MPA, moderate-intensity physical activity; VPA, vigorous-intensity physical activity; MVPA moderate to vigorous-intensity physical activity.

TABLE 3 | Technical specifications of the accelerometer settings of the selected articles.

Author	Instrument/device	Days of assessment	Wear time	Body site	Sampling frequency	Epoch length	Metrics	Cut-points
Weiler et al., 2015	GENEActiv triaxial wrist accelerometer (Activinsights Limited, Cambridge, UK)	7 consecutive days	≥ 500 min/day of continuous wear time during waking hours of the off-training period	Wrist (dominance not specified)	50 Hz	Not specified	Activity counts	Not specified
Clemente et al., 2016	ActiGraph accelerometer wGT3X-BT (Actigraph Corp, Shalimar, FL, USA)	Seven consecutive days	24 h/day, apart from water-based activities. Sixty minutes without activity (zero counts) was considered non-wear time and not included in the data treatment.	Wrist (dominance not specified)	Not specified	Collected at 10-s epochs, subsequently collapsed into 60-s epochs	Activity counts	SB ≤100 cpm; LPA = 100–1,951 cpm; MPA = 1,952–5,724 cpm; VPA ≥ 5,725.
Sperlich et al., 2017	Wrist-worn multisensory device Microsoft Band II (Microsoft Corporation, Redmond, Washington, USA)	31 consecutive days, with 21 weekdays, and 10 weekend days	≥ 480 min/day of continuous wear time during waking hours of the off-training period	Wrist (dominance not specified)	Not specified	Microsoft Band II stores the data of mean hourly energy expenditure online	Proprietary algorithm	Not specified
Exel et al., 2018	ActiGraph GT9X Link (Actigraph Corp, Pensacola, FL, USA)	15 consecutive days	≥ 600 min/day of continuous wear time during waking hours of the off-training period	Hip, on the dominant side	30 Hz	Collapsed into 60-s epochs	Activity counts	SB = 0–180 counts·15s^{-1}, LPA = 181–756 counts·15s^{-1}, MPA = 757–1,111 counts·15s^{-1}, VPA = ≥1,112 counts·15s^{-1}
Sufrinko et al., 2018	ActiGraph GT3X+, (Actigraph Corp, Pensacola, FL, USA)	20.2 ± 9.7 days	24 h/day, apart from water-based activities. Non-wear time was identified and re- moved using the Troiano algorithm in Actilife Soft- ware v6.13.3 (Actigraph Corp, Pensacola, Florida)	Non-dominant wrist	Not specified	Collapsed into 60-s epochs	Activity counts	Not specified
Exel et al., 2019	ActiGraph GT9X Link (Actigraph Corp, Pensacola, FL, USA)	7 consecutive days	9–12 h/day of waking hours; removed during water-based activities and competitions.	Dominant wrist	30 Hz	Raw acceleration data	Euclidean Norm Minus One (ENMO)	intensity-specific cut-points calculated according to Hildebrand et al., 2014
Ala-Kitula et al., 2019	Polar A300 -activity monitor (Polar Electro Oy, Finland)	9 consecutive days, including 7-week days and 2 weekend days	Minimum wear time for valid data not specified.	Non-dominant wrist	Not specified	Not specified	Proprietary algorithm	Not specified

SB, sedentary behavior; PA, physical activity; LPA, light-intensity physical activity; MPA, moderate-intensity physical activity; VPA, vigorous-intensity physical activity.

DISCUSSION

The aim of this scoping review was to answer to the following scientific questions: (1) how active/inactive are competitive athletes out of training and are there differences in their PBs on the basis of competitive level, age categories, and settings? (2) Do off-training PBs affect recovery, performance, health, physical fitness, and career in competitive athletes and by means of which mechanisms? (3) What strategies can be implemented in competitive athletes to improve recovery by using off-training PBs, apart from sleep? The main results of the present article reveal the paucity of information about off-training PBs while awake in competitive athletes, although the number of articles increased in the last years (**Figure 3**), as well as about the association of the PBs with physiological/psychological markers of health, performance, and recovery. Furthermore, the results of this scoping review indicate that there are no studies with an experimental design that might link off-training PBs while awake with recovery, performance, health, and physical fitness. Moreover, there are no studies that attempted to improve these aspects by means of manipulation of the off-training PBs while awake. Finally, the large variability in the eligible studies concerning the populations and the contexts, the instruments and their settings, as well as data processing procedures, make comparison and generalization of the results difficult. Notwithstanding, in the following paragraphs, a discussion is provided on the few emerging evidence and their implications regarding PBs in athletes and future directions for off-training lifestyle research in competitive athletes are indicated.

How Active/Inactive Are Competitive Athletes out of Training?

Overall, the included studies agree on the following points: first, athletes spend too much time in sedentary pursuits, which make them comparable to the general population (Clemente et al., 2016); secondly, most of them meet PA recommendations, although the younger athletes seems to face more difficulties in doing so (Exel et al., 2018), especially in the days when they do not train (Ala-Kitula et al., 2019). In recent years, scientific studies have indicated that too much sitting is harmful for health, independent of PA. However, Ekelund et al. (2016) provided evidence suggesting that high PA levels (60-75 min of MVPA/d) are protective against the risk of death associated with prolonged sitting and TV-viewing. As athletes can easily exceed thses levels of PA, health issues should not be a matter of concern for them. However, while the results of this scoping review indicate that most of the athletes meet the international recommendations on PA, some of them such as university athletes and young soccer players (**Table 4**) may not reach the levels suggested by Ekelund et al. (2016) to counteract the harmful effects of SB. Notably, SB are excessive for all the populations investigated in the eligible studies of this scoping review. The only exception is represented by a subgroup of young athletes in the study of Exel et al. (2018) that showed a pattern of activity that was less sedentary and more active compared to the others, despite having been in the same environments (home and school). As suggested by the authors, this subgroup seems to show a resilience to the "sedentarigenic environments" (home and school) and researchers should investigate the reasons of such behavior because they can represent a healthy reference to the others. This is particularly important considering another result from the study of Ekelund et al. (2016): while high levels of PA seem to eliminate the increased risk of mortality associated with prolonged sitting (more than 8 h/d) they only mitigate, not eliminate the risk associated with protracted TV-viewing (more than 5 h/d), a behavior generally performed at home, suggesting that the domains of activities while sitting are as relevant as the amount of exposure to SB. In a recent study, Jones et al. (2019) showed in well-trained athletes that the use of multiple devices in the evening was associated with more perceived difficulty in falling asleep. Considering the already-known importance of good sleep in athletes and that the use of electronic devices can disturb attention and mood, which are also fundamental in sports training and competitive performance (Green et al., 2017), the investigation of the inherent relationships between training, SPA, SB, and sleep during 24 h appears of crucial importance, together with the need to study the domains of PA and SB. These behaviors take place in a *continuum* from sleep to very vigorous PA and can occur in different parts of the day. Recent studies showed that the intervention on one of those behaviors (e.g., introducing moderate-intensity physical exercise) would necessarily have consequences on the others (Izzicupo et al., 2012; Blasio et al., 2018), a phenomenon that can make efforts for improving health vain (Di Blasio et al., 2012). For this reason, several studies have investigated the combined effect of 24-h PBs on health using isotemporal and compositional data analysis techniques (Biswas et al., 2015; Colley et al., 2018; McGregor et al., 2018). This can be even more important for athletes due to the fact that they are involved, generally, in very vigorous training sessions or in mentally-demanding activity (e.g., archery), which can impact on other behaviors during the remaining time of the day. For instance, training sessions and competitions can take place during different periods of the day (e.g., morning session vs. evening session, multiple training sessions during the day, evening competition), and professional athletes can have a large amount of leisure time, while other competitive athletes are employed, possibly having different repercussions on athletes' training recovery and performance. Indeed, it has been recently shown that athletes' rest-activity circadian rhythm differs in accordance to the sport discipline (Vitale et al., 2019b). However, none of the eligible studies in this scoping review investigated the interaction effects of differently combining PBs over 24-h, but rather analyze behaviors in isolation and descriptively. It is plausible, that training load affects PBs during the remainder of the day and vice versa, as well as it is possible that sleep can be affected by training practice, daily PA and SB and their combination. However, such eventuality has not yet been studied. The only study that considered the relationship between different behaviors is the one from Ala-Kitula et al. (2019) where PA preceding soccer practice had a positive correlation with all PA intensity levels during practice. It is known that children with high PA level are active throughout the day compared to less active children (Fairclough et al., 2012). This result suggests that there might be interdependence

TABLE 4 | Daily physical behaviors time.

Authors	Sleep	Sedentary behavior	Light-intensity physical activity	Moderate intensity physical activity	Vigorous-intensity physical activity
			(h/day)		
Júdice et al., 2014	NA	7.70 ± 2.70	NA	NA	NA
Weiler et al., 2015		8.34 ± 0.98	0.93 ± 0.48	1.24 ± 0.47	0.03 ± 0.06
Clemente et al., 2016					
Males	NA	12.29 ± 3.38	5.18 ± 1.91	0.88 ± 0.71	0.09 ± 0.14
Females	NA	12.17 ± 2.30	5.23 ± 1.26	0.79 ± 0.69	0.07 ± 0.11
Sperlich et al., 2017					
Week days	8.18 ± 1.24	11.63 ± 1.25	1.27 ± 1.15	0.76 ± 0.37	0.51 ± 0.44
Weekend days	8.07 ± 1.34	12.49 ± 1.10	0.67 ± 0.43	0.59 ± 0.37	0.53 ± 0.32
McCracken and Dogra, 2018					
Males	NA	4.72 ± 0.41	NA	0.64 ± 0.15	0.80 ± 0.08
Females	NA	5.63 ± 0.51	NA	0.44 ± 0.07	0.87 ± 0.12
Sufrinko et al., 2018	7.06 ± 0.0.69	NA	NA	NA	NA
Exel et al., 2019	NA	9.01 (3.25)	4.0 (2.23)	1.49 (1.4)	0.03 (0.06)
Ala-Kitula et al., 2019					
Training days	NA	NA	2.83 ± 0.85	1.3 ± 0.45	1.08 ± 0.32
Non-training days	NA	NA	3.3 ± 1.0	0.85 ± 0.43	0.42 ± 0.32

NA, not assessed or not available. In the study of Exel et al. (2018) physical behaviors (PBs) were reported as min/h and the devices were worn only during awake time. Since it was not possible to transform accurately PBs time in h/day, the study was not included in the table. Exel et al. (2019) data are presented as median (interquantile range).

between off-training practices and quantity and quality of movements during training. This hypothesis needs to be further addressed.

Screen time exposure and the domains of SB were investigated by McCracken and Dogra (2018): female master athletes exceeded the amount of screen time (more than 4 h/d) determining harmful effects for health and male master athletes were very close to this value (3.42 h/d). Then, hypothetically they can counteract the effects of too much sitting but not the effects of too much screen time. Furthermore, it must be considered that PA and SB were assessed through questionnaires in this study, which underestimate sitting time while overestimating PA, especially in older adults (Dyrstad et al., 2014). Exel et al. (2019) also indicated that master athletes meet global recommendations on PA using accelerometers. However, participants in the first study were about 20 years older than participants in the latter. In the study of Exel et al. (2019), MVPA cumulated in bouts of at least 10 min were similar between groups but runners spent more time in VPA than footballers while the latter shows higher levels of LPA and MPA in comparison to runners. However, such a difference may be attributed to differences in training routines between running and soccer, rather than to off-training PBs. Authors indeed aimed to investigate if both running and soccer are sport activities suitable for master athletes to meet PA recommendations and, for this reason, they did not separate training from non-training PA. Notably, runners were recreational participants while footballers were considered athletes although competing at local level. Nonetheless, runners showed higher VPA levels due to training routine. McCracken and Dogra (2018) also showed that master athletes spent a considering amount of time in other sitting activities at work, during leisure time or driving. This aspect may significantly differ when age category and level are considered. Scholar age athletes spent a considerable amount of time in sedentary activities at school and home, as showed by Exel et al. (2018) and this can represent a problem during the day without training practice because they can fail to reach the recommended level of PA (Ala-Kitula et al., 2019). Finally, athletes can spend a lot of time traveling due to competition schedules (Fowler et al., 2017), with few opportunities to interrupt sitting for long periods. Padilla and Fadel (2017) showed in a series of experiments that prolonged and uninterrupted sitting, a behavior common during long-haul travels or after suffering an injury, is associated with acute lower limbs dysfunction in healthy young subjects. Furthermore, prolonged and uninterrupted sitting can increase sympathetic and renin-angiotensin system activity (Young and Leicht, 2011), as well as plasma fibrinogen, hematocrit, hemoglobin, and red blood cell count, aside from a reduction in plasma volume (Howard et al., 2013). While it remains to be elucidated if an increase in blood viscosity may hinder skeletal muscle blood supply and then recovery, hypercoagulability in athletes and flight travel-associated thrombotic events (not so rare among athletes) (Bishop et al., 2017) are a matter of concern (Meyering and Howard, 2004). Although there are no previous studies that associated vascular alteration due to prolonged sitting with recovery, it is plausible that both energetic restoration and tissue repair are affected by prolonged sitting, due to the importance of muscle blood flow in these processes. Nevertheless, a comprehensive description of PBs of competitive athletes using an observational design, possibly investigating the combined effect of 24-h PBs considering their domains, is first needed to identify specific patterns of activity. Such a knowledge will

be helpful in focusing research aimed to understand if and how off-training PBs can affect recovery, performance, health, physical fitness, and career in competitive athletes and through which mechanisms.

Do Off-Training PBs Affect Recovery, Performance, Health, Physical Fitness, and Career in Competitive Athletes?

Only two studies associated off-training PBs in competitive athletes with variables associated with health or recovery. Júdice et al. (2014) reported sitting time was positively associated with total and trunk adiposity in different groups of elite athletes, while weight class sports athletes, despite sustaining lower training volume, showed the highest values of sitting time and the strongest association between sitting time and adiposity. This association may cause surprise because training is considered an activity with high energy expenditure and elite athletes should train enough to maintain an excellent body composition. Indeed, the sample in the study into consideration reported a weekly training time that was far above the MVPA recommendations for the general population or the highest active group in previous studies that also found an association between fatness and SB (Vandelanotte et al., 2009). However, the lowest weekly training time was reported by weight class sports participants who also showed the strongest association with adiposity, while the two sports groups that performed higher weekly training times (>20 h/week) showed no association. Then, higher levels of MVPA may compensate for the effects of time spent in SB. On the other hand, a higher amount of training in some categories of athletes may "replace" SB, while the ones with lower weekly training time may have a larger amount of leisure time that can be spent in low energy expenditure activities, especially during a preparation period for a competition. However, the energy balance alone cannot be sufficient to explain the positive association between SB and fat mass in athletes. Indeed, both glucose and lipid metabolisms are affected by SB and lack of PA, even in active people and when matching caloric intake for the reduction in energy expenditure or basal metabolism (Bergouignan et al., 2009; Stephens et al., 2011). Notably, the reduction in insulin sensitivity, lead to lower nitric oxide production and release from endothelial cells, which ends with reduced blood flow to the skeletal muscle (Wagenmakers et al., 2016). It is also possible that athletes involved in weight class sports have less energy due to energy restriction practices and for this reason are more prone to engage in SB. There is evidence that the homeostatic system tries to preserve energy to compensate both high training energy expenditure and low energy intake, reducing consciously or unconsciously SPA (Garland et al., 2011). Moreover, a negative energy balance may also affect physiological functions to preserve energy by reducing the calories spent from the immune and reproductive systems (Pontzer et al., 2015). As a consequence, both low testosterone levels (Trexler et al., 2014), especially in combat sports, and immune response (Ghaemi et al., 2014) may impair performance and recovery in athletes when they underwent weight management. Immune function affects behavior, including movement: elevated circulating cytokines induce a set of behaviors referred to as "sickness" behavior, which involves mood, and behavior changes (e.g., excessive sleep, asthenia, lack of appetite) that in normal conditions support resolution of systemic inflammation but in athletes with poor rest, injuries, and competitive pressure may lead to the overtraining syndrome (Smith, 2000) Although not yet demonstrated, measuring PBs may represent a valid tool for monitoring the risk of overtraining as well as recovery after training or injuries. Preliminary evidence in this direction emerge from one of the studies included in this scoping review which found an association between the levels of PA and the course of recovery following sport-related concussion (Sufrinko et al., 2018). Sport-related concussion itself has a significant neuroinflammatory component which could explain both the association between PA levels and post-concussion recovery as well as the highest sedentary time in weight class sports, represented by combat sports in the study of Júdice et al. (2014), although in the absence of evident concussion. It is important to underline that sitting time in that study was assessed by questionnaires. Then, although they indicated an association that was somewhat surprising considering the investigated population, future studies need to investigate the association between off-training PBs and body composition, vascular adaptations, tissue repair after training and competition, as well as with physiological and psychological indices of recovery. Furthermore, the putative mechanisms (**Figure 4**) should be extensively investigated to better address intervention protocols during off-training periods in athletes to promote repair and recovery.

Can Off-Training Physical Behaviors be Considered as a Recovery Strategy?

No studies in our search used off-training PBs while awake as a strategy to improve recovery, training adaptation or performance. Then, unfortunately, current literature does not allow us to know if an active off-training lifestyle can offset fatigue, accelerate the return to "baseline" body function and to the reference performance levels, or even accelerate tissue repair. The following discussion is therefore based on evidence from studies examining recovery strategies other than off-training PBs. It can be hypothesized that if active recovery can do it, then, a certain amount of SPA should be beneficial for recovery. However, active recovery does not seem to be a universal solution to foster regeneration: in energetic terms, active recovery seems to be superior to passive recovery when performance must be repeated in a short period (< 30 min) and when exercise involves a significant anaerobic contribution (Franchini et al., 2003; Heyman et al., 2009). Active recovery favors an earlier decrease in lactatemia (Bangsbo et al., 1994) and it allows a faster return to resting pH values than passive recovery (Fairchild et al., 2003). This reduces exercise-induced acidosis, promotes co-transport of lactate and H^+ ions from intramuscular medium and the blood, and then it should preserve neuromuscular function during anaerobic tasks that are performed close to each other. Interestingly, active recovery involving large muscle mass is beneficial also when the event

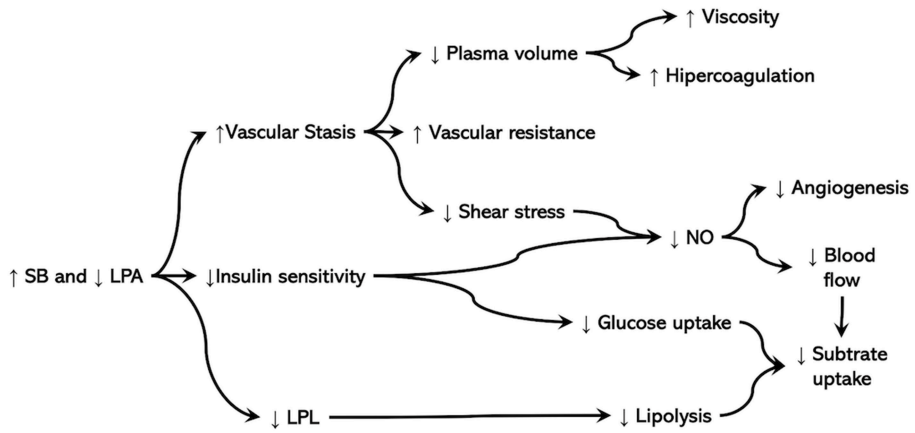

FIGURE 4 | SB, Sedentary behavior; LPA, light-intensity physical activity; LPL, lipoprotein lipase; NO, nitric oxide. ↑, indicates an increase in the amount or activity; ↓, indicates a decrease in the amount or activity. Prolonged and uninterrupted SB and reduced physical activity during the day, mainly represented by LPA, may potentially affect recovery through the vascular and metabolic mechanisms indicated in the figure.

does not involve the same muscle groups that exerted the recovery task (climbing vs. pedaling) (Heyman et al., 2009). Furthermore, aerobic training involving a large muscle mass determines higher circulating levels of VEGF (Izzicupo et al., 2017) that can improve local blood flow in resting muscle. Notably, oxygen availability (McMahon and Jenkins, 2002) and aerobic fitness (Buchheit and Ufland, 2011) are crucial for a fast phosphocreatine resynthesis between short-term events such as repeated sprints. In contrast, depleted glycogen restoration seems to benefit more when using passive recovery (Choi et al., 1994), probably because active recovery further mobilizes glycogen stores. In term of recovery from muscle damage rather than energy restoration, results are contrasting: several studies focused on active recovery after training with both better (Gill, 2006; Tufano et al., 2012) and worse (Sherman et al., 1984; Suzuki, 2004) effects when compared to passive recovery. Overall, active recovery seems to be more effective after eccentrically-based activity (Tufano et al., 2012) and when duration is very short (Gill, 2006). On the other hand, active recovery in the days following an exhaustive competition (i.e., marathon race) seems to be detrimental (Sherman et al., 1984). These results suggest that the amount, intensity, and timing of active recovery are crucial, and, in some cases, it can be better to simply rest rather than perform prolonged active recovery. Probably, while sitting too much may impair local blood flow to the muscle and metabolic efficiency, passive rest can simply be necessary to allocate the energy to the anabolic processes taking place after training and during recovery. Under this point of view, also the intensity of the activities performed during recovery can be important. Several studies suggested that LPA is sufficient to promote positive effects on health (Chastin et al., 2018), however, our knowledge about physiological adaptations to low-intensity work as a recovery strategy in athletes is very limited to date. Hypothetically, sitting interruption and LPA, both implemented off-training and during daily activities, should be considered a promising approach to the problem. Since few minutes seems to be better than longer active recovery interventions, just frequent interruptions of SB through LPA can both promote the positive effects of active recovery, on the one hand, and counteract the side effect of prolonged sitting, on the other one. Furthermore, LPA may be implemented after the main meals to avoid the detrimental metabolic effects of post-prandial prolonged sitting (Thorp et al., 2014) as well as during off-season or after suffering an injury, because some athletes may be very inactive in these phases, compromising the ability to maintain specific fitness components and return to optimal conditions. The activity levels of athletes during these conditions, though, need to be investigated in the future using reliable and standardized methods.

However, at this point, an important consideration must be made regarding the nature of rest and SB: although rest can be performed in a sedentary way, it should be no more or less than the body needs to recover, while, implicitly, SB is harmful because it is over-prolonged. This distinction is a very difficult challenge that, in our opinion, scientific research will have to face in the next years by designing intervention studies in which PBs is modified via education (or other means) to improve athletes' lifestyles and habits.

This is a scoping review to present and mapping the current state of evidence specific to off-training PBs while awake in competitive athletes. The main limitation of this article is that we were not able to answer to part of the formulated scientific questions. Furthermore, both objective and subjective PBs measurement methods were included into the study. Although in our opinion such a choice was necessary to map current understanding on PBs in competitive athletes, the risk of collapsing data that are not directly comparable is high. Nonetheless, this article suggests that at the moment the athlete's lifestyle management is based on nothing more than simple recommendations that are not based in scientific evidence, due to the paucity of indication relative to off-training

PBs in athletes. The 3ST project aims to fill the gap in current literature, first by means of descriptive researches, secondly investigating the putative mechanisms of the eventual effects of SB and SPA on recovery and performance and finally, designing intervention studies in which PBs are modified via education (or other means) to improve athletes' lifestyles and habits. This scoping review article aims at motivating the sport scientists, practitioners and athletes to involve themselves in this new research topic that, based on the limited evidence, can be the next stage to promote better lifestyles along with longer and more successful careers. The results of the 3ST project and future researches related to this topic will be useful to go beyond anecdotal recommendation on lifestyle for athletes and, possibly, allow the implementation of a conceptual model on how athletes should pursue PBs to improve recovery and performance, as well as specific 24-h hygiene recommendations on PBs for athletes.

AUTHOR CONTRIBUTIONS

PI and FYN: conceptualization and writing-original draft. PI and FFR: data curation and formal analysis. PI, FYN, FFR, and EK: methodology. PI, BG, and FYN: investigation. BG and ADB: visualization. PI, ADB, BG, FFR, EK, and FYN: writing-review and editing. ADB: fundings.

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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.2019.00448/full#supplementary-material

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frontiers in Physiology

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Heart Rate Variability Monitoring During Strength and High-Intensity Interval Training Overload Microcycles

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Objective: In two independent study arms, we determine the effects of strength training (ST) and high-intensity interval training (HIIT) overload on cardiac autonomic modulation by measuring heart rate (HR) and vagal heart rate variability (HRV).

Methods: In the study, 37 well-trained athletes (ST: 7 female, 12 male; HIIT: 9 female, 9 male) were subjected to orthostatic tests (HR and HRV recordings) each day during a 4-day baseline period, a 6-day overload microcycle, and a 4-day recovery period. Discipline-specific performance was assessed before and 1 and 4 days after training.

Results: Following ST overload, supine HR, and vagal HRV (Ln RMSSD) were clearly increased and decreased (small effects), respectively, and the standing recordings remained unchanged. In contrast, HIIT overload resulted in decreased HR and increased Ln RMSSD in the standing position (small effects), whereas supine recordings remained unaltered. During the recovery period, these responses were reversed (ST: small effects, HIIT: trivial to small effects). The correlations between changes in HR, vagal HRV measures, and performance were weak or inconsistent. At the group and individual levels, moderate to strong negative correlations were found between HR and Ln RMSSD when analyzing changes between testing days (ST: supine and standing position, HIIT: standing position) and individual time series, respectively. Use of rolling 2–4-day averages enabled more precise estimation of mean changes with smaller confidence intervals compared to single-day values of HR or Ln RMSSD. However, the use of averaged values displayed unclear effects for evaluating associations between HR, vagal HRV measures, and performance changes, and have the potential to be detrimental for classification of individual short-term responses.

Conclusion: Measures of HR and Ln RMSSD during an orthostatic test could reveal different autonomic responses following ST or HIIT which may not be discovered by supine or standing measures alone. However, these autonomic changes were not consistently related to short-term changes in performance and the use of rolling averages may alter these relationships differently on group and individual level.

Keywords: orthostatic test, cardiac autonomic nervous system, fatigue, recovery, individual response, multivariate analysis, resistance training, overreaching

INTRODUCTION

Efficient training provides sufficient exercise stimuli to enhance athletes' performance capacities while avoiding sustained non-functional overreaching or underrecovery (Meeusen et al., 2013; Kellmann et al., 2018). Continuous athlete monitoring may provide information that can be used to balance stress and recovery, ultimately increasing the performance readiness of the athlete and minimizing the risk of illness and injury (Halson, 2014; Schwellnus et al., 2016; Soligard et al., 2016; Bourdon et al., 2017; Coutts et al., 2018; Heidari et al., 2019). A variety of tools, such as psychometric questionnaires (Kellmann, 2010; Saw et al., 2016), blood-borne markers (Urhausen et al., 1995; Fry and Kraemer, 1997; Urhausen and Kindermann, 2002; Meeusen et al., 2013), heart rate (HR)-based measures (Achten and Jeukendrup, 2003; Aubert et al., 2003; Bosquet et al., 2008; Meeusen et al., 2013; Buchheit, 2014; Bellenger et al., 2016a), and (submaximal or non-fatiguing) performance tests (Urhausen and Kindermann, 2002; Meeusen et al., 2013; Claudino et al., 2017), have been discussed for their potential as surrogate markers for assessing fatigue, recovery, or performance. Ideally, so that they can be used frequently in sports practice, these measures are non-fatiguing, easy to administer, inexpensive, and sensitive to performance changes and can provide immediate feedback (Starling and Lambert, 2018).

Monitoring the status of the autonomic nervous system (ANS) with HR-based measures [HR and HR variability (HRV) indices] is an attractive option for testing due to its non-invasiveness and time-efficiency when performed for an entire training group or team. Technological developments within the last few decades have enabled practitioners to use portable devices to obtain accurate beat-by-beat recordings (Achten and Jeukendrup, 2003; Quintana et al., 2012; Buchheit, 2014) as well as software and smart phone applications (Flatt and Esco, 2013; Perrotta et al., 2017; Plews et al., 2017) to obtain (almost) live feedback on HR and HRV indices [HR(V)] in the field. In applied sports research and practice, HR (or average R-R interval) and the time-domain HRV marker RMSSD (root mean square of successive differences between adjacent beat to beat intervals) are commonly measured in a supine, seated, or standing position and may indicate training and fatigue status (Buchheit, 2014; Schmitt et al., 2015a; Bellenger et al., 2016a; Thorpe et al., 2017). However, as ANS activity, and therefore HRV indices, are determined by multiple factors (Sandercock et al., 2005; Buchheit, 2014; Fatisson et al., 2016), it remains difficult to interpret HR(V) measures in isolation. This may contribute to the partially contradictory findings in the literature (Buchheit, 2014; Schneider et al., 2018). To overcome some of these limitations, it has been proposed that researchers use a combination of supine and standing recordings during an orthostatic test to discriminate between different fatigue patterns (Bosquet et al., 2008; Schmitt et al., 2013, 2015a,b; Hottenrott and Hoos, 2017) and use rolling averages to assess adaptation to training (Le Meur et al., 2013; Plews et al., 2014; Flatt and Esco, 2015).

Individuals' HR(V) responses likely differ with training context (i.e., training phase and history, exercise modality and intensity, and the time course of response) (Stanley et al., 2013; Buchheit, 2014; Schmitt et al., 2015a). The majority of relevant scientific reviews focus on either endurance-trained athletes (Bosquet et al., 2008; Bellenger et al., 2016a) or non-athletic populations (Kingsley and Figueroa, 2016; Bhati et al., 2018). Based on this background and to extend insights into training context-specific HR(V) responses, we conducted two independent, similarly designed training trials using whole-body strength training (ST) or high-intensity interval training (HIIT) overload microcycles in well-trained athletes. This study aims to (1) determine the mean changes in HR and HRV measures during active orthostatic tests following ST and HIIT overload and subsequent short-term recovery; (2) evaluate the association between changes in HR, HRV, and performance; (3) classify individuals' HR(V) responses; and (4) analyze whether the use of single-day vs. two-day to four-day average HR values affects the results of the three main analyses.

Abbreviations: 1RM, one-repetition maximum; ANS, Autonomic nervous system; CL, Confidence limits; ddiff, standardized mean difference using SD of differences; dpre, standardized mean difference using between-subject Pre-test SD; HIIT, High-intensity interval training; HR, Heart rate; HRV, Heart rate variability; HR(V), Heart rate and heart rate variability; Ln RMSSD, Natural logarithm of the RMSSD; Ln RMSSD/RR, Ln RMSSD to R-R interval ratio; MVIC, Maximum voluntary isometric contraction; Pre, Baseline testing 1 day prior training microcycle; Post1, Follow-up testing 1 day post overload; Post4, Follow-up testing 4 days post overload; RMSSD, square root of the mean squared differences of successive normal R-R intervals; RSA, Repeated sprint ability; SD, Standard deviation; ST, Strength training; SWC, Smallest worthwhile change; TE, Typical error; V_{IFT}, Peak velocity during the 30-15 Intermittent Fitness Test.

MATERIALS AND METHODS

Participants

Initially, 55 athletes were recruited, of which 51 met the inclusion criteria and 45 competed the study (5 participants did not meet compliance criteria, one dropout due to injury). From the original samples of 23 (9 female, 14 male) individuals performing

ST and 22 (11 female, 11 male) individuals performing HIIT, only 19 (7 female, 12 male) and 18 (9 female, 9 male), respectively, participants provided a sufficient quantity of resting HR recordings to be included in this investigation (we set a minimum of three baseline recordings and a maximum of one missing recording during recovery). The general subject characteristics are presented in **Table 1**. Preliminary health examinations, including resting and exercise electrocardiograms, confirmed the absence of cardiovascular, pulmonary, or orthopedic diseases.

The following inclusion criteria were used for the ST group: estimated one-repetition maximum (1RM) for a parallel squat of at least 80% of body mass for females and 120% of body mass for males and a minimum of 3 years of lower-body strength training with at least two strength training sessions per week. The inclusion criteria for the HIIT group were as follows: peak velocity during the 30-15 Intermittent Fitness Test (V_{IFT}) of at least 16 km/h for females and 19 km/h for males, and a minimum of 5 years of team sport training.

The investigation was approved by the ethics committee of the medical faculty of the Ruhr University Bochum and was conducted according to the guidelines of the Declaration of Helsinki. All subjects participated in the study voluntarily, were free to withdraw without penalty at any time, and provided written informed consent. Participation was rewarded with 100 € at the end of the investigation.

Experimental Design

A repeated-measures study was used to investigate the effects of short-term fatigue and recovery on resting HR and HRV measures [HR(V)]. The investigation comprised a 3-day rest period, baseline testing (Pre), a 6-day overload microcycle, and a 4-day recovery period, which included follow-up testing at 1 (Post1) and 4 (Post4) days post-training (**Figure 1**). Overload was induced by either intensive whole-body ST or HIIT in two independent study arms. Health examination (incl. survey of medication and nutritional supplementation), determination of peak oxygen consumption ($\dot{V}O_2$peak)and familiarization trials for training and testing procedures were conducted 1 week before baseline testing. Discipline-specific maximum effort tests were used as criterion measures to assess fatigue- and recovery-related changes in performance. HR(V) measures were recorded daily during the main 14-day study period (including the rest period, overload training, and recovery). An overview of the experimental design is shown in **Figure 1**.

The analyses presented below were part of an extensive investigation protocol evaluating the ability of different potential surrogate markers to assess fatigue- and recovery-related changes in criterion performance at various overload training camps (i.e., cycling-based endurance training, running-based HIIT, whole-body ST) using a consistent design. The results regarding the performance tests (Wiewelhove et al., 2015; Hammes et al., 2016; Raeder et al., 2016), blood-borne markers (Hecksteden et al., 2016), psychological measures (Hitzschke et al., 2017), and muscle mechanical properties (de Paula Simola et al., 2016) have already been published. Due to insufficient HR(V) baseline recordings, the cycling-based endurance training study arm could not be considered for analyses. Data is provided as **Supplementary Material**.

Training Program

The training microcycles were designed to induce functional overreaching and decrease discipline-specific criterion performance 1 day after training, with the effects reversed on the fourth day post-overload. Two training sessions were performed per day, and on the fourth day of training, the morning session was substituted by an intermediate test of surrogate measures, resulting in a total of 11 training sessions. **Table 2** presents a general overview of the training schedules, and more details can be found in other reports (Wiewelhove et al., 2015; Raeder et al., 2016).

ST combined multi-joint high-resistance training and maximal eccentric strength training, focusing mainly on lower-body exercises (i.e., parallel squats). The training sessions lasted approximately 90 min. They started with a standardized dynamic warm-up, followed by lower-body exercises, and ended with a combination of upper-body, core, hamstring, and back exercises. Preceding the main training exercises, participants performed specific warm-up sets of 5 to 3 repetitions at 50% and 70% of individual maximum performance, respectively. Exercise intensity was standardized in relation to the estimated maximal dynamic strength (1RM) or maximum effort (**Table 2**).

HIIT included straight-line runs, straight-line sprints, and shuttle runs performed on an outdoor 400 m Tartan track. Training sessions lasted approximately 35 min. They started with a standardized 10-min continuous warm-up consisting of 40-m shuttle runs at approximately 60–70% of participants' maximum HR, followed by four 40-m acceleration sprints. Exercise intensity was standardized in relation to peak velocity during the 30–15 Intermittent Fitness Test (V_{IFT}) or maximum effort (**Table 2**).

Procedures

Performance Measures

A detailed description of the testing procedures can be found in the original publications regarding ST (Raeder et al., 2016) and HIIT (Wiewelhove et al., 2015). On the testing days Pre, Post1, and Post4, participants in the ST overload group were subjected to maximum dynamic and isometric strength tests. Criterion performance was measured by participants' 1RM for parallel squats. On the main testing days, participants in the HIIT overload group were subjected to maximum intermittent shuttle-run test and a repeated sprint ability (RSA) test. RSA was defined as the criterion performance measure.

Incremental treadmill test

An incremental treadmill test (Ergo ELG2, Woodway GmbH, Weil am Rhein, Germany) using a breath-by-breath gas collection system (ZAN600USB, nSpire Health GmbH, Oberthulba, Germany) was employed to measure $\dot{V}O_2$peak in order to characterize the participants aerobic capacity. Initial velocity was set at 8 km/h, with 2 km/h increments introduced every 3 min and a constant incline of 0.5% until voluntary exhaustion. The highest 30 s mean value was defined as the $\dot{V}O_2$peak.

TABLE 1 | Subject characteristics.

	Age	Height	Weight	BMI	$\dot{V}O_2$ peak	PTRS	Training volume	
	(years)	(cm)	(kg)	(kg/m^2)	(ml/kg/min)	(km/h)	(n/week)	(h/week)
STRENGTH TRAINING								
female ($n = 7$)	25.0 ± 1.5	167.0 ± 4.9	62.0 ± 7.0	22.2 ± 1.4	45.5 ± 4.5	14.5 ± 1.3	4.1 ± 1.3	7.9 ± 3.5
male ($n = 12$)	24.1 ± 2.2	179.9 ± 5.4	77.8 ± 6.7	24.0 ± 1.7	56.6 ± 4.8	16.6 ± 0.8	4.2 ± 1.5	7.2 ± 3.1
overall ($n = 19$)	24.4 ± 2.0	175.2 ± 8.2	71.9 ± 10.2	23.3 ± 1.8	52.5 ± 7.1	15.8 ± 1.5	4.2 ± 1.4	7.4 ± 3.2
HIGH-INTENSITY INTERVAL TRAINING								
female ($n = 9$)	27.1 ± 3.6	171.3 ± 3.8	64.0 ± 5.1	21.8 ± 1.4	53.1 ± 5.0	15.1 ± 1.6	4.0 ± 1.8	5.3 ± 3.6
male ($n = 9$)	27.0 ± 2.1	180.8 ± 5.4	73.7 ± 6.7	22.6 ± 2.7	63.5 ± 8.8	17.7 ± 2.0	4.3 ± 1.7	6.7 ± 3.5
overall ($n = 18$)	27.1 ± 2.8	176.1 ± 6.7	68.8 ± 7.6	22.2 ± 2.1	58.3 ± 8.8	16.4 ± 2.2	4.2 ± 1.7	6.0 ± 3.5

Data are shown as mean ± standard deviation. BMI, body mass index; $\dot{V}$ O_2peak, peak oxygen uptake; PTRS, peak treadmill running speed.

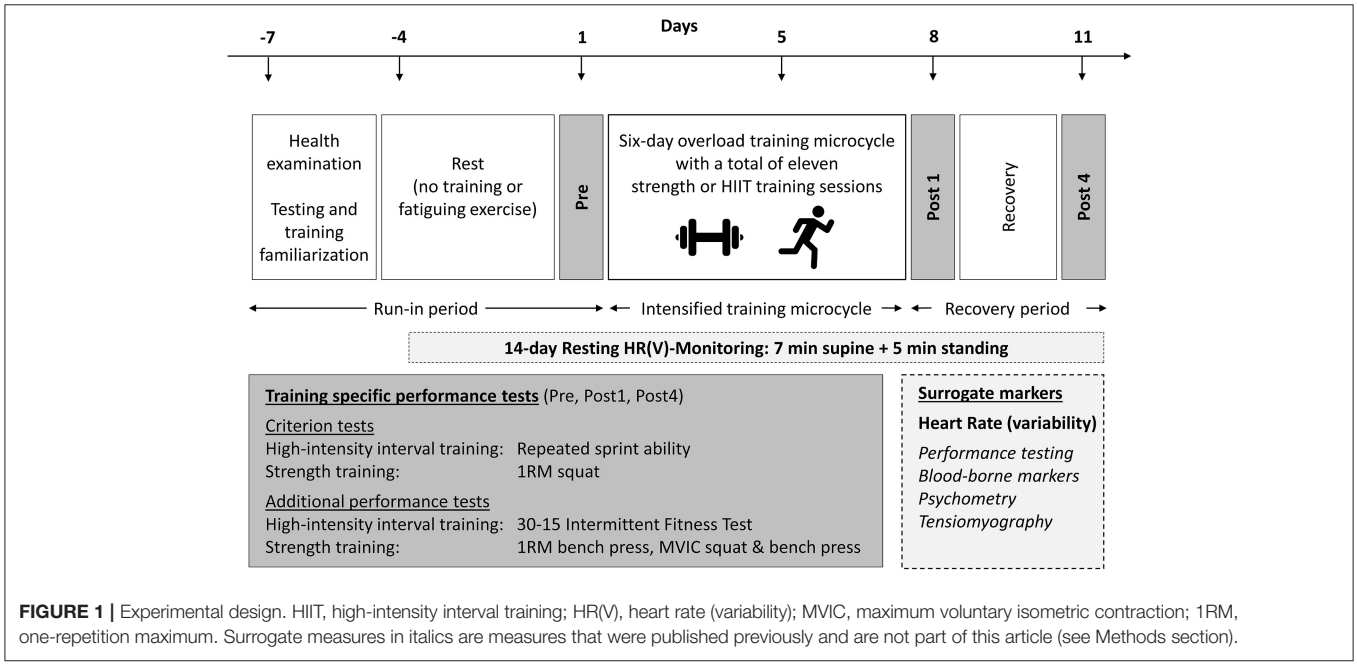

FIGURE 1 | Experimental design. HIIT, high-intensity interval training; HR(V), heart rate (variability); MVIC, maximum voluntary isometric contraction; 1RM, one-repetition maximum. Surrogate measures in italics are measures that were published previously and are not part of this article (see Methods section).

Maximum voluntary isometric contraction

Participants' maximum voluntary isometric contraction (MVIC) force output for parallel squat and bench press exercises was determined using a Multitrainer 7812-000 testing device similar to a Smith machine (Kettler Profiline, Ense-Parsit, Germany) and the corresponding user software (DigiMax, version 7.X). Joint angles were set at 90° using a goniometer and the corresponding testing device position was kept constant throughout the study. Following two submaximal practice trials at ∼50 and 70% of participants' MVIC, the participants were asked to produce a 3-s MVIC with initial slow force development. MVIC test performance was defined as the mean force of two attempts separated by a rest of 2 min.

One-repetition maximum

Participants' maximum dynamic strength was assessed 60 min after MVIC testing for parallel squat (ST criterion performance measure) and bench press exercises using a Smith rack machine (Technogym, Cesena, Italy). Squat depth was standardized using an integrated linear transducer that produced acoustic stimuli to mark the turning point of the motion. Participants completed two warm-up sets of 5 and 3 repetitions at 50 and 70% of their individual 5–10 RM, respectively. Using a formula by Brzycki (Maud and Foster, 2006), participants' 1RM was estimated based on the heaviest 5–10 RM lift within a maximum of three testing sets separated by rests of 3 min. Tests were stopped when the subjects were unable to raise the barbell using a proper technique or when the supervisors' help was required. The reliability of the 1RM squat test was previously investigated in our laboratory and was determined to be high [1RM (kg), $n = 38$, ICC = 0.96, TE 5.2 (Raeder et al., 2016)]. Participants' 1RM performance was later used to calculate the exercise intensity of the training protocols.

Repeated sprint ability

Participants' RSA (HIIT criterion performance measure) was determined using a non-motorized treadmill (Force 3.0,

TABLE 2 | Overload training microcycles.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
STRENGTH TRAINING						
a.m.	FW squats	FW squats	FW squats	Intermediate	FW squats	FW squats
	TD bench press	DS bench press	DS bench press	testing	DS bench press	DS bench press
	Hamstrings, back & core	Hamstrings, back & core	Hamstrings, back & core		Hamstrings, back & core	Hamstrings, back & core
p.m.	EO squats	TD squats	EO squats	TD squats	EO squats	TD squats
	TD squats	FW squats	TD squats	FW squats	TD squats	FW squats
	EO bench press	TD bench press	EO bench press	TD bench press	EO bench press	TD bench press
	Hamstrings, back & core	Hamstrings, back & core	Hamstrings, back & core	Hamstrings, back & core	Hamstrings, back & core	Hamstrings, back & core

Protocol	Volume (sets × repetitions)	Intensity (% 1RM)	Rest
TD	4 × 6	85	3 min
DS	1 × 6 (+3 drop sets)	85 (70-55-40)	30 s
EO	4 × 6	100 ECC-70 CON	3 min
FW	4 × 6 (+2 acc reps)	Maximum effort	3 min

HIGH-INTENSITY INTERVAL TRAINING						
a.m.	Straight-line runs	Straight-line runs	Straight-line runs	Intermediate	Straight-line runs	Straight-line runs
	4 × 4 min, 80% V_{IFT}	7 × 2 min, 85% V_{IFT}	4 × 4 min, 80% V_{IFT}	testing	4 × 4 min, 80% V_{IFT}	7 × 2 min, 85% V_{IFT}
	(r = 3 min)	(r = 2 min)	(r = 3 min)		(r = 3 min)	(r = 2 min)
p.m.	Straight-line sprints	40m-shuttle runs	Straight-line sprints	40m-shuttle runs	Straight-line sprints	40m-shuttle runs
	4 × 6 × 5 s, all out	2 × 12 × 30 s, 90% V_{IFT}	4 × 6 × 5 s, all out	2 × 12 × 30 s, 90% V_{IFT}	4 × 6 × 5 s, all out	2 × 12 × 30 s, 90% V_{IFT}
	(r = 25 s; R = 5 min)	(r = 30 s; R = 3 min)	(r = 25 s; R = 5 min)	(r = 30 s; R = 3 min)	(r = 25 s; R = 5 min)	(r = 30 s; R = 3 min)

FW, flywheel YoYo squat; TD, traditional multiple sets; DS, drop sets; EO, eccentric overload; 1RM, one-repetition maximum; ECC, eccentric; CON, concentric; acc reps, acceleration repetitions; VIFT, peak running speed obtained in the 30-15 Intermittent Fitness Test; r, passive recovery between intervals; R, passive recovery between series. Raeder et al. (2016), Wiewelhove et al. (2015) *doi: 10.1371/journal.pone.0139801.t002*

Woodway GmbH, Weil am Rhein, Germany). The participants completed a standardized warm-up prior to the trial. The test consisted of six 4-s maximal sprints beginning from a standing position with passive recovery of 20 s between sprints. The highest velocities measured for each sprint were recorded, and the mean peak velocity was calculated. The reliability of the RSA test was previously investigated in our laboratory and was determined to be high [mean peak velocity (m/s), $n = 17$, ICC = 0.92, TE 0.1 (Wiewelhove et al., 2015)].

Intermittent aerobic performance

Maximum intermittent aerobic performance was assessed based on participants' peak running speed (V_{IFT}) during the 30-15 Intermittent Fitness Test (Buchheit, 2008). The test was conducted on an outdoor Tartan track. Participants were tasked with running back and forth between two lines set 40 m apart. The shuttle runs were 30 s with 15 s of passive recovery between each run. The initial running speed was set at 8 km/h, with stepped increases of 0.5 km/h every 45 s. Running speed was declared using audio signals, and V_{IFT} was defined as the velocity of the last completed stage. V_{IFT} was later used to calculate the exercise intensity of the training protocols.

Heart Rate and Heart Rate Variability

Every morning, an active orthostatic test (7 min supine, 5 min standing) was performed after participants' awoke and emptied their bladder throughout the main 14-day study period. During recordings, the participants were asked to leave their eyes open, breathe calmly, and avoid movement. A general briefing and written guidelines for the orthostatic test were provided before the beginning of the study (see **Supplementary Material** for details). R-R series were recorded using Polar RS800cx heart rate monitors (Polar Electro, Kempele, Finland), and the data were transferred to the software Polar Pro Trainer 5 (version 5.40.170, Polar Electro, Kempele, Finland). Polar files (.hrm) were then exported and used for further processing. HR, the natural logarithm of RMSSD (Ln RMSSD) and the Ln RMSSD to R-R interval ratio (Ln RMSSD/RR = Ln RMSSD divided by the mean R-R interval) were calculated during the last 5 min for which participants were supine and the 5-min standing measurements using Kubios (version 2.2, Biosignal Analysis and Medical Imaging Group, University of Eastern Finland, Finland) (Tarvainen et al., 2014). The Ln RMSSD, which is considered to be measure of vagal-mediated HRV (at least in the acute-term) (Malik et al., 1996; Carter et al., 2003), was chosen as the primary HRV marker because it features high reliability (Al Haddad et al., 2011) and is less affected by different breathing patterns compared to spectral analysis (Penttilä et al., 2001; Saboul et al., 2013). Additionally, average HR was calculated (Plews et al., 2013; Buchheit, 2014) to further allow comparison of whether HRV is more sensitive to overload- and recovery-related changes than HR. It remains unclear whether HRV is more sensitive than HR to changes in athletes' training status (Billman et al., 2015b; Schneider et al., 2018). Finally, as previously proposed,

the Ln RMSSD/RR was determined to gain further insights into the association between HRV and average HR (Plews et al., 2013; Buchheit, 2014; Billman et al., 2015b; Trimmel et al., 2015, Editorial).

Data Analysis and Statistical Analysis

Data are presented as mean ± standard deviation (SD) unless otherwise specified. For our statistical analyses, we used Microsoft Excel 2016 (Microsoft Office 365, Version 1810, Microsoft Corp., Redmond, WA, USA) for basic calculations and descriptive statistics and the free open-source software JASP (Version 0.8.6, Amsterdam, Netherlands) (JASP Team, 2018) for inferential procedures. The Shapiro-Wilks test was used to verify the assumed normal distribution of data. Statistical analyses are provided as JASP files (.jasp) in the **Supplementary Material**.

The day-to-day reliability of resting HR(V) indices were assessed using specifically designed spreadsheets (Hopkins, 2015b). The typical error (TE) was selected as the reliability statistic of interest and was calculated by dividing the standard deviation (SD) of day-to-day differences (SD) by $\sqrt{2}$ pooled for the four-day baseline period (i.e., the differences between days 1 and 2, 2 and 3, and 3 and 4) (Hopkins et al., 2001). The group-based smallest worthwhile change (SWC) was defined as 0.2 × between-subject SD for pooled baseline measurements (Hopkins et al., 2009). TE and SWC were calculated as absolute and percentage values. The spreadsheets used for the assessment are provided as **Supplementary Material**.

Differences between the three main time points (Pre, Post1, and Post4) were tested by repeated measures analysis of variance (ANOVA; repeated factor: time; grouping factor: sex) including sex as grouping factor to determine possible sex differences in HR(V) responses (Aubert et al., 2003; Sandercock et al., 2005). The violation of sphericity was adjusted by Greenhouse-Geisser correction. Bonferroni-adjusted p-values (p_{bonf}) are reported for pairwise comparisons. The level of significance was set at $p \leq 0.05$. Further, paired t-tests were used to calculate 90% confidence limits (CL) for the mean differences and standardized effect sizes (d). The magnitude of change (d_{pre}) was evaluated using the between-subject Pre-test SD. Then, d_{pre} and 90% CL were calculated in MS Excel and adjusted for the sample size using the following formula (Cumming, 2012, p. 294):

$$d \text{ unbiased} = \left(1 - \frac{3}{4\,df - 1}\right) \times d \quad (1)$$

where df is the degree of freedom of the SD estimate ($df = n-1$). The threshold values for d_{pre} were >0.2 (small), >0.6 (moderate), and >1.2 (large) (Hopkins et al., 2009).

To evaluate the consistency of within-subject changes (d_{diff}) between the different measures used within our study, mean differences were standardized according to the SD of differences (Dankel and Loenneke, 2018). Then, d_{diff} and 90% CL were calculated with JASP.

The Pearson correlation (r) and 90% CL were used to evaluate the associations between changes in HR(V) and performance measures as well as between HR and Ln RMSSD. In accordance with previous analyses (Plews et al., 2014), percentage changes from the previous values were correlated for changes between Pre, Post1, and Post4 with JASP. Individual HR and Ln RMSSD time series were correlated with Microsoft Excel. Threshold values for r were >0.1 (small), >0.3 (moderate), and >0.5 (large) (Hopkins et al., 2009).

The mean differences and correlations were calculated for changes in daily HR(V) measures and the 2-, 3-, and 4-day rolling averages. When the 90% confidence intervals overlapped small positive and negative values, the effects were deemed *unclear* (Hopkins et al., 2009).

Individual responses were classified as *likely* to be increased or decreased when changes exceeded the TE. Therefore, this category (i.e., "likely") includes changes for which the approximate 50% confidence interval associated with the observed change (i.e., ±TE) does not include zero change (Hopkins, 2004; Swinton et al., 2018). If changes in ±TE occurred, individual responses were classified as *unclear*. Group-based TE (Wiewelhove et al., 2015; Raeder et al., 2016) was used to assess performance changes, and individual TE (i.e., 4-day baseline SD) was used to classify changes in HR(V) indices. Subsequently, 3 × 3 tables were created to descriptively evaluate the categorial agreement between changes in performance (i.e., criterion) and HR(V) measures (i.e., surrogate) as well as the agreement between changes in HR and Ln RMSSD when using single-day values or 4-day averages, respectively. The evaluation of categorical agreement (i.e., 3 × 3 tables) refers to the commonly proposed threshold-based approaches to assess individual HR(V) changes, for example using the TE or SWC as cut-off values for decision-making (Plews et al., 2013; Buchheit, 2014), and is aimed to complement the assessment of continuous association (i.e., correlations) between HR(V) and performance measures.

RESULTS

Statistically significant sex differences were apparent (main effect of sex: $p \leq 0.05$) in the performance data (ST, HIIT) as well as the standing HR (ST, HIIT) and Ln RMSSD (ST) values. In absence of clear interactions between time and sex, statistical analyses were conducted only for pooled data.

Baseline Recordings and Missing Data

During the baseline period, one supine and/or standing HR(V) recording was missing for three ST and four HIIT participants. Another HR(V) recording for the day following Post1 was missing for one ST participant. This missing data slightly affected the 3- and 4-day rolling averages, but not the degrees of freedom for the analyses.

Descriptively, the day-to-day reliability of HR and Ln RMSSD, expressed as the typical error (TE in %), was larger when the participants were standing compared to supine in the ST group (HR: +2.1%; Ln RMSSD: +1.3%), HIIT group (HR: +2.3%; Ln RMSSD: +5.1%), and pooled data of the ST and HIIT groups (HR: +2.0%; Ln RMSSD: +3.2%) (**Table 3**). Baseline HR (i.e., 4-day average) was slightly lower for the HIIT group compared to the ST group in a supine position [difference: −3 bpm, smallest worthwhile change (SWC): 1–2 bpm]. However, it was slightly higher when participants were in a standing position (+2 bpm). Differences in Ln RMSSD remained below the SWC (**Tables 3–5**).

TABLE 3 | Day-to-day reliability and smallest worthwhile change for resting heart rate (variability) measures during the 4-day baseline period.

		Typical error						Smallest worthwhile change					
SUPINE RECORDINGS													
HR	ST	3	(3; 4)	bpm	5.7	(4.8; 7.1)	%	1	(1; 2)	bpm	2.5	(2.0; 3.5)	%
	HIIT	3	(2; 4)	bpm	4.9	(4.2; 6.3)	%	2	(1; 2)	bpm	2.6	(2.0; 3.6)	%
	pooled	3	(3; 4)	bpm	5.4	(4.8; 6.3)	%	2	(1; 2)	bpm	2.6	(2.1; 3.2)	%
Ln RMSSD	ST	0.24	(0.20; 0.29)	ms	6.0	(5.1; 7.5)	%	0.11	(0.08; 0.15)	ms	2.7	(2.1; 3.7)	%
	HIIT	0.21	(0.17; 0.26)	ms	6.1	(5.1; 7.7)	%	0.11	(0.09; 0.16)	ms	3.1	(2.4; 4.3)	%
	pooled	0.22	(0.20; 0.26)	ms	6.0	(5.3; 7.0)	%	0.11	(0.09; 0.13)	ms	2.8	(2.4; 3.5)	%
Ln RMSSD/RR	ST	0.21	(0.18; 0.26)	$\times 10^3$	5.2	(4.4; 6.4)	%	0.09	(0.07; 0.12)	$\times 10^3$	2.1	(1.7; 2.9)	%
	HIIT	0.28	(0.23; 0.35)	$\times 10^3$	7.4	(6.2; 9.4)	%	0.09	(0.07; 0.12)	$\times 10^3$	2.3	(1.8; 3.2)	%
	pooled	0.26	(0.23; 0.30)	$\times 10^3$	6.7	(5.9; 7.8)	%	0.09	(0.07; 0.11)	$\times 10^3$	2.2	(1.9; 2.8)	%
STANDING RECORDINGS													
HR	ST	6	(5; 8)	bpm	7.8	(6.6; 9.7)	%	2	(2; 3)	bpm	2.6	(2.1; 3.7)	%
	HIIT	6	(5; 8)	bpm	7.2	(6.1; 9.2)	%	3	(2; 4)	bpm	3.5	(2.7; 4.9)	%
	pooled	6	(5; 7)	bpm	7.5	(6.6; 8.7)	%	2	(2; 3)	bpm	3.0	(2.5; 3.8)	%
Ln RMSSD	ST	0.24	(0.21; 0.30)	ms	7.2	(6.1; 9.0)	%	0.12	(0.09; 0.16)	ms	3.6	(2.9; 5.1)	%
	HIIT	0.27	(0.23; 0.34)	ms	11.1	(9.4; 14.2)	%	0.14	(0.11; 0.20)	ms	5.6	(4.4; 8.0)	%
	pooled	0.25	(0.22; 0.29)	ms	9.1	(8.1; 10.6)	%	0.13	(0.11; 0.16)	ms	4.7	(3.9; 5.9)	%
Ln RMSSD/RR	ST	0.28	(0.24; 0.35)	$\times 10^3$	6.6	(5.6; 8.2)	%	0.12	(0.09; 0.16)	$\times 10^3$	2.7	(2.1; 3.7)	%
	HIIT	0.22	(0.19; 0.28)	$\times 10^3$	6.5	(5.5; 8.2)	%	0.13	(0.11; 0.19)	$\times 10^3$	3.6	(2.8; 5.1)	%
	pooled	0.25	(0.23; 0.29)	$\times 10^3$	6.5	(5.7; 7.5)	%	0.13	(0.11; 0.16)	$\times 10^3$	3.2	(2.7; 4.0)	%

Data are shown as mean values and 90% confidence interval. Reliability statistics were calculated for baseline measurements using specifically designed spreadsheet (Hopkins, 2015b). HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; Ln RMSSD/RR, Ln RMSSD to R-R interval ratio; ST, strength training (n = 19); HIIT, high-intensity interval training (n = 18), pooled (n = 37). Typical error: Standard deviation of day-to-day differences divided by $\sqrt{2}$ pooled for the 4-day baseline period. Smallest worthwhile change: 0.2 × pooled between-subject SD for baseline measurements.

Performance

Participants' 1RM performance in the squat exercise (i.e., criterion) was slightly decreased at Post1 by −4.4 kg (90% CL, −7.8; −1.0 kg) and was increased at Post4 by +4.0 kg (1.0; 7.1 kg). A statistically non-significant main effect was revealed for time ($p = 0.097$). Maximum dynamic and isometric bench press performance showed statistically significant but marginal changes over time (main time effects: $p \leq 0.005$, $|d_{pre}| \leq 0.10$; **Table 4**). Repeated sprint ability and maximum intermittent aerobic performance showed small changes over time (main time effects: $p \leq 0.005$, $|d_{pre}| \geq 0.27$). The mean peak velocity in the RSA test (i.e., criterion) was decreased at Post1 by −0.18 m/s (−0.23; −0.12 m/s) and was increased at Post4 by +0.18 m/s (0.08; 0.27 m/s; **Table 5**).

Time Course of HR(V) Measures

For the ST group, daily resting HR increased above the SWC from second day of training to Post1 when participants were in a supine position (effect magnitude range for single day and 2- to 4-day averaged values, ΔPre to Post1: $d_{pre} = 0.36$ to 0.50) and decreased at Post4 (ΔPost1 to Post4: $d_{pre} = -0.30$ to −0.40). The mean supine Ln RMSSD showed an inverse response (ΔPre to Post1: $d_{pre} = -0.43$ to −0.51; ΔPost1 to Post4: $d_{pre} = 0.19$ to 0.32; **Table 4**). For the HIIT group, daily HR was decreased beyond the SWC from the fourth day of training in the standing position (ΔPre to Post1: $d_{pre} = -0.50$ to −0.59) and increased after Post1 (ΔPost1 to Post4: $d_{pre} = 0.09$ to 0.31). The mean standing Ln RMSSD showed an inverse response (ΔPre to Post1: $d_{pre} = 0.38$ to 0.47; ΔPost1 to Post4: $d_{pre} = -0.06$ to −0.20; **Table 5**). Changes in Ln RMSSD/RR were unclear for both groups and for both recording positions (see **Supplementary Tables 1, 2** for detailed results).

An overview of the time course of mean HR(V) measures is presented in **Figure 2** and the mean changes are presented in **Tables 4–5** (see **Supplementary Tables 1–2** for extensive results). Averaged HR(V) values yielded more precise interval estimates for mean changes (i.e., smaller confidence intervals and larger d_{diff}) compared to single-day values (**Figure 3**, **Tables 4–5**, **Supplementary Figure 1**, **Supplementary Tables 1, 2**).

Association Between Changes in Performance and HR(V)

For both the ST and HIIT groups, the majority of correlation analyses between performance and HR(V) changes were unclear, or inconsistent for changes from Pre to Post1 compared to changes from Post1 to Post4. Moreover, the effect of using average HR(V) values compared to single-day values remained unclear for estimate precision (confidence intervals) and the magnitude (r) of correlations. The detailed results of the analyses are presented in **Supplementary Figures 2, 3** and **Supplementary Tables 3, 4**.

TABLE 4 | Performance and heart rate (variability) measures at Pre, Post1, Post4, and changes between testing days for the strength training microcycle (7 female, 12 male).

	Pre	Post1	Post4	Δ Pre to Post1				Δ Post1 to Post4				Time
	Mean ± SD	Mean ± SD	Mean ± SD	Δ ± SD	d_{pre} (90% CL)	d_{diff}	p_{bonf}	Δ ± SD	d_{pre} (90% CL)	d_{diff}	p_{bonf}	*p*
1RM squat (kg)												
Female	69.1 ± 11.6	66.9 ± 12.0	71.5 ± 15.6									
Male	117.2 ± 23.3	111.4 ± 23.8	115.1 ± 20.3									
Overall	99.5 ± 30.8	95.0 ± 29.7	99.1 ± 28.3	−4.4 ± 8.6	−0.14 (−0.24; −0.03)	−0.52	0.109	4.0 ± 7.6	0.13 (0.03; 0.22)	0.53	0.097	0.097
MVIC squat (N)												
Female	952 ± 120	912 ± 81	945 ± 124									
Male	1632 ± 413	1681 ± 370	1705 ± 419									
Overall	1381 ± 472	1398 ± 481	1425 ± 505	17 ± 187	0.03 (−0.12; 0.18)	0.09	1.000	27 ± 191	0.06 (−0.10; 0.21)	0.14	1.000	0.599
1RM bench (kg)												
Female	41.9 ± 5.6	41.9 ± 6.7	43.3 ± 7.5									
Male	96.2 ± 16.1	94.2 ± 17.8	97.4 ± 16.7									
OVERALL	76.2 ± 29.9	75.0 ± 29.7	77.4 ± 30.1	−1.2 ± 3.2	−0.04 (−0.08; 0.00)	−0.38	0.356	2.5 ± 3.2	0.08 (0.04; 0.12)	0.78	0.010	0.038
MVIC bench (N)												
Female	562 ± 52	539 ± 59	556 ± 62									
Male	1210 ± 197	1164 ± 217	1191 ± 234									
Overall	971 ± 357	933 ± 355	957 ± 366	−38 ± 54	−0.10 (−0.16; −0.04)	−0.70	0.021	24 ± 37	0.06 (0.02; 0.10)	0.63	0.039	0.031
SUPINE RECORDING												
HR (bpm)												
Single-day	60 ± 8	64 ± 9	61 ± 7	3 ± 7	0.40 (0.06; 0.74)	0.46	0.176	−3 ± 7	−0.34 (−0.65; −0.02)	−0.43	0.236	0.169
2-day avg	61 ± 8	64 ± 9	61 ± 7	3 ± 6	0.36 (0.08; 0.64)	0.50	0.125	−3 ± 5	−0.40 (−0.63; −0.17)	−0.69	0.024	0.048
3-day avg	61 ± 7	64 ± 8	61 ± 7	3 ± 5	0.44 (0.19; 0.68)	0.70	0.021	−3 ± 3	−0.40 (−0.58; −0.22)	−0.87	0.004	0.006
4-day avg	60 ± 7	64 ± 8	62 ± 7	3 ± 4	0.50 (0.28; 0.73)	0.88	0.004	−2 ± 2	−0.30 (−0.39; −0.20)	−1.28	<0.001	0.005
Ln RMSSD (ms)												
Single-day	4.32 ± 0.45	4.08 ± 0.61	4.20 ± 0.46	−0.24 ± 0.42	−0.51 (−0.87; −0.16)	−0.57	0.069	0.12 ± 0.45	0.26 (−0.12; 0.64)	0.27	0.758	0.074
2-day avg	4.28 ± 0.48	4.06 ± 0.57	4.23 ± 0.47	−0.22 ± 0.30	−0.43 (−0.67; −0.20)	−0.73	0.016	0.16 ± 0.30	0.32 (0.08; 0.56)	0.54	0.092	0.018
3-day avg	4.30 ± 0.48	4.08 ± 0.54	4.22 ± 0.45	−0.22 ± 0.23	−0.44 (−0.62; −0.26)	−0.98	0.001	0.14 ± 0.24	0.28 (0.09; 0.47)	0.58	0.063	0.001
4-day avg	4.31 ± 0.46	4.09 ± 0.51	4.18 ± 0.47	−0.22 ± 0.18	−0.45 (−0.60; −0.30)	−1.19	<0.001	0.09 ± 0.11	0.19 (0.10; 0.28)	0.86	0.004	<0.001
STANDING RECORDING												
HR (bpm)												
Single-day	83 ± 10	83 ± 10	85 ± 11	−1 ± 11	−0.07 (−0.51; 0.37)	−0.06	1.000	3 ± 11	0.26 (−0.16; 0.67)	0.25	0.887	0.200
2-day avg	83 ± 9	82 ± 8	83 ± 9	0 ± 6	−0.04 (−0.28; 0.20)	−0.07	1.000	1 ± 7	0.13 (−0.17; 0.44)	0.18	1.000	0.150
3-day avg	82 ± 9	82 ± 8	83 ± 8	0 ± 5	0.01 (−0.21; 0.22)	0.01	1.000	0 ± 5	0.03 (−0.19; 0.25)	0.05	1.000	0.672
4-day avg	82 ± 9	82 ± 8	83 ± 8	1 ± 5	0.07 (−0.14; 0.29)	0.14	1.000	0 ± 4	0.03 (−0.13; 0.19)	0.07	1.000	0.584
Ln RMSSD (ms)												
Single-day	3.41 ± 0.46	3.31 ± 0.64	3.22 ± 0.52	−0.09 ± 0.43	−0.19 (−0.54; 0.17)	−0.21	1.000	−0.10 ± 0.45	−0.21 (−0.57; 0.16)	−0.22	1.000	0.160
2-day avg	3.37 ± 0.49	3.35 ± 0.51	3.27 ± 0.52	−0.03 ± 0.19	−0.05 (−0.20; 0.10)	−0.13	1.000	−0.07 ± 0.29	−0.14 (−0.37; 0.08)	−0.26	0.837	0.234
3-day avg	3.37 ± 0.52	3.35 ± 0.55	3.31 ± 0.52	−0.02 ± 0.18	−0.05 (−0.17; 0.08)	−0.14	1.000	−0.04 ± 0.21	−0.06 (−0.22; 0.09)	−0.16	1.000	0.529
4-day avg	3.41 ± 0.52	3.35 ± 0.54	3.31 ± 0.53	−0.06 ± 0.17	−0.11 (−0.24; 0.02)	−0.34	0.460	−0.03 ± 0.15	−0.06 (−0.17; 0.05)	−0.21	1.000	0.190

Heart rate (variability) measures are provided as single-day values and 2-day to 4-day rolling averages. Data are shown as mean ± SD and 90% confidence limits. 1RM, estimated one-repetition maximum; MVIC, maximum voluntary isometric contraction; HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; d_{pre}, standardized mean difference using sample-size adjusted, between-subject SD at Pre; d_{diff}, standardized mean difference using SD of differences; p_{bonf}, Bonferroni adjusted p-values.

TABLE 5 | Performance and heart rate (variability) measures at Pre, Post1, Post4, and changes between testing days for the high-intensity interval training microcycle (9 female, 9 male).

	Pre	Post1	Post4	Δ Pre to Post1				Δ Post1 to Post4				Time
	Mean ± SD	Mean ± SD	Mean ± SD	Δ ± SD	d_{pre} (90% CL)	d_{diff}	p_{bonf}	Δ ± SD	d_{pre} (90% CL)	d_{diff}	p_{bonf}	*p*
RSA (m/s)												
Female	4.53 ± 0.19	4.32 ± 0.20	4.57 ± 0.38									
Male	5.49 ± 0.31	5.34 ± 0.28	5.44 ± 0.27									
Overall	5.01 ± 0.55	4.83 ± 0.58	5.01 ± 0.55	−0.18 ± 0.13	−0.31 (−0.40; −0.22)	−1.37	<0.001	0.18 ± 0.23	0.30 (0.14; 0.47)	0.75	0.016	0.005
V_{IFT} (km/h)												
Female	17.4 ± 1.8	16.8 ± 1.3	17.3 ± 2.1									
Male	20.2 ± 1.3	19.3 ± 1.4	20.0 ± 1.4									
Overall	18.8 ± 2.1	18.1 ± 1.9	18.6 ± 2.2	−0.8 ± 0.7	−0.35 (−0.49; −0.22)	−1.06	<0.001	0.6 ± 0.8	0.27 (0.12; 0.41)	0.76	0.016	0.001
SUPINE RECORDING												
HR (bpm)												
Single-day	59 ± 8	57 ± 9	55 ± 8	−1 ± 4	−0.15 (−0.34; 0.05)	−0.30	0.667	−2 ± 4	−0.24 (−0.45; −0.03)	−0.47	0.182	0.004
2-day avg	58 ± 9	59 ± 8	55 ± 8	0 ± 3	0.05 (−0.09; 0.18)	0.14	1.000	−3 ± 3	−0.38 (−0.50; −0.26)	−1.28	<0.001	<0.001
3-day avg	58 ± 8	59 ± 9	56 ± 8	1 ± 3	0.16 (0.01; 0.31)	0.43	0.252	−3 ± 2	−0.40 (−0.50; −0.31)	−1.73	<0.001	<0.001
4-day avg	57 ± 7	59 ± 8	56 ± 8	1 ± 3	0.19 (0.05; 0.34)	0.56	0.090	−3 ± 2	−0.39 (−0.48; −0.29)	−1.69	<0.001	<0.001
Ln RMSSD (ms)												
Single day	4.34 ± 0.59	4.38 ± 0.46	4.32 ± 0.51	0.04 ± 0.34	0.06 (−0.17; 0.29)	0.11	1.000	−0.06 ± 0.31	−0.10 (−0.30; 0.11)	−0.19	1.000	0.733
2-day avg	4.26 ± 0.62	4.30 ± 0.50	4.38 ± 0.48	0.04 ± 0.23	0.06 (−0.08; 0.21)	0.18	1.000	0.07 ± 0.27	0.12 (−0.05; 0.28)	0.28	0.765	0.167
3-day avg	4.30 ± 0.56	4.28 ± 0.52	4.37 ± 0.48	−0.02 ± 0.18	−0.04 (−0.17; 0.09)	−0.13	1.000	0.09 ± 0.23	0.15 (−0.01; 0.31)	0.39	0.339	0.182
4-day avg	4.31 ± 0.52	4.26 ± 0.53	4.37 ± 0.46	−0.05 ± 0.18	−0.09 (−0.22; 0.04)	−0.27	0.784	0.11 ± 0.21	0.20 (0.04; 0.36)	0.51	0.138	0.074
STANDING RECORDING												
HR (bpm)												
Single-day	84 ± 13	76 ± 11	80 ± 13	−8 ± 11	−0.56 (−0.88; −0.25)	−0.74	0.018	4 ± 10	0.31 (0.02; 0.61)	0.44	0.242	0.004
2-day avg	84 ± 13	76 ± 10	80 ± 13	−8 ± 8	−0.59 (−0.82; −0.36)	−1.06	<0.001	4 ± 9	0.27 (0.01; 0.52)	0.43	0.253	<0.001
3-day avg	84 ± 13	76 ± 10	79 ± 12	−8 ± 9	−0.56 (−0.81; −0.31)	−0.91	0.004	2 ± 7	0.17 (−0.02; 0.36)	0.36	0.423	<0.001
4-day avg	84 ± 13	77 ± 9	78 ± 11	−7 ± 9	−0.50 (−0.76; −0.24)	−0.80	0.011	1 ± 6	0.09 (−0.08; 0.26)	0.22	1.000	0.002
Ln RMSSD (ms)												
Single-day	3.20 ± 0.59	3.48 ± 0.57	3.36 ± 0.53	0.29 ± 0.36	0.47 (0.23; 0.70)	0.80	0.011	−0.12 ± 0.36	−0.20 (−0.44; 0.04)	−0.34	0.485	0.007
2-day avg	3.16 ± 0.63	3.44 ± 0.51	3.34 ± 0.53	0.27 ± 0.35	0.41 (0.20; 0.63)	0.78	0.012	−0.09 ± 0.39	−0.14 (−0.39; 0.10)	−0.24	0.964	0.015
3-day avg	3.15 ± 0.64	3.44 ± 0.49	3.36 ± 0.52	0.28 ± 0.38	0.42 (0.18; 0.65)	0.73	0.020	−0.07 ± 0.29	−0.11 (−0.28; 0.07)	−0.26	0.879	0.008
4-day avg	3.18 ± 0.64	3.43 ± 0.49	3.39 ± 0.52	0.25 ± 0.37	0.38 (0.15; 0.60)	0.69	0.029	−0.04 ± 0.24	−0.06 (−0.20; 0.09)	−0.16	1.000	0.009

Heart rate (variability) measures are provided as single-day values and 2-day to 4-day rolling averages. Data are shown as mean ± SD and 90% confidence limits. RSA, mean peak velocity of the repeated sprint ability test; V_{IFT}, peak running speed of the 30–15 Intermittent Fitness Test; HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; d_{pre}, standardized mean difference using sample-size adjusted, between-subject SD at Pre; d_{diff}, standardized mean difference using SD of differences; p_{bonf}, Bonferroni adjusted p-values.

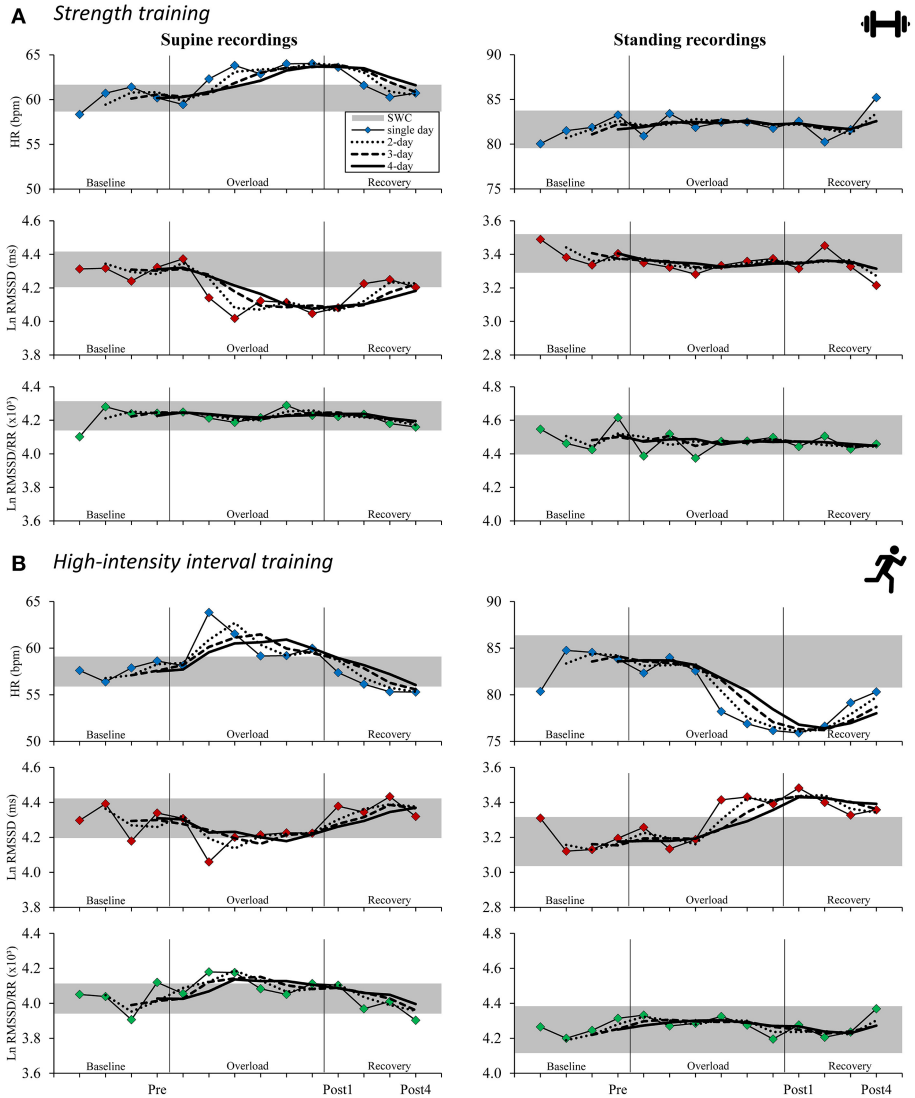

FIGURE 2 | Time course for supine (left) and standing (right) mean resting heart rate (variability) measures in the strength training **(A)** and the high-intensity interval training **(B)** study arms for isolated daily values and rolling 2–4-day averages. Gray horizontal bar: smallest worthwhile change (0.2 × pooled between-subject SD for baseline measurements; see **Table 3** for details); single-day: thin lines with colored markings; 2-day rolling average: dotted lines; 3-day rolling average: dashed lines; 4-day rolling average: bold lines. HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; Ln RMSSD/RR, Ln RMSSD to R-R interval ratio.

Individual HR(V) Responses

Individual HR(V) responses are presented as single-day and 4-day average values and in reference to the TE as a threshold for response classification for the recording position that was sensitive to group changes (ST: supine recordings; HIIT: standing recordings).

Within the ST group (supine recordings, $n = 19$), Pre to Post1 changes in single-day HR were likely (i.e., beyond ± individual TE) increased in 10 athletes and likely decreased in 3 athletes. Ln RMSSD was likely decreased in 9 athletes and increased in 1 athlete. Of the 10 athletes whose squat 1RM (criterion performance) decreased below group-based TE (4.9%), 5 had likely increased HR and 5 had likely decreased Ln RMSSD. Regarding Pre to Post1 changes in 4-day HR(V) averages, 10 athletes showed likely increased HR and 1 athlete showed likely decreased HR. Ln RMSSD was likely decreased in 8 athletes. Of the 10 athletes whose squat 1RM decreased, 6 had likely increased HR and 4 had likely decreased Ln RMSSD. When classifying individual responses in three categories (likely increased, unclear, likely decreased), changes in squat 1RM and HR(V) agreed with the direction of group changes in the HR of 8 and 9 athletes (single-day and 4-day average, respectively) and in the Ln RMSSD of 9 and 7 athletes (single-day and 4-day average, respectively). In **Table 6**, blue and gray values represent agreement.

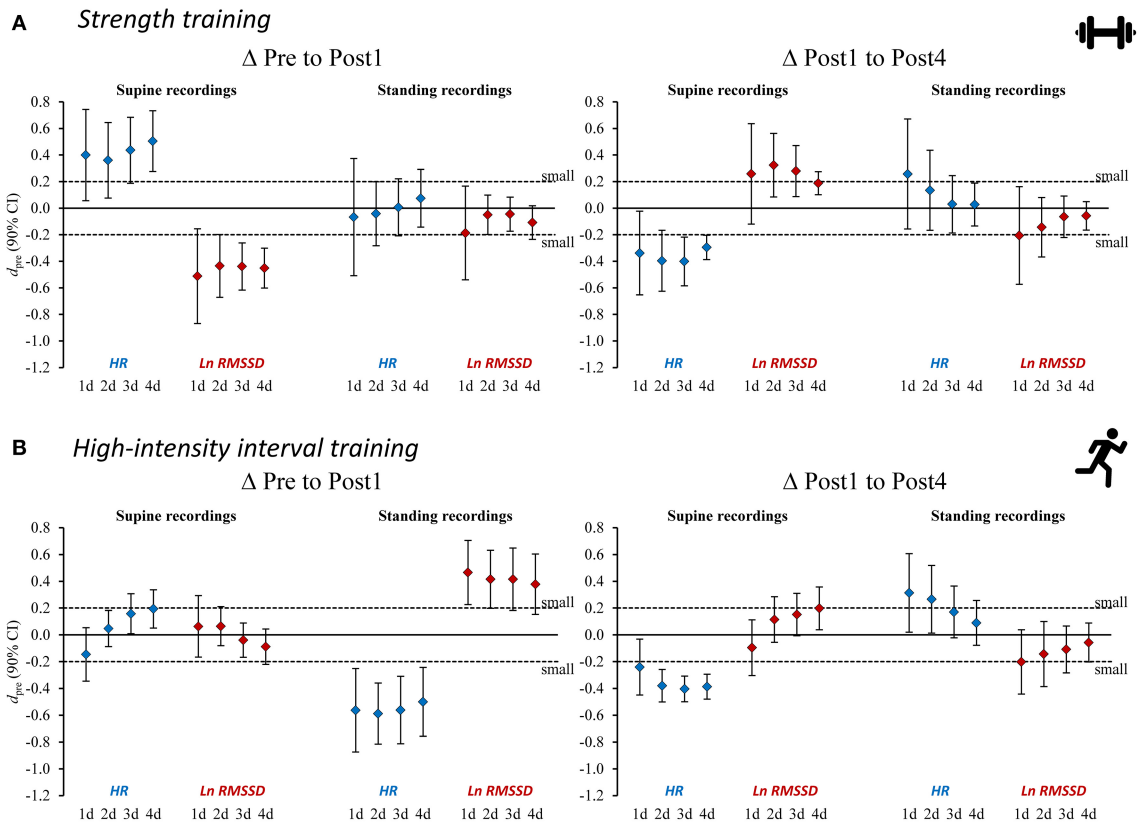

FIGURE 3 | Standardized mean differences (d_{pre}) for changes in heart rate (variability) measures from Pre to Post1 and from Post 1 to Post4 for **(A)** strength training and **(B)** high-intensity interval training. Heart rate (variability) measures are provided as single-day values and 2–4-day rolling averages. HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals.

For the HIIT group (standing recordings, $n = 18$), Pre to Post1 changes in single-day HR was likely decreased in 12 athletes and likely increased in 2 athletes. Ln RMSSD was likely increased in 9 athletes and decreased in 2 athletes. Of the 15 athletes whose RSA (criterion performance) decreased below group-based TE (1.8%), 10 had likely decreased HR and 8 had likely increased Ln RMSSD. Regarding Pre to Post1 changes in 4-day average HR(V), 9 athletes showed likely decreased HR and 1 athlete showed likely increased HR. Ln RMSSD was likely increased in 8 athletes and likely decreased in 2 athletes. Of the 15 athletes whose RSA decreased, 8 had likely decreased HR and 8 had likely increased Ln RMSSD. When classifying individual responses in three categories (likely increased, unclear, likely decreased), changes in RSA and HR(V) agreed with the direction of group changes in the HR of 11 and 10 athletes (single-day and 4-day average, respectively) and in the Ln RMSSD of 10 and 11 athletes (single-day and 4-day average, respectively). In **Table 6**, red and gray values represent agreement. Individual responses within the HIIT group (standing recordings) are detailed in **Figure 4**.

In the ST group (supine recordings), categorial associations between changes in HR and Ln RMSSD showed agreement (i.e., likely increased HR and likely decreased Ln RMSSD, and vice versa) for 11 of 19 athletes (ΔPre to Post1, ΔPost1 to Post4) according to single-day measures. The correlations between HR and Ln RMSSD were moderate to large for changes between main testing days ($r = -0.33$ to -0.77) and large for individual time series (mean $r = -0.61$ to -0.67). In the HIIT group (standing recordings), categorial associations between changes in single-day HR and Ln RMSSD showed agreement for 13 of 18 athletes (ΔPre to Post1) and 12 of 18 athletes (ΔPost1 to Post4). Correlations between HR and Ln RMSSD were trivial to moderate for changes in supine recordings ($r = -0.35$ to 0.06), large for changes in standing recordings ($r = -0.58$ to -0.76), and large for individual time series (mean $r = -0.50$ to -0.78). Most of the time, use of 4-day averages resulted in higher overall categorial agreement and mostly increased agreement for *unclear* changes (i.e., HR and Ln RMSSD changes within ±TE). In **Table 7**, blue and gray values indicate agreement. A full account of individual responses is provided in the **Supplementary Figures 4, 5** and **Supplementary Tables 5–7**.

DISCUSSION

The purpose of this study was to characterize changes in HR and vagal HRV in response to ST and HIIT overload

TABLE 6 | Example 3 × 3 tables for individual response classification for changes from Pre to Post1 of criterion performance and resting heart rate (variability) measures using single-day and 4-day average values.

Strength training

			Supine recordings					
			HR			Ln RMSSD		
Single-day	1RM		−	o	+	−	o	+
	−	10	2	3	5	5	4	1
	o	7		2	5	3	4	
	+	2	1	1		1	1	
4-day average			HR			Ln RMSSD		
	1RM		−	o	+	−	o	+
	−	10	1	3	6	4	6	
	o	7		3	4	4	3	
	+	2		2			2	

High-intensity interval training

			Standing recordings					
			HR			Ln RMSSD		
Single-day	RSA		−	o	+	−	o	+
	−	15	10	3	2	2	5	8
	o	3	2	1			2	1
	+							
4-day average			HR			Ln RMSSD		
	RSA		−	o	+	−	o	+
	−	15	8	6	1	2	5	8
	o	3	1	2			3	
	+							

Data in the 3 × 3 tables represent the number of observed individual responses classified for 9 response types (3 response types for criterion and heart rate (variability) measures, respectively). HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; 1RM, one-repetition maximum in the squat; RSA, repeated sprint ability; –, observed reduction > typical error (TE); o, observed changes < TE; +, observed increase > TE. Group-based TE were used for criterion measures (see Wiewelhove et al., 2015; Raeder et al., 2016) and individual TE was used for heart rate measures (individual SD during 4-day baseline period).

and short-term recovery. The main finding was that the HR(V) patterns identified during active orthostatic testing displayed altered supine HR(V) following ST while standing measures remained stable. Following HIIT, standing HR(V) measures were deflected while supine measures remained unchanged. Both continuous and categorical associations between changes in HR(V) indices and performance changes were weak or unclear. Further, our data suggested that the use of rolling averages may improve statistical sensitivity to group changes compared to the use of single-day HR(V). However, in the present study, average values appeared to be detrimental for assessing individual short-term responses when using the TE as a response threshold, as the magnitude of day-to-day changes was reduced. This may compromise sensitivity by decreasing signal-to-noise ratio, resulting in a decreased magnitude of change compared to the baseline variability.

Evidence of Autonomic Modulation in Response to Short-Term ST and HIIT Overload

During ST overload, on average, supine HR increased, and supine Ln RMSSD decreased. The effects were reversed during the recovery period. Standing HR(V) recordings remained unchanged. These observations suggest small, reversible, decreased parasympathetic activity (increased HR and decreased Ln RMSSD) for the supine resting condition. However, autonomic responsiveness to orthostatic stress remained unaffected suggesting that ST overload did not impair parasympathetic withdrawal and/or sympathetic activation in response to standing up. As previously reported, overload induced substantial changes in perceived stress and recovery (Hitzschke et al., 2017), creatine kinase and c-reactive protein (Hecksteden et al., 2016), and jump performance (Raeder et al., 2016). The findings concerning the supine recordings align with the results of a recent review on the effects of resistance exercise and training on HRV (Kingsley and Figueroa, 2016). This suggests a prolonged decrease in parasympathetic modulation in young, healthy adults following acute whole-body resistance exercise. For example, vagal HRV (i.e., high frequency power) was reduced for at least 24 h after trained weightlifters performed whole-body resistance exercises for 2 h (Chen et al., 2011). In addition, the weightlifters had impaired weightlifting performance and increased creatine kinase concentrations. Unfortunately, previous studies on HRV responses to acute resistance exercise in strength-trained subjects have either used substantially less session volume (González-Badillo et al., 2016; Pareja-Blanco et al., 2017) or reported only acute effects within a few hours after exercising (Kingsley et al., 2014; Figueiredo et al., 2015a; Figueiredo et al., 2015b).

In response to the HIIT microcycle, supine HR(V) recordings remained unchanged on average. However, standing HR decreased and standing Ln RMSSD increased, with reverse changes occurring during recovery. Similar to ST, HIIT overload induced substantial changes in perceived stress and recovery (Hitzschke et al., 2017), creatine kinase (Hecksteden et al., 2016), and jump performance (Wiewelhove et al., 2015), as previously reported. In combination with stable HR(V) in a supine position, the changes associated with a standing position reflect an attenuated dynamic cardiovascular response to quickly changing from a supine to standing position. These findings may be attributed to the so-called saturation phenomenon that occurs in the supine position and reduced vagal withdrawal and/or reduced sympathetic activity during orthostatic stress. The saturation phenomenon indicates a loss of the relationship between HR and vagal HRV. It was suggested to be ascribed to saturation of acetylcholine receptors at the myocyte level, which may further suppress respiratory heart modulation and thus reduce vagal HRV measures at low HR (Buchheit, 2014). The saturation phenomenon is generally associated with low HR—it can occur at an HR of ∼60 bpm or lower (Kiviniemi et al., 2004; Plews et al., 2012, 2013). Additionally, it may be partially related to aerobic capacity (Kiviniemi et al., 2004). This

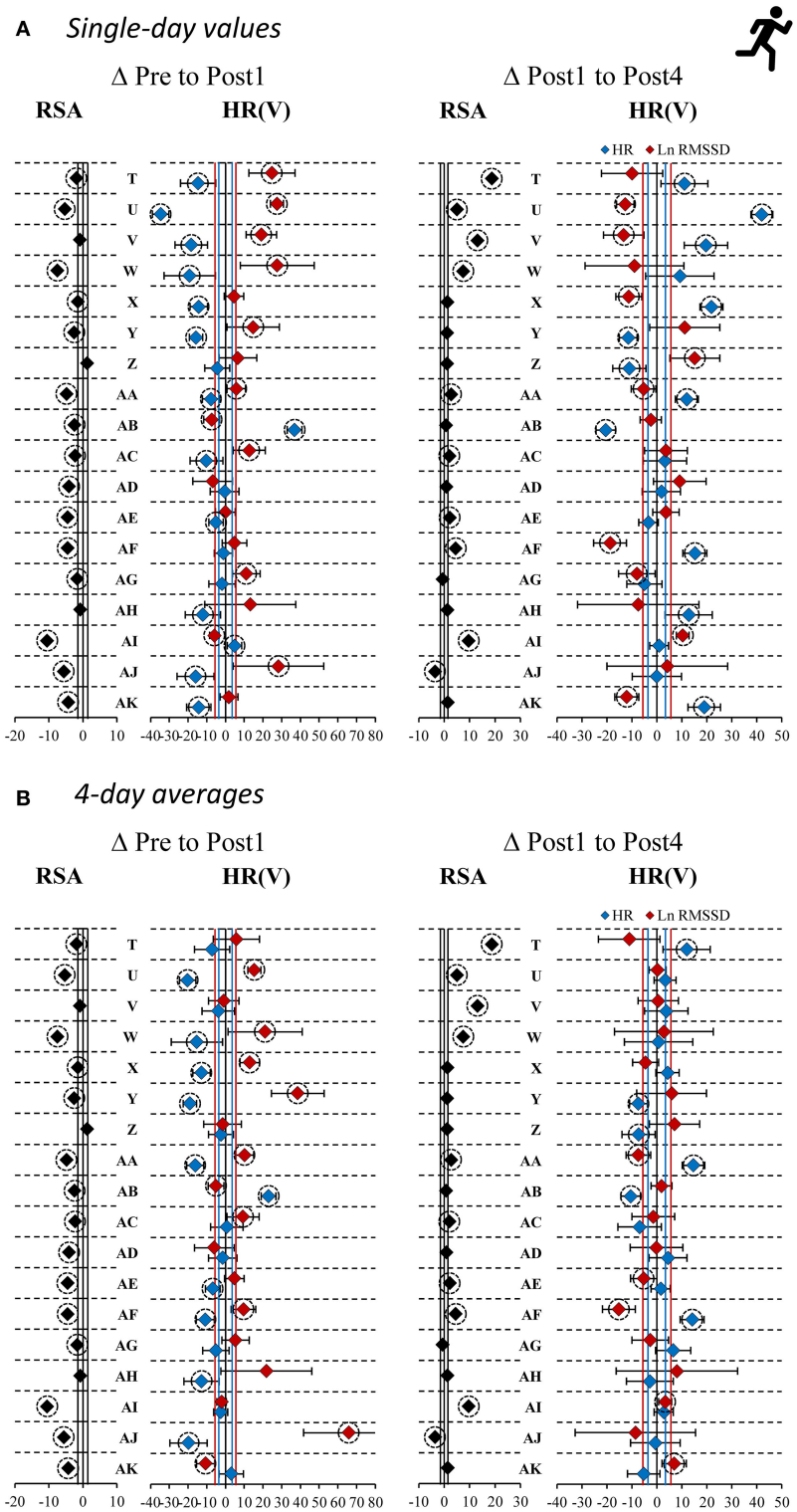

FIGURE 4 | Individual responses as percentage changes in criterion performance and standing heart rate (variability) measures [HR(V)] for high-intensity interval training overload. HR(V) measures are provided as as **(A)** single-day values and **(B)** 4-day rolling averages. RSA, repeated sprint ability; HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals. Vertical lines: group-based typical error (TE) for RSA (black), smallest worthwhile change (see **Table 3**) in HR (blue), and Ln RMSSD (red). Error bars: individual TE (4-day baseline SD). Dashed circles: changes exceed ±TE.

TABLE 7 | Example of 3 × 3 tables for individual response classification for changes from Pre to Post1 and Post1 to Post4 of resting heart rate and Ln RMSSD using single-day and 4-day average values.

Strength training

Supine recordings

	Δ Pre to Post1				Δ Post1 to Post4			
		Ln RMSSD				Ln RMSSD		
Single-day	HR	−	o	+	HR	−	o	+
	−	1	2		−		1	7
	o	1	4	1	o	3	4	
	+	7	3		+		4	
4-day average		Ln RMSSD				Ln RMSSD		
	HR	−	o	+	HR	−	o	+
	−		1		−		1	2
	o	2	6		o		15	1
	+	6	4		+			

High-intensity interval training

Standing recordings

	Δ Pre to Post1				Δ Post1 to Post4			
		Ln RMSSD				Ln RMSSD		
Single-day	HR	−	o	+	HR	−	o	+
	−		4	8	−		2	1
	o		3	1	o	1	5	1
	+	2			+	6	2	
4-day average		Ln RMSSD				Ln RMSSD		
	HR	−	o	+	HR	−	o	+
	−		2	7	−		3	
	o	1	6	1	o	1	9	2
	+	1			+	2	1	

Data in the 3 × 3 tables represent the number of observed individual responses classified for 9 possible response types (3 response types for heart rate and Ln RMSSD, respectively). HR, heart rate; Ln RMSSD, natural logarithm of root mean square of successive differences between adjacent beat to beat intervals; –, observed reduction > typical error (TE); o, observed changes < TE; +, observed increase > TE. Individual TE was used for heart rate measures (individual SD during 4-day baseline period).

hypothesis (i.e., the presence of saturation in the supine position) is supported by the supine baseline HR of 57 bpm (i.e., 4-day average) for the group and the fact that 14 of 18 athletes showed supine baseline HR below 60 bpm. The evidence indicated that HRV saturation was likely in four athletes (athletes U, Z, AA, AF), as both supine HR and Ln RMSSD were decreased post-overload, but not in a standing position (**Supplementary Table 6**, **Supplementary Figure 4**). Although it has been suggested that parasympathetic reactivation is primarily intensity-dependent and it may take at least 48 h for vagal HRV to be fully restored after high-intensity aerobic exercise, higher aerobic fitness may accelerate post-exercise cardiac autonomic recovery (Stanley et al., 2013). For example, vagal HRV following a HIIT session (i.e., 6 × 3 min intervals above ventilatory threshold 2) returned to the pre-exercise level within a few hours in trained and highly trained subjects ($\dot{V}O_2$max: 60 ± 5, and 72 ± 5 ml/min/kg, respectively) (Seiler et al., 2007). This suggests that vagal HRV indices might even overcompensate within the hours or days following very intense or prolonged exercise (Hottenrott and Hoos, 2017). This hypothesis is supported by previous studies, where increased Ln RMSSD were observed after 2 to 3 weeks of endurance-based overload training (Le Meur et al., 2013; Bellenger et al., 2016b), where average between-session recovery was likely smaller than 24 h. Another possible explanation for the reduced HR and increased Ln RMSSD in standing position following overload, could be altered sympathetic nervous system activity (e.g., due to reduced catecholamine excretion or desensitization of cardiac beta-adrenergic receptors) (Lehmann et al., 1998), which would become more evident after an increase in sympathetic activity in response to orthostatic stress.

The results agree with a meta-analysis (Bellenger et al., 2016a) on the effects of adaptation to endurance training on markers of autonomic HR regulation. Similar to the effects in the HIIT group, this meta-analysis reported a small increase in RMSSD following short training periods (i.e., 2 or 3 weeks), which led to decreased exercise performance. In addition, previous studies reported that standing HRV may be more sensitive to training-related changes compared to supine HRV as orthostatic stress may overcome possible saturation effects (Le Meur et al., 2013; Bellenger et al., 2016b).

To our knowledge, this is the first study to report HR(V) responses to ST and HIIT overload and subsequent short-term recovery measured with daily active orthostatic tests. We observed different HR(V) patterns during the orthostatic tests in response to the two different training modes, which might reflect activity- and/or fatigue-specific autonomic modulations (Schmitt et al., 2015a). Changing from a supine position to an active standing position causes a stress response due to the gravitational shift of blood from the central venous system to the lower extremities. This leads to severe vagal withdrawal as well as an increase in sympathetic-mediated vasomotor activity in order to preserve arterial blood pressure and avoid cerebral hypoperfusion (Buchheit et al., 2009a; Hottenrott and Hoos, 2017). Since supine and standing HR and HRV measures are influenced by the involvement of different cardiopulmonary receptors, we performed both supine and standing recordings. The postural HR(V) profiles were fully independent and non-exchangeable in elite endurance athletes (Schmitt et al., 2015a,b). Our observations provide some support for these arguments, as only combining supine and standing recordings enabled us to identify possible vagal saturation during HIIT overload and to describe different autonomic modulations depending on the training modes.

In summary, the observed differences in within-group HR(V) changes between study arms could be caused by various factors. On the one hand, differences in demands and in acute responses between training modalities may be responsible for different HR(V) patterns. In general, endurance-based (dynamic) exercise is suggested to mainly induce volume load on the cardiac cavities, whereas ST (i.e., static exercise) induces mainly pressure

load (Aubert et al., 2003; Barbier et al., 2006). Further, HIIT is characterized by substantially greater amount of aerobic metabolism compared to ST (Barbier et al., 2006), and we assume higher total energy expenditure in response to HIIT. It was also suggested that HIIT exercise reduces arterial stiffness, while vigorous ST exercise increases arterial stiffness (Li et al., 2015; Way et al., 2019). HIIT may also induce acute plasma volume expansion (Green et al., 1984; Buchheit et al., 2009b). From a plausibility point of view, the described training responses could explain the observed within-group changes and between-group differences in orthostatic HR(V) regulation to some degree. However, we are not aware of studies providing direct evidence for such relationships, and overload training studies using active orthostatic tests in ST-trained subjects are entirely missing (see Kingsley and Figueroa, 2016; Bhati et al., 2018). On the other hand, the between-group differences could also be ascribed to differences in subject characteristics between study arms, such as higher average aerobic capacity in HIIT participants (**Table 1**) and possible additional training-specific functional (e.g., autonomic regulation) or structural adaptations (e.g., cardiac hypertrophy or changes in intrinsic HR) (Dickhuth et al., 1987; Achten and Jeukendrup, 2003; Billman et al., 2015a; Boyett et al., 2017; Flannery et al., 2017). In general, training-related HRV changes are frequently considered to be a result of altered autonomic HR regulation. However, in the absence of supportive physiological measurements, it remains speculative whether and to which degree the observed changes within our study were caused by different functional changes in response to varying exercise demands, or differences in training history-related adaptation and aerobic fitness between study groups.

Lack of a Clear Association Between Changes in Performance and HR(V) After ST and HIIT Overload Microcycles

Overall, the continuous associations (i.e., correlations) between changes in HR, HRV and performance were mainly unclear (i.e., the confidence intervals display a substantial overlap with positive and negative effects) or inconsistent in direction from Pre to Post1 and Post1 to Post4. Categorical associations (i.e., 3 × 3 tables) also showed weak agreement between changes in HR(V) and criterion performance when using the TE as a threshold value. Therefore, short-term changes in HR(V) may be a poor surrogate marker for discipline-specific performance following strenuous ST or HIIT microcycles at the individual level. These findings seem plausible for several reasons. First, previously reported correlation analyses between criterion performance and other possible surrogate measures (i.e., perceived stress and recovery, blood-borne markers, muscle contractile properties, and non-fatiguing performance tests) also revealed unclear (i.e., not statistically significant) associations (Wiewelhove et al., 2015; Hecksteden et al., 2016; Raeder et al., 2016; Hitzschke et al., 2017), and these measures show a more direct theoretical relationship to discipline-specific performance compared to HR(V). Secondly, Plews et al. (2014) observed only moderate correlations between changes in Ln RMSSD and running performance in trained triathletes following a three-week overload period. Thus, we conclude that either the overload training stimulus was too low or short in duration or that the selected (criterion) performance tests were too noisy (i.e., they had a suboptimal signal-to-noise ratio) to reveal clear associations.

In summary, HR(V) measures may indeed reflect training- and recovery-induced autonomic modulations, which could affect athletes' performance. In addition, it has been proposed that cardiac vagal modulation may rather indicate an athlete's capacity to adapt to (aerobic) exercise stimuli and is therefore a prerequisite for performance-related adaptation (Hautala et al., 2009). However, an athlete's ANS status is only one factor contributing to the complex nature of fatigue and performance, and it is unlikely that a single marker can accurately display changes in such multidimensional constructs (Meeusen et al., 2013; Bourdon et al., 2017; Coutts et al., 2018; Kellmann et al., 2018; Schneider et al., 2018).

Using Rolling HR(V) Averages May Attenuate Sensitivity to Individual Short-Term Changes in Autonomic Modulation

The use of average HR(V) improved the sensitivity to group changes compared to the use of single-day values, as indicated by the reduced width of confidence intervals for the d_{pre} and increased d_{diff} effect sizes (**Figure 3**, **Tables 4**, **5**, **Supplementary Figure 1**, **Supplementary Tables 1, 2**). This supports previous proposals to use rolling averages (Le Meur et al., 2013; Plews et al., 2014). Visual inspection of the individual HR(V) response panels (**Figure 4**, **Supplementary Figures 4–5**) suggests lower percentage changes in average values for several athletes. In addition, the number of athletes showing both unchanged HR and Ln RMSSD for average values was increased (**Table 7**, **Supplementary Table 6**). The approach we used to classify the HR(V) response indicated inverse effects at the individual level compared to the group level. Although this finding relies solely on descriptive evaluation, it appears reasonable, as averaging daily values attenuates day-to-day change. However, this can cause important information to be lost, especially since cardiac autonomic recovery may occur within 24 h in trained athletes (Seiler et al., 2007; Stanley et al., 2013). On the other hand, however, averaging HR(V) may be necessary to reduce the measurement error for daily HR(V) changes. This controversial issue could be explored by future studies.

LIMITATIONS AND STRENGTHS OF THE STUDY

Several factors limit the generalizability of results beyond the utilized study model. We primarily focused on determining the changes in HR(V) that occur following ST and HIIT overload in trained subjects. It remains unclear whether HR(V) also provides valuable information in more moderate, normal training environments or in highly trained athletes, as training and recovery responses may be diminished and accelerated, respectively, in these circumstances. In addition, adaptive responses to intensified training may be characterized by changes in the magnitude of day-to-day fluctuation in Ln RMSSD (Flatt and Howells, 2019), but this was not assessed in the current

study. Further, as previously discussed in detail (Hecksteden et al., 2016), the comparability of HR(V) responses to ST vs. HIIT is constrained by the generic challenge of matching workload and fatigue levels between the different modes of exercise. Moreover, recruitment of performance-matched control groups for the (in total) three different study arms was not feasible due to limited time and resources. To verify our findings, randomized (crossover) trials with a priori optimized sample size are desired. Another limitation is that, despite an initial survey of medication and nutritional supplementation, it was neither possible for us to control the intake of HR(V) influencing medication throughout the study period, nor the consumption of caffeinated beverages immediately prior to orthostatic testing. Finally, as HR(V) is an indirect measure of cardiac autonomic modulation, interpretations regarding underlying physiological mechanisms should be treated with caution.

We tried to overcome several limitations by assessing the fatigue responses using criterion performance tests and utilizing repeated testing [i.e., daily HR(V) recordings] with a single-subject A-B-A withdrawal design (Kinugasa et al., 2004; Barker et al., 2011). The latter enabled us to describe HR(V) changes more precisely by considering individual day-to-day variation, which is not possible in a simple pre-post design. Furthermore, week-to-week reliability was determined beforehand in our laboratory (Wiewelhove et al., 2015; Raeder et al., 2016). It indicates random variation in criterion performance and may partially be a substitute for the presence of control groups (Hopkins, 2015a; Hecksteden et al., 2018). To our knowledge, comparative assessment of daily resting HR(V) for ST and HIIT during active orthostatic tests in a methodologically consistent design is a unique approach that offers novel insights into HR(V) responses.

CONCLUSION

Daily HR(V) monitoring with active orthostatic tests displayed altered supine HR(V) measures for ST- and altered standing HR(V) measures for HIIT short-term overload. HR(V) measures remained unchanged in the respective other recording position. These autonomic patterns may not be discovered by supine or standing measures alone. However, HR(V) changes were not consistently related to short-term performance changes, which limits their usefulness as surrogate measures for ST or HIIT performance in overload microcycles. Moreover, the use of rolling averages may attenuate the sensitivity to individual short-term autonomic modulations, despite improving sensitivity at the group level. To provide further guidance for sports practice, future studies should utilize repeated intervention designs (Hecksteden et al., 2015) with appropriate baseline recordings to determine the consistency of acute and short-term HR(V) responses, as previously done for long-term adaptation to training (Plews et al., 2013).

PRACTICAL APPLICATIONS

The combined assessment of HR(V) measures in supine rest and following quickly standing up using orthostatic tests can provide unique and more comprehensive insights into athletes' autonomic HR regulation compared to either supine or standing recordings in isolation. Although divergent autonomic patterns might be observed following various training demands, it should be acknowledged that HR(V) measures may not mimic individual short-term performance changes. Furthermore, based on our results, we encourage practitioners to (also) analyze single-day HR(V) measures when assessing short-term training responses. Finally, day-to-day variability and training response varies substantially between athletes, which further complicates HR(V)-guided training prescription. In summary, we believe that it is still advisable first to gain experience at an individual level through pure observation in order to avoid inappropriate training adjustments due to overly simplistic training-response models.

ETHICS STATEMENT

The investigation was approved by the ethics committee of the medical faculty of the Ruhr University Bochum and was conducted according to the guidelines of the Declaration of Helsinki. All subjects participated in the study voluntarily, were free to withdraw without penalty at any time, and provided written informed consent.

AUTHOR CONTRIBUTIONS

CS prepared the original manuscript, figures and tables and analyzed the data. CS, AAF, and OH interpreted the results. TW, CR, AAF, OH, LH, and AF assisted with writing and editing the manuscript, figures and tables. TW and CR performed the experiment. OS calculated the heart rate variability indices from raw data files. TW, CR, MK, TM, MP, and AF conceived and designed the experiment.

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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.2019.00582/full#supplementary-material

Table 1.xlsx | Supplementary Tables.

Data Sheet 1.xlsx | Data Sheet.

Data Sheet 2.zip | Data analysis and statistical analysis.

Data Sheet 3.docx | Instructions heart rate recordings.

Image 1.pdf | Supplementary Figures.

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The Effect of Phase Change Material on Recovery of Neuromuscular Function Following Competitive Soccer Match-Play

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Aim: Cryotherapy is commonly implemented following soccer match-play in an attempt to accelerate the natural time-course of recovery, but the effect of this intervention on neuromuscular function is unknown. The aim of the present study was to examine the effect of donning lower-body garments fitted with cooled phase change material (PCM) on recovery of neuromuscular function following competitive soccer match-play.

Methods: Using a randomized, crossover design, 11 male semi-professional soccer players wore PCM cooled to 15°C (PCM_{cold}) or left at ambient temperature (PCM_{amb}; sham control) for 3 h following soccer match-play. Pre-, and 24, 48, and 72 h post-match, participants completed a battery of neuromuscular, physical, and perceptual tests. Maximal voluntary contraction force (MVC) and twitch responses to electrical (femoral nerve) and magnetic (motor cortex) stimulation (TMS) during isometric knee-extension and at rest were measured to assess central nervous system (CNS) (voluntary activation, VA) and muscle contractile (quadriceps potentiated twitch force, $Q_{tw,pot}$) function. Fatigue and perceptions of muscle soreness were assessed via visual analog scales, and physical function was assessed through measures of jump [countermovement jump (CMJ) height and reactive strength index (RSI)] performance. A belief questionnaire was completed pre- and post-intervention to determine the perceived effectiveness of each garment.

Results: Competitive soccer match-play elicited persistent decrements in MVC, VA measured with femoral nerve stimulation, $Q_{tw,pot}$, as well as reactive strength, fatigue and muscle soreness ($P < 0.05$). Both MVC and VA were higher at 48 h post-match after wearing PCM_{cold} compared with PCM_{amb} ($P < 0.05$). However, there was no effect of PCM on the magnitude or time-course of recovery for any other neuromuscular, physical function, or perceptual indices studied ($P > 0.05$). The belief questionnaire revealed that players perceived that both PCM_{cold} and PCM_{amb} were moderately effective in improving recovery, with no difference between the two interventions ($P = 0.56$).

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Conclusion: Although wearing cooled PCM garments improved MVC and VA 48 h following match-play, the lack of effect on measures of physical function or perceptual responses to match-play suggest that PCM offers a limited benefit to the recovery process. The lack of effect could have been due to the relatively small magnitude of change in most of the outcome measures studied.

Keywords: central nervous system, cryotherapy, fatigue, peripheral, recovery

INTRODUCTION

Association football (soccer) is an intermittent-sprint sport which imposes high physiological, neuromuscular and cognitive demands (Mohr et al., 2005). During a typical match, players cover 10–13 km, with 2–3 km covered at high intensities, and a diverse range of high-intensity movements performed, such as accelerating, decelerating, changing direction, impacts and tackles (Mohr et al., 2003). An inexorable consequence of these demands is fatigue, defined as a sensation of tiredness and weakness underpinned and/or modulated by a multitude of physiological and psychological processes (Thomas et al., 2018). The fatigue which occurs as a result of soccer match-play persists post-exercise, and can take days to resolve (Rampinini et al., 2011). Nevertheless, in most top professional leagues, it is normal procedure for teams to compete in three successive games during a 7 days period at several stages throughout a season, often with as little as 48–72 h recovery between games. Due to the demanding nature of soccer match-play and the congested fixture schedules in the modern-day game, understanding the etiology of fatigue, the time-course of recovery, and strategies to alleviate fatigue and expedite recovery are pertinent issues (Nedelec et al., 2012, 2013).

When implementing recovery strategies aimed at alleviating fatigue and accelerating recovery, it is imperative to understand the stressors causing reductions in performance and delayed recovery before applying the intervention (Howatson et al., 2016). While the fatigue which persists in the days following soccer match-play is multifactorial and complex, impairment in maximal voluntary contraction (MVC) strength, which can take up to 72 h to resolve (Brownstein et al., 2017), is likely an important contributor to post-match fatigue. In turn, impairments in MVC strength are underpinned by a multitude of processes, and are often attributed to impairments in neuromuscular function, measured as deficits in contractile function and/or the capacity of the central nervous system (CNS) to activate muscle (Gandevia, 2001). Using neurostimulation techniques, a recent study from our laboratory examined the effect of soccer match-play on neuromuscular function in the days post-match, and demonstrated substantial impairments in contractile and CNS function which required up to 48 h to recover (Brownstein et al., 2017). In turn, it was further hypothesized that the protracted impairments in contractile and CNS function were likely a consequence of the repeated eccentric contractions associated with match-play and the subsequent muscle damage and inflammatory response which ensues (Ascensao et al., 2008; Brownstein et al., 2017). A number of factors would support this suggestion. Firstly, it is known that soccer match-play induces considerable muscle damage and a prolonged inflammatory response which can persist for several days post-exercise (Ispirlidis et al., 2008; Fatouros et al., 2010). Secondly, while impairments in contractile and CNS function can also occur due to metabolic influences (Allen et al., 2008), many of the metabolic mechanisms thought to interfere with neuromuscular function dissipate rapidly following exercise cessation. For example, following exercise that imposes large metabolic but little mechanical demand, recovery is substantially faster than exercise that is mechanically demanding (Skurvydas et al., 2016). In addition, the mechanical stress imposed on muscle fibers during eccentric based exercise has been shown to elicit prolonged impairments in the excitation-contraction coupling process (Souron et al., 2018), as well as residual deficits in voluntary activation which can take days to resolve (Goodall et al., 2017). As such, it is a plausible assumption that the impaired neuromuscular function which persists for several days following soccer match-play is primarily a consequence of muscle damage and the associated inflammatory response, and strategies to alleviate the negative effects of muscle damage and inflammation could thus be suitable to accelerate recovery following competitive soccer match-play.

The precise mechanisms of exercise-induced muscle damage (EIMD) are complex and remain to be fully elucidated. However, muscle damage has previously been simplified into two general areas; the initial event that occurs during the exercise bout (termed "primary damage"), and the secondary events that propagate damage through factors associated with inflammation (termed "secondary damage") (Howatson and van Someren, 2008; Owens et al., 2018). While the inflammatory response that ensues following EIMD is thought to be crucial in orchestrating muscle repair and recovery (Butterfield et al., 2006), the secondary damage associated with inflammation is suggested to further exacerbate impairments in muscle function (Pizza et al., 2005). As such, a common target of interventions is to alleviate the negative effects associated with the inflammatory response in an attempt to expedite the recovery process (Howatson et al., 2010; Rowsell et al., 2011).

A common post-exercise recovery strategy is cryotherapy, which is regularly implemented following soccer match-play, and is supposed to attenuate post-exercise reductions in functional capacity and athletic performance (Nedelec et al., 2013). While the precise underlying mechanisms remain to be elucidated, cryotherapy is purported to reduce muscle temperature and attenuate inflammation and oxidative stress (White and Wells, 2013). A recently implemented form of cryotherapy that has produced encouraging results as a recovery aid is phase change

material; PCM (Clifford et al., 2018; Kwiecien et al., 2018; McHugh et al., 2018). Phase change material is a substance with a high heat fusion, which melts and solidifies at certain temperatures. When frozen PCM is convectively heated, for example, through exposure to the human body, it will continuously absorb heat until all material has changed from solid to liquid. As such, PCM can maintain low temperatures within the tissues of the target limb for sustained periods. The application of PCM has many logistical and practical benefits due to being easily transportable, the lower level of thermal discomfort compared with cryotherapy, and capacity to maintain low temperatures for a prolonged period of time (Kwiecien et al., 2018). A recent study applied cold PCM to the quadriceps for 3 h following competitive soccer match-play and found reduced muscle soreness and accelerated recovery of MVC (Clifford et al., 2018), findings which have since been corroborated (McHugh et al., 2018).

Despite the promising results of recent studies (Clifford et al., 2018; Kwiecien et al., 2018; McHugh et al., 2018), more evidence is required to substantiate the efficacy of PCM as a recovery intervention and to gain mechanistic insight into the potential benefits of PCM on recovery. Accordingly, the aim of the present study was to examine the effect of wearing cold PCM garments on recovery of neuromuscular function, as well as physical and perceptual measures following soccer match-play. It was hypothesized that wearing cold PCM garments would expedite recovery of impaired neuromuscular function and attenuate muscle soreness, possibly by reducing the negative effects associated with the acute inflammatory response on contractile and CNS function.

MATERIALS AND METHODS

Participants

After receiving ethical approval from the Northumbria University Faculty of Health and Life Sciences Ethics committee in accordance with the ethical standards established in the Declaration of Helsinki, fifteen male semi-professional soccer players from Level eight of the English Football League, gave written informed consent to participate in the study. Throughout the data collection period, four players sustained injuries which prevented them from completing the study, leaving eleven participants in total (three defenders, five midfielders, three attackers; 22 $\pm$ 1 years; stature 1.80 $\pm$ 0.10 m; mass 78 $\pm$ 8 kg). Players trained three to four times a week, in addition to at least one competitive match. The participants competitive season ran from August to May, with testing taking place in the mid-season phase of the players training year. Participants were required to refrain from physical activity and alcohol consumption for the duration of the study and in the 48 h prior to data collection and abstain from caffeine consumption for the 12 h prior to each experimental visit.

Design

The study employed a randomized cross-over design to assess the effectiveness of PCM on recovery in the days following competitive soccer match-play. Participants visited the laboratory prior to commencement of the data collection period for habituation to the measurement tools employed in the study. For the experimental trials, participants were required to visit the laboratory prior to and 24, 48, and 72 h following two competitive soccer matches. The pre-match visit took place 24 h before the fixtures. On one occasion, players wore shorts fitted with PCM (Glacier Tek; USDA BioPreferred PureTemp, Plymouth, MN) that was either cooled (PCM_{cold}) or left ambient (PCM_{amb}), which served as a sham control. The order of the conditions was randomized using an online randomizer[1]. Phase change material was applied to the quadriceps and hamstring muscle groups, and was worn for 3 h post-match. To ensure compliance with the intervention, away fixtures in which the team were required to travel back for $\geq$3 h were selected. The two fixtures were separated by 4–8 weeks. During each experimental visit, participants completed assessments of neuromuscular, physical, and perceptual function to ascertain the effect of PCM on recovery.

Procedures

Practice Trial

Prior to the experimental trials, participants attended the laboratory for habituation with the study procedures. This involved an explanation of the methods employed in the study, before participants performed a practice trial consisting of the neuromuscular, physical and perceptual measures employed in the study (described below).

Experimental Trials

Competitive Soccer Match

Participants visited the laboratory 24 h prior to each match for pre-match measurements (described in detail below). On the subsequent day, players completed a 90 min soccer match within their competitive league consisting of two 45 min halves interspersed by a 15 min recovery interval. In total, the study took place across six matches, with five participants investigated following games one and two, three participants investigated following games three and four, and three participants investigated following games five and six. All fixtures took place on a grass pitch at either 13:00 (games one, two, and six) or 14:00 (games three, four, and five). Players were required to play a minimum of 70 min per match in order to be included in the experiment. The activity profiles and heart rates of the players were measured throughout the games using GPS with built in heart rate monitors (Polar Team Pro, Polar Electro Oy, Finland), and compared between games in order to ensure the physical and physiological demands of the matches in each condition were similar.

Phase Change Material

Prior to the post-match application of PCM_{cold}, the temperature of the blocks was cooled and maintained in a freezer at 15°C, while PCM_{amb} were stored >22°C. When traveling to the fixtures, PCM_{cold} were stored in an insulated storage container.

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The PCM blocks worn over the quadriceps were 32 cm in length and 13 cm in width, while the blocks worn over the hamstrings were 16 cm in length and 13 cm in width. Two blocks were worn on the quadriceps and hamstring muscles inside compression shorts, with blocks placed over the medial and lateral parts of both muscle groups. The PCMs were applied within 30 min post-exercise, and were worn while traveling back from the matches on the team bus.

Outcome Measures

A range of neuromuscular, physical and perceptual measures were assessed 24 h pre-match, and 24, 48, and 72 h post-match. Details of these measures are provided below.

Perceptual Responses

Participants completed the "*Elite Performance Readiness Questionnaire*" (Dean et al., 1990) at each time point, a measure of performance readiness consisting of 10 subjective measures of fatigue, soreness, motivation to train, anger, confusion, depression, tension, alertness, confidence, and sleep. Participants drew a vertical line on a 100 mm horizontal line in response to questions used for each measure, such as "how fatigued do you feel?" "how sore do your muscles feel?" and "how motivated to train do you feel?" Each scale was anchored with verbal descriptors "not at all" to "extremely." Perceptual measures were assessed at each time-point prior to commencing the warm-up. In addition, similar to a previous study (Clifford et al., 2018) participants completed a questionnaire in which they rated how effective they felt the cold and ambient PCM were going to be for recovery prior to the intervention (pre-match), and how effective they felt they were in improving recovery at the end of the intervention (72 h post-match). The belief questionnaire consisted of a Likert scale from 1 "not effective at all" to 5 "extremely effective."

Assessment of Neuromuscular Function

Measures of neuromuscular function were assessed at each time-point with electrical stimulation of the femoral nerve and TMS of the contralateral motor cortex at rest and during voluntary contractions of the right knee-extensors. The neuromuscular assessment began with two practice MVCs to ensure potentiation of subsequent evoked measures, followed by three ∼3 s MVCs, all separated by 30 s. During these 3 MVCs, paired motor nerve stimulation (100 Hz) was delivered when peak force plateaued, and ∼2 s after the MVC to measure voluntary activation (VA), with a single pulse electrical stimuli delivered 5 s post-MVC to assess potentiated quadriceps twitch force ($Q_{tw,pot}$) of the knee-extensors. The average of the 3 MVCs was included in the analysis. Single-pulse TMS was subsequently delivered during two sets of five 3–5 s contractions at 100, 87.5, 75, 62.5, and 50% MVC, with 5 s rest between contractions and 10 s rest between sets, to determine VA_{TMS}.

Force and electromyographical recordings

The evoked quadriceps force and electromyographic (EMG) responses of the *rectus femoris* (RF) to TMS of the primary motor cortex, and electrical stimulation of the femoral nerve, were used to assess neuromuscular function. A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) recorded muscle force (N) during an isometric voluntary contraction of the knee extensors. During contractions, participants sat with hips and knees at 90° flexion, with a load cell fixed to a custom-built chair and attached to the participants right leg, superior to the ankle malleoli, with a noncompliant cuff. Electromyographic activity from the RF and *biceps femoris* (BF) was recorded from surface electrodes (Ag/AgCl; Kendall H87PG/F, Covidien, Mansfield, MA, United States) placed 2 cm apart over the belly of each muscle, with a reference electrode placed on the patella. The placement of the EMG electrodes was based on SENIAM guidelines (Hermens et al., 2000). Electrode placement was marked with indelible ink to ensure consistent placement throughout the study, with the areas cleaned and shaved prior to electrode placement. The electrodes recorded electrical activity in the RF and BF, with the signal processed to permit analysis of the root-mean-square (RMS) amplitude for sub-maximal and MVCs, the maximal compound muscle action potential (M_{max}) from the electrical stimulation of the femoral nerve, and the motor evoked potential (MEP) elicited by TMS. Signals were amplified: gain × 1,000 for EMG and × 300 for force (CED 1902; Cambridge Electronic Design, Cambridge, United Kingdom), band-pass filtered (EMG only: 20–200 Hz), digitized (4 kHz; CED 1401, Cambridge Electronic Design) and analyzed offline. Further details on these methods are provided below.

Motor nerve stimulation

Motor nerve stimulation was used for the measurement of contractile function, muscle membrane excitability and VA. Single and paired electrical stimuli (100 Hz) were administered using square wave pulses (200 μs) via a constant-current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, United Kingdom) using self-adhesive surface electrodes (CF3200, Nidd Valley Medical Ltd., North Yorkshire, United Kingdom). Electrical stimuli were first administered to the motor nerve at rest in 20 mA step-wise increments from 20 mA until the maximum quadriceps twitch amplitude (Q_{tw}, N) and M_{max} (mV) were elicited. To ensure a consistent, supramaximal stimulus and account for any activity-induced changes in axonal excitability, the resulting stimulation intensity was increased by 30% (198 ± 38 mA). The peak-to-peak amplitude and area of the electrically evoked maximal compound action potential (M_{max}) was used as a measure of membrane excitability. In addition, the following mechanical measures of muscle contractility were derived from the single pulse potentiated twitch response: contraction time (CT, time to peak twitch tension), maximum rate of force development (MRFD, maximal linear incline of the force response calculated at 100 ms epochs), maximal rate of relaxation (MRR, maximal linear decline of the force response calculated at 100 ms epochs), and one half relaxation time.

Voluntary activation with TMS

Single-pulse TMS was delivered over the motor cortex via a concave double cone coil using a Magstim 200^2 stimulator

(The Magstim Company Ltd., Whitland, United Kingdom). The junction of the double cone coil was aligned tangentially to the sagittal plane, with its center 1–2 cm to the left of the vertex and was oriented to induce current in the posterior-to-anterior direction. The optimal coil placement was determined at the start of each trial as the position that elicited the largest MEP in the RF, with a concurrent small MEP in the BF during a light voluntary contraction (10% MVC). The optimal position was marked with indelible ink to ensure consistent placement throughout the study. To determine VA with TMS (VA_{TMS}), single pulse TMS was delivered during brief (3–5 s) contractions at 100, 87.5, 75, 62.5, and 50% MVC, separated by 5 s of rest (Dekerle et al., 2018). This procedure was repeated two times, with 15 s between each set. The stimulation intensity was set at the stimulator output that elicited the maximum superimposed twitch force during a 50% MVC (Thomas et al., 2017), and did not differ between conditions (PCMcold 66 ± 10% vs. PCMamb 68 ± 8%, respectively, $P = 0.57$), or across the 4 time-points ($P = 0.49$). The stimulator output activated a large proportion of the KE motoneuron pool at baseline, with no difference between PCM_{cold} (67 ± 24% M_{max} amplitude) or PCM_{amb} (61 ± 13%, $P = 0.38$). Small co-activation of the antagonist muscle (BF) was observed in response to TMS and did not differ between PCM_{cold} (0.85 ± 0.37 mV) or PCM_{amb} (0.86 ± 0.36 mV, $P = 0.96$) or across the 4 time-points ($P = 0.51$).

Assessment of Physical Function

Participants completed a battery of assessments to measure physical function in variables relevant to optimal soccer performance. All measures of physical function were performed following the neuromuscular assessment and the completion of a standardized warm-up. An optical timing system (Optojump Next, Microgate, Milan, Italy) was used to measure jump height (cm) during a countermovement jump (CMJ), and reactive strength index (RSI) during a drop jump (DJ). For CMJ, participants started from an erect position with hands akimbo. On verbal command, participants made a downward countermovement before jumping vertically for maximum height. For reactive strength index (DJ-RSI), participants were instructed to step off a 30 cm box, before jumping vertically for maximum height as soon as possible after landing, maintaining hands akimbo throughout. To ensure the DJ-RSI was assessing fast stretch-shortening cycle function, a maximum ground contact time of 200 ms was allowed during each jump, with participants given visual feedback on each ground contact time and jump height after each jump (Thomas et al., 2017). Reactive strength index ($cm \cdot s^{-1}$) was calculated as the ratio between jump height (cm) and ground contract time (s). All participants were given three attempts at each jump with 60 s between each repetition. For CMJ and DJ-RSI, the best of the three attempts was included in the analysis.

Match-Play Physical Performance and Intensity

During the games, GPS with built in HR monitors (Polar Team Pro, Polar Electrophase Oy, Finland) were used to assess total distance (TD), high-intensity running (HIR, distance covered at running velocities higher than 15 $km \cdot h^{-1}$), total accelerations ($>1\ m \cdot s^{-2}$), total decelerations ($>-1\ m \cdot s^{-2}$), and mean and peak HR (Akenhead et al., 2013). These variables were compared between games to ensure the physical and physiological demands of the matches in each condition were similar.

Data Analysis

Voluntary activation was assessed through the interpolated twitch technique and was quantified by comparing the amplitude of the superimposed twitch force (SIT) with the potentiated twitch force (100 Hz) delivered 2 s following the MVC at rest using the following equation: Motor nerve VA (%) = $[1 - (SIT/Q_{tw,pot}) \times 100]$. VA_{TMS} was assessed during two sets of contractions at 100, 87.5, 75, 62.5, and 50% MVC according to Dekerle et al. (2018), and the regression between SIT amplitude and contraction intensity was extrapolated to the y intercept to obtain an estimated resting twitch (ERT, Todd et al., 2003). The regression analysis confirmed a linear relationship at each time-point (r^2 range = 0.89 ± 0.04–0.93 ± 0.06). The estimated resting twitch (ERT) was calculated as the *y*-intercept of the linear regression between the mean amplitude of the SIT force evoked by TMS at each contraction intensity. Subsequently, VA_{TMS} was quantified using the equation $[1 - (SIT/ERT) \times 100]$. The peak-to-peak amplitude of evoked MEP and M_{max} were measured offline.

Reproducibility Coefficients

Typical error as a coefficient of variation (CV, %) and intraclass correlation coefficients ($ICC_{3,1}$) between the two baseline visits were calculated to quantify the reproducibility of neuromuscular and physical function measures. Reproducibility coefficients were as follows: MVC (ICC = 0.97, CV = 1.7%), VA with motor nerve stimulation (ICC = 0.85, CV = 2.8%), M_{max} (ICC = 0.91, CV = 4.3%), $Q_{tw,pot}$ (ICC = 0.84, CV = 4.3%), VA_{TMS} (ICC = 0.81, CV = 4.1%),MRFD (ICC = 0.89, CV = 0.9%), MRR (ICC = 0.80, CV = 2.6%), CT (ICC = 0.60, CV = 8.1%), and $RT_{0.5}$ (ICC = 0.71, CV = 11.1%), CMJ (ICC = 0.97, CV = 6.6%), DJ-RSI (ICC = 0.87, CV = 7.7%).

Statistical Analysis

Data are presented as mean ± SD. A two-way repeated measures ANOVA with 2 treatment levels (PCM_{cold} vs. PCM_{amb}) with 4 time points (Pre-, 24, 48, and 72 h post-match) was performed. Normality of the data was assessed using the Shapiro–Wilks test. Assumptions of sphericity were explored and controlled for all variables using the Greenhouse-Geisser adjustment, where necessary. In the event of a significant interaction effect (treatment × time), Bonferonni *post hoc* analysis was performed to locate where the differences lie. Paired sample *t*-tests were used to assess differences in match-running and heart rate variables between the two conditions. The belief questionnaire was analyzed using the Wilcoxon signed-rank test. To estimate the magnitude of the treatment effects, Cohen's *d* effect sizes (ES) were calculated with the magnitude of effects considered either small (0.20–0.49), medium (0.50–0.79), or large (>0.80). All data were analyzed using Statistical Package for

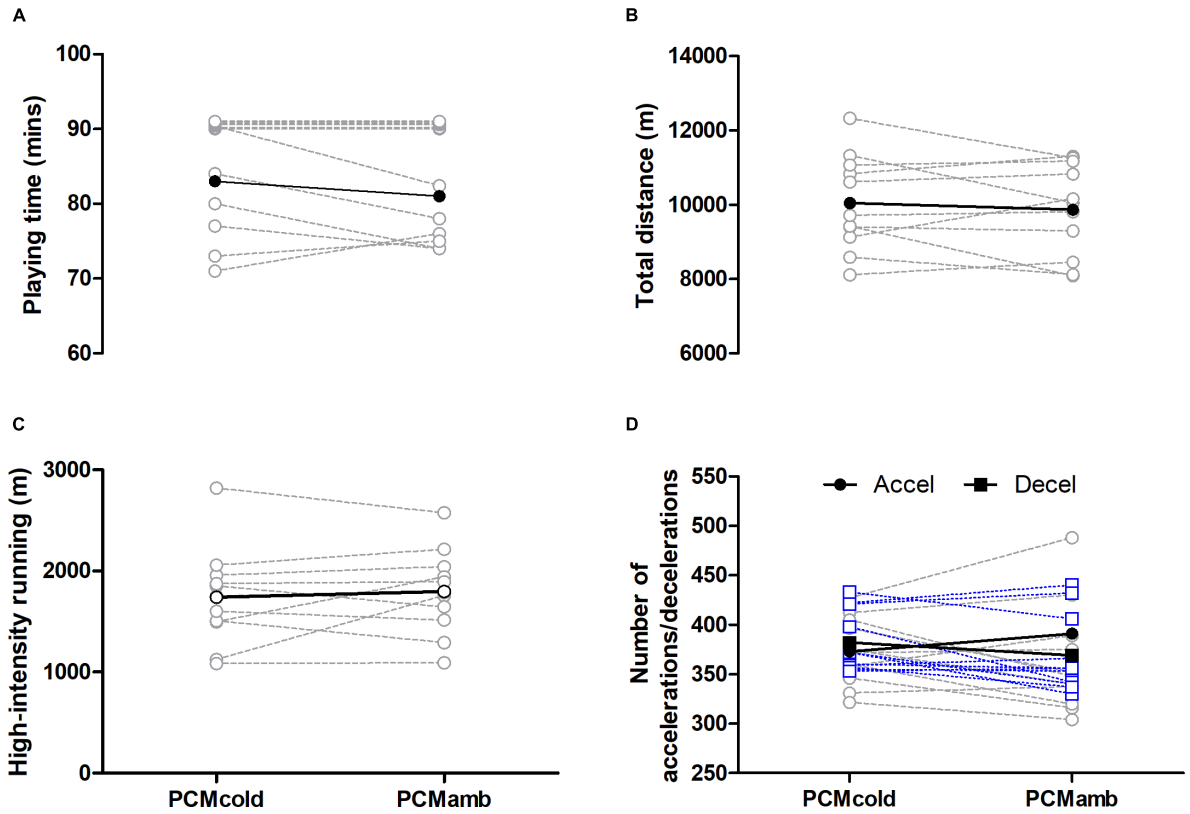

FIGURE 1 | Playing time **(A)**, total running distance **(B)**, high-intensity running **(C)** and number of accelerations (dashed gray line) and decelerations (dashed blue line; **D**) measured during soccer match-play for two conditions (PCM_{cold} vs. PCM_{amb}; $n = 11$). Values corresponding to 90 min in **(A)** are offset to prevent overlapping for clarity.

Social Sciences (SPSS version 22.0). Statistical significance was accepted at $P < 0.05$.

RESULTS

Match Performance and Intensity

Match activity variables are displayed in **Figure 1**. No differences in playing time, match activity, or heart rate variables (mean HR 167 ± 9 and 165 ± 5, peak HR 192 ± 7 and 195 ± 9 for PCM_{cold} and PCM_{amb}, respectively) were found between the two conditions ($P \geq 0.10$). Players were required to play at least 70 min in order to be included in the intervention; no players were excluded on this criterion. In terms of treatment order, six players wore PCM_{amb} first and five players wore PCM_{cold}.

Perceptual Responses

Perceptual responses from the Elite Performance Readiness Questionnaire can be viewed in **Table 1**. Soccer match-play elicited fatigue [$F_{(3,\ 30)} = 18.62$, $P < 0.001$] and soreness [$F_{(3,30)} = 17.99$, $P < 0.001$] which persisted up to 72 h relative to baseline (all $P \leq 0.03$). No effects of PCM were observed for any of the perceptual responses [$F_{(3,\ 30)} = \leq 0.65$, $P \geq 0.59$]. Analysis of the belief questionnaire revealed no differences in the perceived effectiveness of the two treatments either pre- or post-intervention ($P = 0.56$; **Table 2**).

Neuromuscular Function

Neuromuscular function variables are depicted in **Figure 2**. Soccer match-play elicited declines in MVC force [$F_{(3,\ 30)} = 6.26$, $P < 0.01$], VA measured with motor nerve stimulation [$F_{(3,30)} = 5.05$, $P < 0.01$], and $Q_{tw,pot}$ [$F_{(3,\ 30)} = 3.09$; $P = 0.03$], with impairments in MVC and $Q_{tw,pot}$ persisting for up to 72 h post-match (all $P \leq 0.04$), and reductions in VA persisting for up to 48 h post-match ($P = 0.03$). VA_{TMS} did not change at any time-point [$F_{(3,\ 30)} = 2.662$, $P = 0.129$]. Similarly, no time effect was found for M_{max} [range 4.4–4.8 mV, $F_{(3,\ 30)} = 0.808$, $P = 0.524$], or any measure of muscle contractility [$F_{(3,\ 30)} \leq 0.768$, $P \geq 0.547$; **Figure 3**]. No treatment × time interactions were observed for any of the neuromuscular variables ($F \leq 2.73$, $P \geq 0.18$). However, a main effect of treatment was found for both MVC [$F_{(1,\ 10)} = 6.254$, $P = 0.03$] and VA with motor nerve stimulation [$F_{(1,\ 10)} = 5.47$, $P = 0.04$]. *Post hoc* analyses revealed that for both MVC and VA, there was no difference between PCM_{cold} and PCM_{amb} at 24 h (MVC: $P = 0.28$; motor nerve VA: $P = 0.61$), while at 48 h, MVC ($P = 0.03$; $d = 0.26$) and VA ($P = 0.01$; $d = 0.66$) were higher in the PCM_{cold} compared with the PCM_{amb} condition.

TABLE 1 | Perceptual responses measured through a visual analog scale (mm) at pre-, and 24, 48, and 72 h post-match (n = 11) for two conditions (PCM_{cold} vs. PCM_{amb}).

	PCM_{cold}				PCM_{amb}			
	Pre-	**24 h**	**48 h**	**72 h**	**Pre-**	**24 h**	**48 h**	**72 h**
Fatigue	15.2 ± 11.8	55.5 ± 17.7**	37.3 ± 21.7*	23.7 ± 9.2	20.9 ± 18.0	51.7 ± 21.0**	41.0 ± 13.6*	24.0 ± 14.6
Soreness	18.6 ± 13.5	53.9 ± 17.7**	40.2 ± 16.1**	20.8 ± 18.3	23.5 ± 20.7	52.1 ± 19.6**	51.8 ± 18.2**	28.4 ± 19.1
Motivated to train	74.4 ± 20.2	51.6 ± 21.4	66.8 ± 14.4	67.6 ± 18.6	71.8 ± 23.6	45.2 ± 18.6	57.2 ± 24.5	64.8 ± 25.3
Anger	11.8 ± 9.4	12.9 ± 10.9	7.5 ± 4.5	7.7 ± 6.9	10.5 ± 9.7	14.6 ± 18.6	8.5 ± 7.1	7.1 ± 6.4
Confusion	18.6 ± 13.5	53.9 ± 17.7	40.2 ± 16.1	20.8 ± 18.3	23.5 ± 20.7	52.1 ± 19.6	51.8 ± 18.2	28.4 ± 19.1
Depression	8.5 ± 6.6	16.0 ± 17.2	8.1 ± 5.7	7.2 ± 4.8	7.7 ± 7.2	8.5 ± 6.6	8.9 ± 8.1	8.9 ± 6.2
Tension	20.5 ± 15.9	33.5 ± 25.1	17.6 ± 14.0	14.5 ± 8.6	18.9 ± 16.2	30.8 ± 22.9	25.0 ± 15.3	18.8 ± 15.6
Alertness	68.4 ± 16.7	46.5 ± 23.2	60.5 ± 17.0	63.9 ± 24.4	66.5 ± 22.4	54.5 ± 20.1	65.4 ± 18.8	65.6 ± 14.9
Confidence	65.6 ± 21.6	71.5 ± 12.3	66.8 ± 22.1	74.5 ± 10.6	71.9 ± 15.3	71.1 ± 12.0	70.7 ± 16.0	75.6 ± 12.5
Sleep	67.1 ± 18.1	63.5 ± 27.5	65.9 ± 18.1	64.2 ± 25.1	72.7 ± 24.4	56.5 ± 27.4	66.5 ± 15.2	63.5 ± 25.4

*Values are mean ± SD. Significant differences in comparison with baseline indicated by *$p < 0.05$, **$p < 0.01$.*

Physical Function

Physical function variables are displayed in **Figure 4**. Although a main effect for time on CMJ height was observed [$F_{(3,\ 30)} = 5.01$, $P = 0.03$], *post hoc* analysis revealed no significant differences relative to baseline (**Figure 4A**). Soccer match-play results in reductions in RSI [$F_{(3,\ 30)} = 7.45$, $P = 0.02$] which persisted for up to 48 h ($P = 0.02$; **Figure 4B**). There was no effect of PCM on any of the physical function variables [treatment × time $F_{(3,30)} \geq 1.05$, $P \geq 0.20$].

DISCUSSION

The aim of the present study was to examine the effect of wearing cold PCM garments on recovery of neuromuscular function, physical function and perceptual measures following soccer match-play. It was hypothesized that wearing cold PCM garments would expedite recovery of impaired neuromuscular function and attenuate muscle soreness, possibly by reducing the negative effects associated with the acute inflammatory response on contractile and CNS function. Although there was no difference in the change in any of the neuromuscular, physical function or perceptual indices over time, as indicated by the lack of a treatment × time interaction, MVC and VA were higher 48 h post-match after wearing PCM_{cold} compared with PCM_{amb}, with the between-treatment differences at these time-points greater than the measurement error. Nevertheless, PCM_{cold} had no apparent effect on contractile function (measured through evoked responses to electrical stimulation at rest), physical function, and fatigue or soreness in the days following match-play. These findings suggest that while wearing cold PCM garments could attenuate the magnitude of impairments in MVC and the ability of the CNS to activate the knee extensors, the lack of effect on measures of physical performance or perceptual responses during the recovery period post-soccer match-play implies that PCM offers a limited benefit to the recovery process.

TABLE 2 | Percieved effectiveness of the PCM garments for recovery before and after the intervention measured using a 1–5 Likert scale.

	PCM_{cold}	PCM_{amb}
Pre-match	3.6 ± 0.5	3.0 ± 0.6
72 h post-match	3.3 ± 0.9	3.0 ± 1.0

Fatigue and Impairments in Neuromuscular Function Following Competitive Match-Play

The magnitude of impairments in the maximal force generating capacity of the muscle and the time-course of recovery in the present study was similar to that observed following competitive match-play in a study conducted by Rampinini et al. (2011), but less than was observed by Brownstein et al. (2017), in which MVC remained 11% below baseline at 24 h post. Specifically, MVC was reduced at 24 (PCM_{cold} 5.2%, PCM_{amb} 7.5%) and 48 h (PCM_{amb} 4.3%), before recovering by 72 h post-match. Similarly, $Q_{tw,pot}$ was reduced at 24 (PCM_{cold} 8.0%, PCM_{amb} 6.7%), and 48 h (PCM_{cold} 4.2%, PCM_{amb} 3.4%), before recovering by 72 h post-match. Voluntary activation measured with motor nerve stimulation was reduced at 24 h (PCM_{cold} 1%, PCM_{amb} 6%) before recovering by 48 h post-match. In addition, physical function measured through the DJ-RSI was impaired for up to 72 h post-match, while analysis of perceptual responses indicate that fatigue and muscle soreness persisted for up to 72 h post-match. Furthermore, the reduction in MVC, one of the most widely used indicators of EIMD (Goodall et al., 2017), along with the increase in muscle soreness for up to 72 h post-match, indicates that the competitive soccer matches involved in the study elicited muscle damage. The occurrence of muscle damage was likely a consequence of the high volume of decelerations recorded throughout the matches along with the numerous other eccentric actions associated with soccer match-play. Given that recovery of contractile and CNS function has been shown to occur rapidly following exercise that is metabolically, but not mechanically demanding (Skurvydas et al., 2016), it is likely that the prolonged impairments in $Q_{tw,pot}$ and VA in the present study were a consequence of the muscle damage incurred

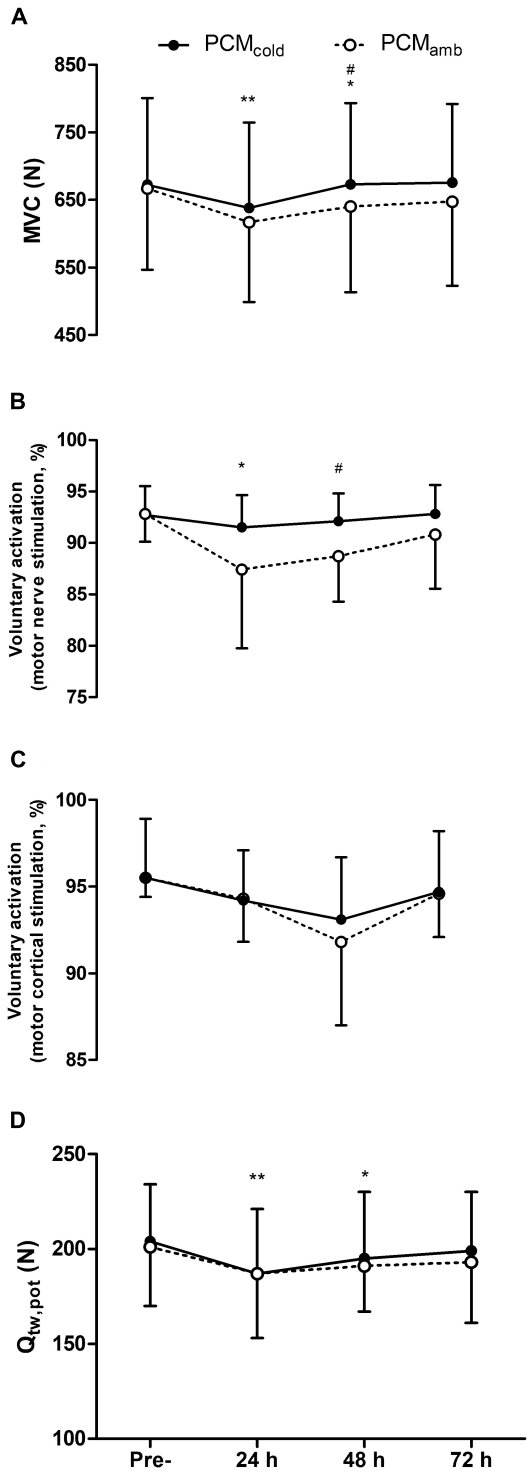

FIGURE 2 | Maximal voluntary contraction force (MVC, **A**), voluntary activation measured with femoral nerve stimulation **(B)**, voluntary activation measured using motor cortical stimulation **(C)**, and quadriceps potentiated twitch force ($Q_{tw,pot}$, **D**) measured at pre-, 24, 48, 72 h post-competitive soccer match-play for two conditions (PCM_{cold} vs. PCM_{amb}; $n = 11$). Values are mean ± SD. Significant between-treatment differences indicated by $^{\#}p < 0.05$. Significant differences in comparison with baseline indicated by $^{*}p < 0.05$ and $^{**}p < 0.01$.

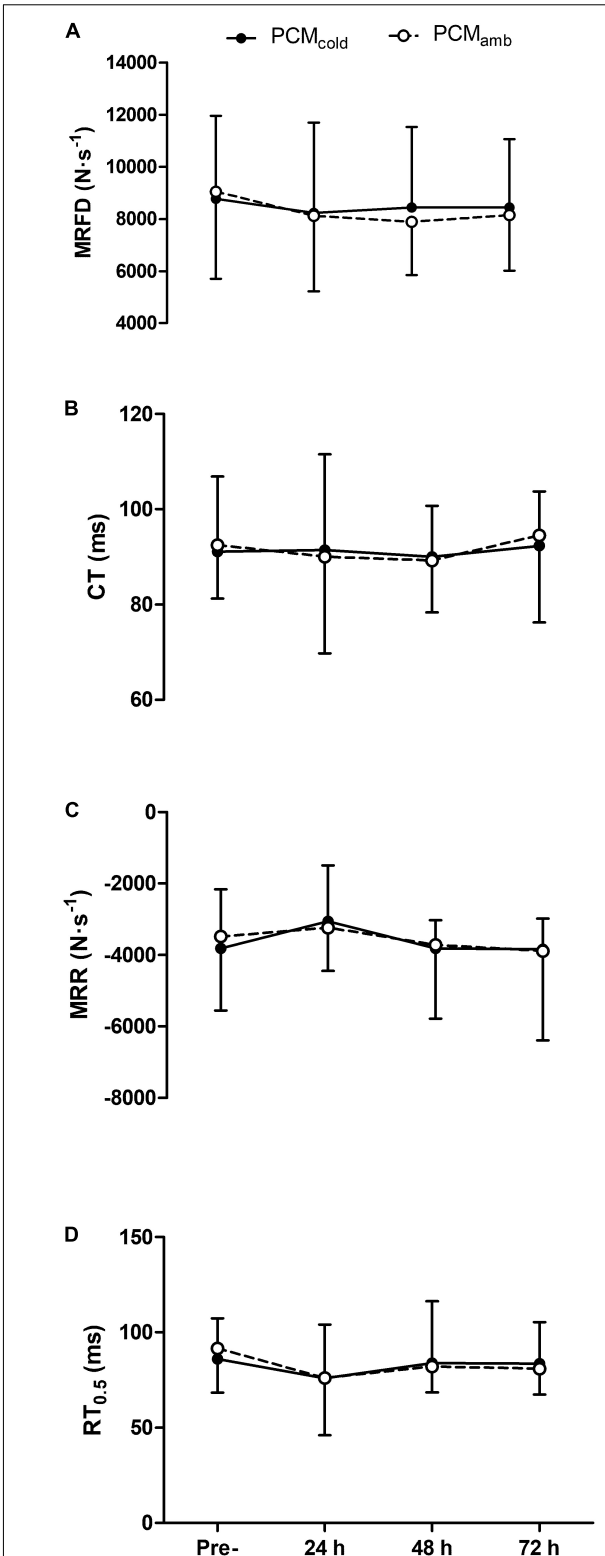

FIGURE 3 | Maximum rate of force development (MRFD, **A**), contraction time (CT, **B**), maximum rate of relaxation (MRR, **C**) and half-relaxation time ($RT_{0.5}$, **D**) measured from the electrically evoked quadriceps potentiated twitch ($Q_{tw,pot}$) assessed at pre-, 24, 48, 72 h post-competitive soccer match-play for two conditions (PCM_{cold} vs. PCM_{amb}; $n = 11$). Values are mean ± SD.

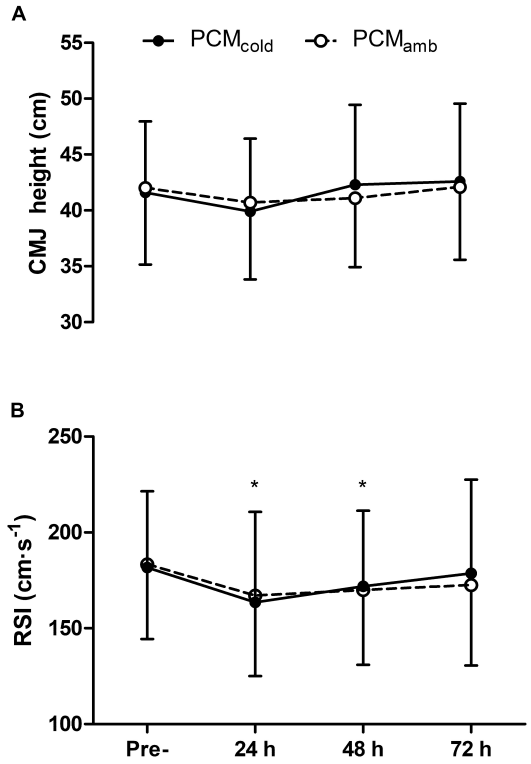

FIGURE 4 | Countermovement jump height (CMJ, **A**) and reactive strength index (RSI, **B**) measured at pre-, 24, 48, 72 h post-competitive soccer match-play for two conditions (PCM_{cold} vs. PCM_{amb}; $n = 11$). Values are mean ± SD. Significant differences in comparison with baseline indicated by $^{*}p < 0.05$.

during match-play along with the inflammatory response which ensues thereafter.

Effect of PCM_{cold} on Neuromuscular Function

The decline in the maximum force generating capacity of the quadriceps was attenuated following PCM_{cold}, as indicated by the higher MVC at 48 h post compared with PCM_{amb}. Furthermore, the between-treatment differences in the magnitude of reduction in MVC between baseline and 24 (2.3%) and 48 h (4.3%) was greater than the measurement error obtained from the two baseline visits in the present study (1.7%). The attenuated decline in MVC following PCM_{cold} in the present study is in line with recent studies conducted by Clifford et al. (2018) and Kwiecien et al. (2018), which displayed a substantially accelerated recovery of MVC strength in the days following soccer match-play and an eccentric based exercise protocol, respectively. The magnitude of improvement in the present study, however, was lower than that of Clifford et al. (2018), who found a treatment × time interaction and large effect size between the PCM_{cold} and PCM_{amb} condition at 36 h post-match. This could have been due to the substantially lower decline in MVC in the present study (4 ± 5% reduction at 48 h compared with ∼15% at 36 h post-match in the study by Clifford et al. (2018)), possibly owing to participants continuing to train in the days post-match and potentially compounding reductions in MVC in the study by Clifford et al. (2018), while players in the present study refrained from physical activity in the 72 h post-match. Taking this into consideration, it could be suggested that PCM could be a useful tool during periods of heavy training and/or competition, during which impairments in muscle function could be compounded by limited recovery periods.

The ability of the muscle to generate maximum force is influenced by the capacity of the CNS to activate the muscle, and the efficacy of excitation-contraction processes occurring at or distal to the neuromuscular junction to produce force in response to neural input. In the present study, motor nerve VA was greater following PCM_{cold} compared with PCM_{amb} at 48 h, while, similar to MVC, the between-treatment difference in the reduction in VA between baseline and 24 (5.0%) and 48 h (3.8%) was greater than the VA measurement error (2.8%). Considering the higher VA following PCM_{cold} coupled with the lack of effect on contractile function ($Q_{tw,pot}$), this suggests that the beneficial effect of PCM_{cold} on MVC was primarily due to improvements in VA. It is difficult to deduce the precise mechanisms by which PCM_{cold} improved VA in the present study. However, while the mechanisms underpinning the prolonged reduction in VA post-exercise are largely unknown (Carroll et al., 2017), it is well established that muscle damage elicits impairments in VA which can take several days to resolve (Goodall et al., 2017). Factors associated with inflammation are thought to interfere with CNS function, through an increase in firing rate of group III and IV muscles afferents sensitive to various markers of muscle injury (e.g., bradykinin, histamines, and prostaglandins; Pitman and Semmler, 2012). Alternatively, or additionally, increases in the concentration of brain cytokines following eccentric exercise are potent modulators of brain function (Dantzer, 2001), and might also influence recovery of CNS impairment (Carmichael et al., 2006). Given that one of the proposed benefits of cryotherapy is to attenuate inflammation, it is possible that wearing PCM_{cold} post-match could have reduced the inflammatory response and thereby attenuated inflammation-induced perturbations in CNS function. However, given that markers of inflammation were not measured in the present study, this suggestion remains speculative. Further research examining the effects of PCM_{cold} on inflammation concurrent with measures of VA following damaging exercise is thus warranted.

A number of previous studies have shown that muscle damage leads to prolonged impairments in contractile function, as evidenced through protracted reductions in $Q_{tw,pot}$ (Endoh et al., 2005; Goodall et al., 2017). It is likely that the prolonged reductions in $Q_{tw,pot}$ following eccentric based exercise are a consequence of direct myofibrillar damage, disorganization of sarcomeres and interference with cellular Ca^{2+} handling which inhibit the excitation-contraction coupling process (Skurvydas et al., 2016). The lack of change in M_{max}, a measure of neuromuscular transmission, suggests that the prolonged reduction in $Q_{tw,pot}$ in the present study was due to processes beyond the sarcolemma. In addition to measuring M_{max} and $Q_{tw,pot}$, we included measures of muscle contractility (namely, MRFD, CT, MRR, and $RT_{0.5}$) to attempt to provide further

insight into contractile function following soccer match-play. Similar to previous studies (Rampinini et al., 2011; Brownstein et al., 2017), reductions in $Q_{tw,pot}$ persisted despite a lack of change in measures of contractility, suggesting that impairments within the excitation-contraction coupling process which were not detectable through our measures of muscle contractility were responsible for the decline in $Q_{tw,pot}$. While we cannot discern the precise mechanism for the protracted impairment in contractile function, events that occur secondary to the initiation of muscle damage have also been implicated in impairments in excitation-contraction coupling. Specifically, the accumulation of reactive oxygen/nitrogen species has been shown to interfere with SR Ca^{2+} release, which has been attributed to redox modification of ryanodine receptors (Cheng et al., 2016), while reactive oxygen species are also thought to diminish the calcium sensitivity of myofilaments (Moopanar and Allen, 2005; Reid, 2008). In this regard, it was thought that the application of cryotherapy, which has been suggested to inhibit the inflammatory response and limit the generation of reactive oxygen/nitrogen species (White and Wells, 2013), could ameliorate the impairments in contractile function in the days following soccer match-play. However, the application of cold PCM had no effect on recovery of either $Q_{tw,pot}$. The lack of effect of PCM_{cold} on neuromuscular function could have been due to a number of factors. Firstly, whether or not cryotherapy actually reduces inflammation remains equivocal, despite its widespread application (Broatch et al., 2014; Peake et al., 2017). Veritably, studies have neither been consistent nor produced compelling evidence to support the role of cryotherapy in reducing inflammation and improving aspects of recovery (Leeder et al., 2012), and it has been suggested that many of the previously reported benefits of cryotherapy could simply be due to a placebo effect, rather than any physiological effect (Broatch et al., 2014). Despite the promising findings from recent studies using cold PCM as a recovery aid (Clifford et al., 2018; McHugh et al., 2018), and that applying these garments has been shown to reduce muscle temperature (Kwiecien et al., 2018), there is no evidence to suggest that cold PCM reduces inflammation. As such, it is possible that PCM_{cold} had no effect on the inflammatory processes suggested to interfere with contractile and CNS function. Secondly, as alluded to previously, the magnitude of the impairments in $Q_{tw,pot}$ was relatively small, potentially limiting the ability to detect subtle differences between groups. Indeed, it would be reasonable to assume that the benefits of cryotherapy on recovery would only be evident were the impairments in neuromuscular function more substantial than those seen in the present study. Further research to examine the effects of wearing cold PCM on recovery of neuromuscular function following exercise which elicits substantially more damage is probably warranted.

Effect of PCM_{cold} on Physical Function

Despite the improvement in MVC and motor nerve VA with PCM_{cold}, there was no improvement in measures of jump performance (CMJ or DJ-RSI). The lack of effect of PCM_{cold} on physical performance measures might have been due to the relatively modest improvement in MVC and VA in the present study. Thus, the functional relevance and meaningfulness of the differences between PCM_{cold} and PCM_{amb} for MVC and VA are unclear, and could be questioned. Although it is plausible that a reduced capacity of the CNS to activate muscles would impede the ability to perform tasks requiring maximal force production, given that current methods of determining VA are restricted to isometric or isokinetic contractions, it is not possible to accurately qualify the functional consequences of reduced VA on the performance of unconstrained physical tasks relevant to football performance.

Limitations

This study used a competitive soccer match in order to study the effects of the application of cold PCM on recovery in the days post-match. While this approach provides the most ecologically valid means of investigating the effects of a recovery intervention following soccer match-play, one limitation of this method compared with a laboratory simulation is the lack of experimental control over the activity profiles of the players and the high inter-subject variability in match demands. Consequently, it is possible that differences between match-demands could have influenced the magnitude of fatigue and time-course of recovery following the two treatments. However, differences between the time-motion and heart rate variables between the matches were negligible. Furthermore, although simulated match protocols are designed to replicate the physiological demands of competitive matches, many of the neuromuscular, skill and cognitive demands associated with competitive match-play cannot be replicated through match simulations, and the validity of using these protocols when assessing the efficacy of a recovery intervention could thus be questioned. In addition, although no differences were found in the results from the belief questionnaires, on average, participants reported that they believed both PCM_{cold} and PCM_{amb} were "moderately effective" in improving recovery both before and following the intervention. As such, it is possible that a placebo effect could have influenced recovery under both conditions. However, the magnitude of fatigue and the time-course of recovery was similar to that observed following competitive match-play in professional soccer players (Rampinini et al., 2011), suggesting that any placebo effect on the results was negligible. Furthermore, that the participants believed both interventions to be moderately effective could be considered an important finding given that a growing body of evidence indicates that recovery is related to individual preference and perceptions of the intervention (Halson, 2014). Moreover, because local tissue temperature was not measured in the present study, it is unknown whether or not PCM_{cold} had the desired effect in regards to cooling the muscle. Nevertheless, previous work has displayed that PCM_{cold} reduced skin temperature to 22°C for 3 h following eccentric based exercise (Kwiecien et al., 2018). Thus, it is likely that the skin temperature was, similarly, decreased in the present study. Finally, another limitation of the present study was the 4–8 week gap between matches for each condition. Consequently, it is possible that players were in a different phase of the training cycle between the two matches, potentially influencing the magnitude of fatigue and time-course of recovery in response to competitive match-play. However, the majority of fixtures

were separated by 6 weeks or less, with only two matches separated by 8 weeks. As such, it is likely that the influence of the duration between conditions had a negligible effect on the results of the study.

CONCLUSION

The present study showed that applying cooled phase change material to the quadriceps and hamstring muscles for 3 h following soccer match-play resulted in a modest effect on the recovery of MVC and VA, but had no effect on contractile or physical function, or perceptual responses. It is possible that the lack of effect of these garments could be due to the relatively small impairments in contractile and physical function in the days post-match. Despite the limited benefit of PCM on recovery, the results from the belief questionnaires indicated that participants believed the PCM to be moderately effective in improving their recovery following the intervention. This could be considered an important finding given that the efficacy of recovery interventions could be related to individual preference and perceptions of the intervention. Further investigations are warranted to assess whether cold PCM has any effect of neuromuscular function during periods of fixture congestion, when muscle damage could be compounded by the limited recovery periods.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Northumbria University Health and Life Sciences Ethics Committee, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Northumbria University Health and Life Sciences Ethics Committee.

AUTHOR CONTRIBUTIONS

CB, KT, SG, GH, and MM contributed to the conception and design of the work, contributed to the interpretation, and analysis of the data. CB, PA, and JŠ acquired the data for the study. All authors have drafted and revised the intellectual content and revised the final version. All listed authors qualify for authorship.

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Individualized Monitoring of Muscle Recovery in Elite Badminton

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Purpose: Individualized reference ranges for serum creatine kinase (CK) and urea are a promising tool for the assessment of recovery status in high-level endurance athletes. In this study, we investigated the application of this approach in racket sports, specifically for the monitoring of elite badminton players during the preparation for their world championships.

Methods: Seventeen elite badminton players were enrolled of which 15 could be included in the final analysis. Repeated measurements of CK and urea at recovered (R) and non-recovered (NR) time points were used for the stepwise individualization of group-based, prior reference ranges as well as for the evaluation of classificatory performance. Specifically, blood samples were collected in the morning following a day off (R) or following four consecutive training days (NR), respectively. Group based reference ranges were derived from the same data. Error rates were compared between the group-based and individualized approaches using the Fisher exact test.

Results: Error rates were numerically lower for the individualized as compared to the group-based approach in all cases. Improvements reached statistical significance for urea (test-pass error rate: $p = 0.007$; test-fail error rate: $p = 0.002$) but not for CK (p vs. group-based: test-pass error rate: $p = 0.275$, test-fail error rate: $p = 0.291$). Regardless of the chosen approach, the use of CK was associated with lower error rates as compared to urea.

Conclusion and Practical Applications: Individualized reference ranges seem to offer diagnostic benefits in the monitoring of muscle recovery in elite badminton. The lack of significant improvements in error rates for CK is likely due to the large difference between R and NR for this parameter with error rates that are already low for the group-based approach.

Keywords: reference range, Bayesian, fatigue, sport, recovery

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INTRODUCTION

In elite sport, the assessment of recovery status has become an important goal to prevent accumulating fatigue which may lead to maladaptive states such as non-functional overreaching or overtraining and increase the risk of injury. During the last decades numerous fatigue indicators have been described including (but not limited to) subjective ratings, heart rate measures as well

as blood borne markers (Majumdar et al., 1997; Hecksteden et al., 2016, 2017). However, large interindividual variability impedes on the diagnostic accuracy of all fatigue indicators known to date and thereby limits their utility in the assessment of individual athletes (Majumdar et al., 1997; Hecksteden et al., 2016). Individualized reference ranges may offer a solution to this challenge, comparable to the principle of the Athlete Biological Passport (Sottas et al., 2007; Hecksteden et al., 2016). Following this rationale, our group has recently developed a method to gradually individualize group-based (prior) reference ranges of fatigue indicators (Kellmann et al., 2018) resulting in separate "corridors" for recovered (R) and non-recovered (NR) states. In this work we aim to scrutinize transferability of this method to elite badminton by monitoring the preparation phase of one part of the German national team for the world championships 2017.

Following this rationale, we opted to employ the same markers and very similar methodology which have been successfully used in the original publication of the method (Kellmann et al., 2018). Blood borne markers seem particularly promising due to their objectivity, minimal interference with the training process, low technical error of measurement and known physiology (Majumdar et al., 1997; Hecksteden et al., 2017). Among the multitude of blood borne fatigue indicators (Majumdar et al., 1997; Hecksteden et al., 2017), creatine kinase (CK) and urea have been selected for the development of the individualization procedure (Kellmann et al., 2018) as well as for this work for several reasons. From a practical perspective, CK and urea are inexpensive to measure and already known in sports practice. From the physiological perspective, urea as the end product of protein breakdown reflects metabolic strain and ultimately energy balance. It is therefore mainly elevated by high training volumes (Hecksteden et al., 2017). By contrast, serum CK levels increase especially after eccentric muscle contractions, therefore CK is widely used as a marker of training induced muscle strain and recovery. It may therefore be plausibly expected to play an important role in badminton. The appropriateness of the selected markers for badminton is underlined by the previously reported increase in serum CK and urea levels 12 h after badminton specific training (Sottas et al., 2011). However, it has to be kept in mind that the applicability of the individualization algorithm is in principle not limited to CK and urea or even to blood borne markers in general.

Taken together, we report the first application of a recently published method for the individualized monitoring of muscle recovery in elite sports practice, specifically the preparation of badminton players for world championships. We thereby scrutinize the method's transferability from endurance to racket sports as well as into sports practice.

MATERIALS AND METHODS

Experimental Approach to the Problem

The present work employed an observational approach. Due to the importance of the competitive event, any interference with the training process would have been unacceptable for coaches and athletes. As illustrated in **Figure 1**, the study period from April to August 2017 included a learning phase for the derivation of individualized reference ranges (April–early July) and an individualized monitoring phase during the immediate preparation for the world championships (Mid-July–August). According to the published algorithm (Kellmann et al., 2018), individualized reference ranges were derived from values for CK and urea at time points with known recovery status (R or NR, respectively). Considering that a reference classification is needed for the assessment of error rates, time points from the learning phase were also used to assess the performance of the individualization procedure (Kellmann et al., 2018). Of course, this was conducted as a cross-validation, meaning that the data points to be classified were not included in the respective run of the individualization procedure. Therefore, the main results of this work are based on data from the learning phase. Results of the individual monitoring phase are presented graphically to illustrate the potential application in sports practice.

Subjects

Seventeen elite male badminton players, all members of the German national squad and training at the National Training Center of the German Badminton Association and Olympic Training Center Rheinland-Pfalz/Saarland in Saarbrücken, volunteered to participate in this study. Females could not be included because they were training in another center. Each participant was informed about the experimental procedures of the study and provided written informed consent. The study was approved by the local Human Research Ethics Committee (Ärztekammer des Saarlandes, approval no. 228/13 and amendments). Two players had to be excluded due to an insufficient number of time points during the learning phase. Characteristics of the remaining 15 athletes are summarized in **Table 1**.

Procedures

Venous blood samples were collected in the morning before the first training bout of the day. Standard methods were used for venous blood sampling and analysis as previously published (Hecksteden et al., 2017). In particular, CK and urea were analyzed within 60 min by automated routine techniques (UniCell DxC 600 Synchron; Beckman Coulter GmbH, Krefeld, Germany).

Procedures and statistical analyses were conducted in analogy to the initial publication of the method as described in short below.

Reference Classification of Time Points During the Learning Phase

In the learning phase, values for serum CK and urea were obtained during competition-free weeks and adapted to the individual trainings plans of the players. Criteria for the reference classification of R and NR time points are summarized in **Figure 2**. The resulting numbers of time points and players are illustrated in **Figure 3**. Training load and the possible presence of other physical loads was checked for every individual player by reviewing training logs and standardized questions. Personal communication was sought when needed. Additionally,

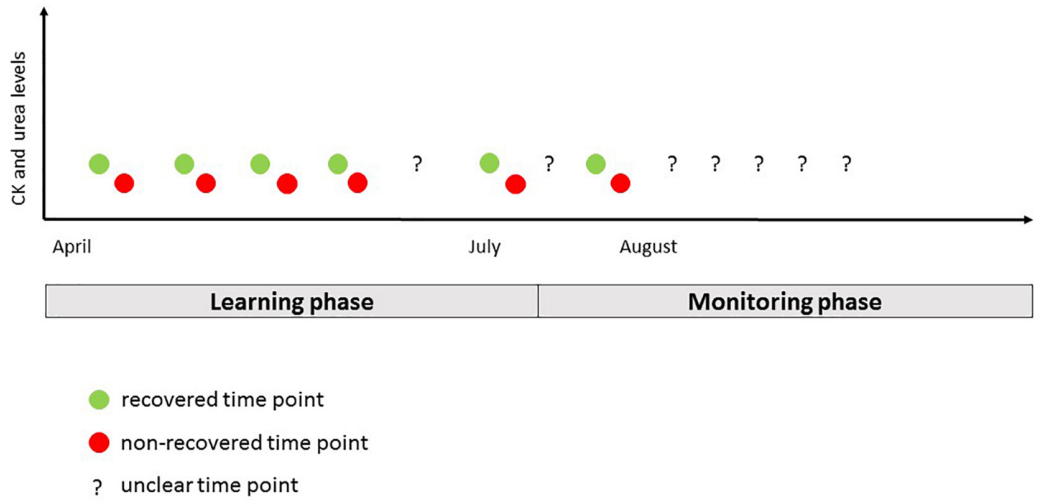

FIGURE 1 | General design.

TABLE 1 | Subject characteristics.

Age (years)	22 ± 3
Height (cm)	183 ± 6
Weight (kg)	78 ± 9
Years playing competitive badminton	10 ± 4
Training volume (h/week)	22 ± 2

Means ± standard deviation.

a validated questionnaire (Acute Recovery and Stress Scale, ARSS; Sottas et al., 2007; Kellmann et al., 2016; Kellmann and Kölling, 2019) was completed by each participant before blood sampling to verify subjectively perceived changes in recovery status. The German version of the ARSS, which was used in this study, contains eight items describing physical, emotional, mental and overall aspects of recovery and fatigue using a seven-point scale.

During the individual monitoring phase blood sampling was conducted on request of the coaches at the same time of day as during the learning phase.

The Individualization Procedure

Details of the individualization procedure including equations, statistical code and sport specific prior distributions have been published previously (Kellmann et al., 2018). In short, for every parameter a group based (prior) distribution is used as starting point. Importantly, relevant differences in the distribution of CK and urea between different sports were not present (Kellmann et al., 2018). Subsequently, individualized (posterior) distributions for NR and R time points are generated by stepwise inclusion of measurements from the individual athlete in the respective state.

For every subject several runs of the individualization procedure (equal to the number time points per fatigue state) are conducted to allow for the classification of all time points. For every run 4 R and 4 NR values were used in chronological order for the deduction of the individualized cut-off value which was then used to classify the following R and NR values, respectively. The principle is illustrated in **Figure 4**.

The principle of the stepwise individualization procedure is illustrated in **Figure 5** which shows the development of the individual "corridors" for one of the athletes. Of note, **Figure 5** also includes the values from the individual monitoring phase.

Classification of Time Points and Calculation of Error Rates

The cut off value for the individualized classification of time points was set at equal distance between the posterior means for R and NR time points as resulting from the forth individualization step. Importantly, no cut-off value was calculated when the posterior mean for R was higher than the posterior mean for NR after the forth individualization step. Rather, the respective run was excluded because the fifth value was considered non-classifiable (Kellmann et al., 2018).

The group-based comparator classification was based on the overall mean for the respective parameter (which, due to the equal number of time points between states is located at equal distance to the group means for R and NR time points). Thereby, the group-based cut-off value was based on the same data that were to be classified to avoid overestimating a potential benefit of the individualization procedure.

Error rates of the individualized and group-based approaches were determined by comparing classifications based on CK and urea with the reference classification. The proportion of data points that were falsely classified as R among all R classifications was defined as test-pass error rate. Test-fail error rate was defined as the proportion of data points falsely classified as NR among all NR classifications.

Statistical Analyses

All statistical analyses were conducted using Statistica software version 13.3 (StatSoft Hamburg). Raw values for CK as well as urea were log-transformed before any further calculations.

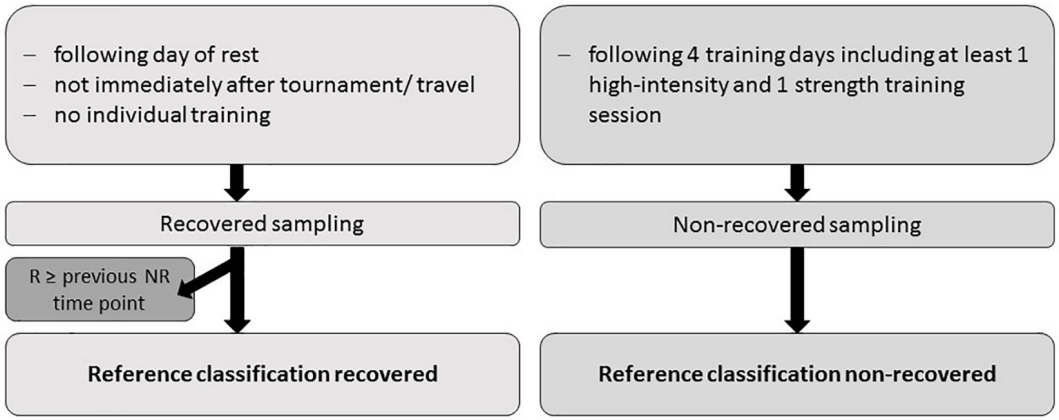

FIGURE 2 | Reference classification of time points. R, recovered; NR, non-recovered.

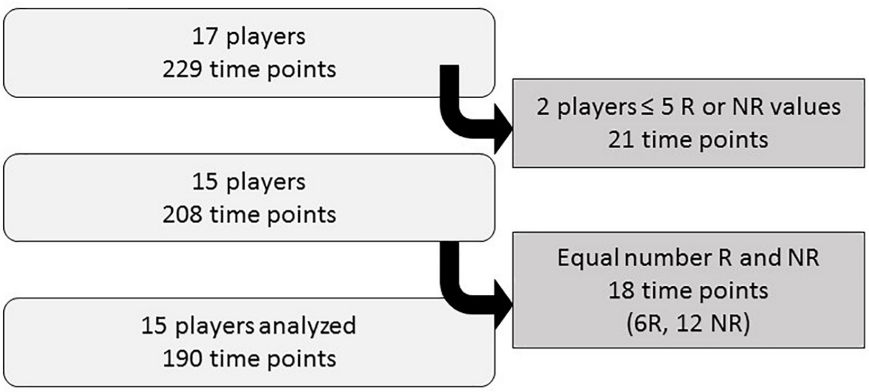

FIGURE 3 | Criteria for data inclusion. R, recovered; NR, non-recovered.

The log-transformed data were normally distributed for either parameter. Results were transformed back to the original scale. Descriptive statistics are reported as means and standard deviations (SD). Differences in CK and urea between R and NR time points were verified on the group level using a mixed linear model analysis. Recovery status was included as a fixed effect, the player's identity and status-by-subject ID interaction were random effects. To analyze the differences in error rates between the individual and the group-based classifications a Fisher's Exact test was conducted. The level of significance was set with an α-error of $p < 0.05$ for all tests.

RESULTS

Mean values for CK and urea were significantly higher for the NR compared to the R time points (CK (U/l): R 164 $\pm$ 106, NR 425 $\pm$ 319, $p < 0.001$; urea (mg/dl): R 35 $\pm$ 7, NR 39 $\pm$ 9, $p < 0.001$). As illustrated in the respective variability plots (**Figure 6**), considerable interindividual variation could be observed for individual mean values as well as for the difference between R and NR states.

Error rates were numerically lower for the individualized as compared to the group-based classification for either parameter and recovery status. However, the difference reached statistical significance only for urea (**Table 2**). Importantly, absolute values for CK error rates were already low for the group-based approach due to the large effect size of the difference between R and NR time points.

The rate of unclassifiable values was 15% ($n = 28$) for urea and 2% ($n = 4$) for CK.

The development of individual corridors over the learning phase as well as measured values from the individual monitoring phase are displayed in **Figure 5**. In this figure, player 2 and 11 are used as examples to illustrate the interindividual differences between reference ranges: CK values of 250 U/l are at the upper range of the NR corridor of player 2, whereas in player 11, this value can be found in the lower NR range. Urea levels do hardly differ between the R and NR states in player 2, but in player 11, urea levels are situated between 30 and 40 mg/dl when the player is recovered and about 50 mg/dl for the NR state. Of note, the urea corridors of player 11 show that values exceeding the clinical reference limit can be the physiological level for a specific, healthy individual. The corridors of each player are provided in

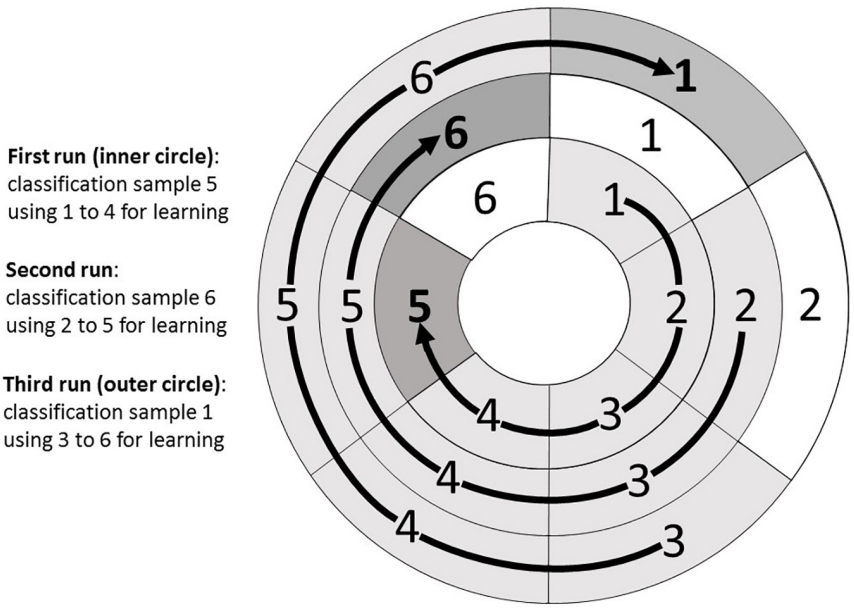

FIGURE 4 | Runs of the individualization procedure (exemplarily for six time points). For every run 4 recovered (R) and 4 non-recovered (NR) values were used in chronological order for the deduction of the individualized cut-off value which was then used to classify the following R and NR values. Values to be classified are marked dark gray, samples for learning are marked light gray.

Supplementary Figure S1. The individualized monitoring in this structured two-step procedure and particularly the visualization of reference ranges as individual corridors (**Figure 5** and **Supplementary Figure S1**) were evaluated as very helpful by the coaches (oral communication).

DISCUSSION

Reduced error rates for the assessment of muscle recovery in elite badminton from the use of a novel procedure are the main result of this study. Individualized reference ranges being derived from time points with known recovery status during the learning phase can be used for the individualized monitoring of athletes during the decisive training phase preceding the event. Although "success" of the individual monitoring phase may not be formally quantified within the framework conditions of the present trial, the individualized monitoring was deemed very helpful by the coaches. Together our results point to the transferability of individualized reference ranges for CK and urea to the monitoring of muscle recovery in elite sports practice, specifically in racket sports.

Regarding the magnitude of improvement in diagnostic accuracy, considerable differences between parameters were observed. While for urea error rates could be almost reduced by 50%, for CK the reduction in error rates (although numerically present) was less pronounced and failed to reach statistical significance. When interpreting the differences in the effect of individualization it should be kept in mind that group-based error rates for CK were already much lower compared to urea, leaving little potential for improvement. The lower group-based error rates for CK are attributable to the large overall CK response in our data which is readily explained by the high proportion of muscular strain in badminton due to the frequent accelerations and decelerations. Obviously, a larger mean difference between states and lower within-state variation both improve diagnostic accuracy of the respective indicator. This consideration also pertains to the higher rate of unclassifiable values for urea as compared to CK.

Moreover, the principle that the usefulness of CK and urea, respectively, for monitoring muscle recovery depends on the relationship of between-state contrast and within-state variation ("individual effect size") also applies on the individual level. Importantly, the variability plots displayed in **Figure 6** illustrate that athletes greatly differ in this respect. The calculation of individualized normal ranges for R and NR time points accounts for the variable between-state contrast by providing an individualized two point calibration of the respective marker. In other words: Measured values are interpreted in relation to two individualized anchor points which represent the extremes of recovery status attained during habitual training cycles. As a result, inferences from a measured CK or urea value on the current recovery status of a particular athlete may be made more confidently as compared to approaches which rely on only one (even if individual) reference. The same applies for interpreting the magnitude of changes in those markers. Taken together, the method applied in this work accounts for two challenging characteristics of blood-borne fatigue markers which so far impede on the assessment of recovery status in athletes (**Figure 6**): (i) differences in habitual levels and (ii) differences in the magnitude of changes in fatigue markers between R and NR time points. While the method employed here is the

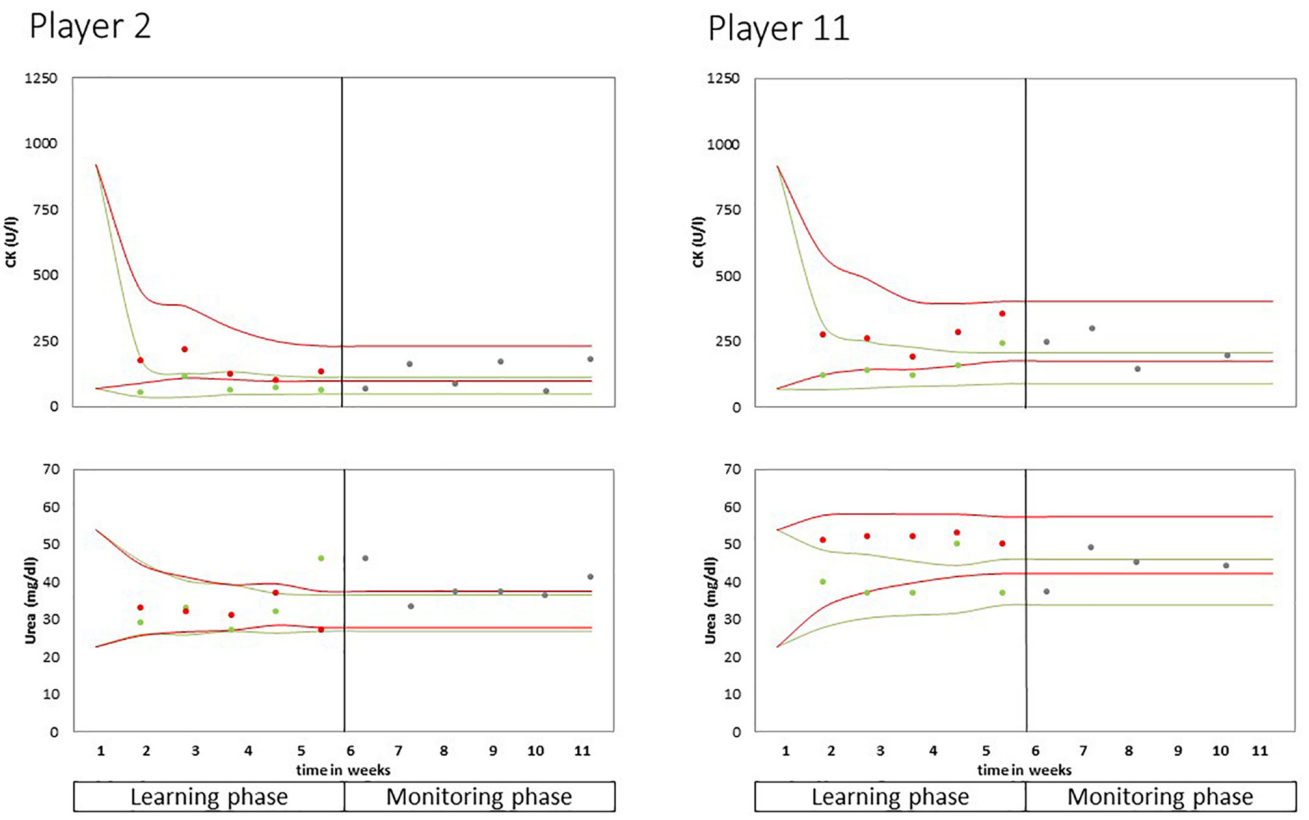

FIGURE 5 | Individual distributions of CK and urea levels.

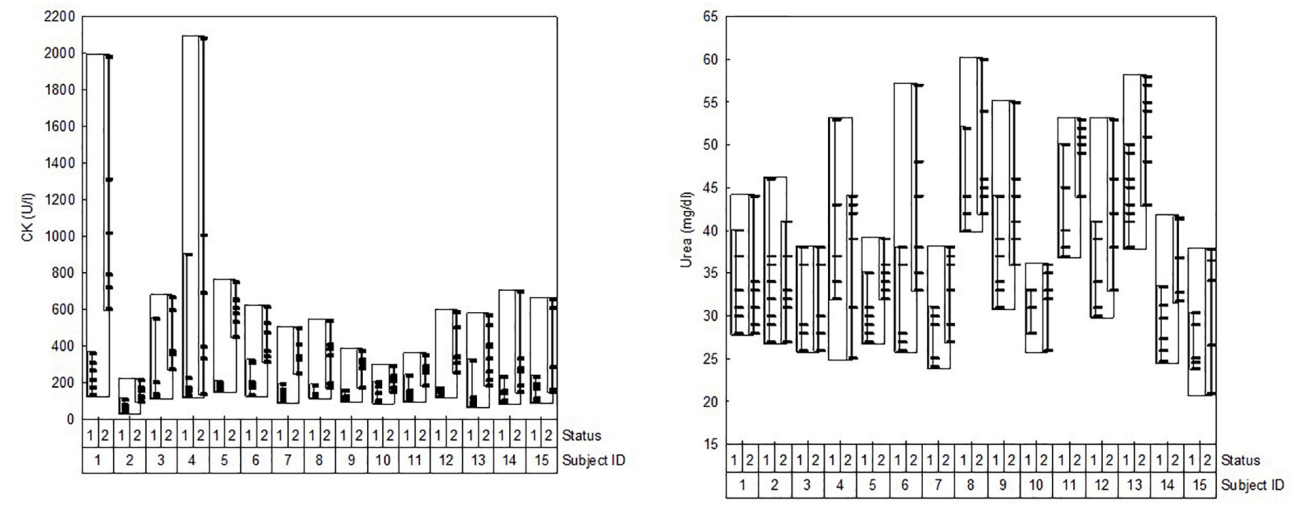

FIGURE 6 | Intra- and individual variabilities of CK and urea. Status: 1 = recovered, 2 = non-recovered.

first one to offer a two point calibration, more elementary methods are available to account for differences in habitual levels of fatigue indicators. Example are *z*-scores based on the individual mean and SD or on the individual mean and the standard error of measurement (Kellmann et al., 2018). However, due to the reliance of standard error on the number of data points, these methods require a high number of individual measurements (a long learning phase). The downside is avoided by the gradual individualization of group-based reference ranges (Hecksteden et al., 2017).

TABLE 2 | Test-pass and test-fail error rates for the group-based and the individualized classification.

	Group-based	Individualized	*p*
CK (U/l)			
Test-pass error rates	15 (16%)	9 (10%)	0.2751
Test-fail-error rates	24 (25%)	17 (18%)	0.2907
Urea (mg/dl)			
Test-pass error rates	41 (43%)	19 (23%)	<0.01
Test-fail-error rates	44 (46%)	19 (23%)	<0.01

Test-pass error rate was defined as the proportion of data points that were falsely classified as recovered among all recovered classifications. Test-fail error rate was defined as the proportion of data points falsely classified as non-recovered among all non-recovered classifications.

From a physiological perspective, elucidating the reasons as well as potential practical implications of the different responses in fatigue indicators to similar training within a single training group (**Figure 6**) is beyond the scope of this study. However, it may be speculated that subject inherent (e.g., biological and training age, muscle fiber distribution, endurance capacity) as well as environmental factors (e.g., nutrition) play a role. The potential efficacy of changing environmental factors in minimizing training induced changes in physiological fatigue indicators (e.g., increasing carbohydrate availability during and after training to minimize protein breakdown and the subsequent increase in serum urea concentration) and ultimately in performance decrements merits further investigation. In any case, the Bayesian rationale implemented in the present method will ensure that the individualized reference range follows changes in the habitual levels of the respective fatigue marker, state, and person over time.

Importantly, transferring the diagnostic potential of advanced analytical approaches (such as individualized reference ranges) to sports practice requires adequate communication of results to athletes and coaches (Meeusen et al., 2013; Bourdon et al., 2017). The visualization of the individual "corridors" (cp. **Figure 5**) seems to be helpful for communicating the interpretation of measured values during the individual monitoring phase – that is for the ultimate purpose of assessing athletes fatigue status when required by the coach. For example, CK levels about 1,000 U/l after high-intensity training are habitual for player 1 but for player 2 this value would be nearly four times higher than his usual NR levels.

Formally assessing the performance of the individualization procedure during the individual monitoring phase has not been possible in this setting. This would have required either a reference classification of time points for the calculation of error rates or hard endpoints plus a large-size comparator arm to assess the ecological validity of the approach (e.g., less health problems or better performance in the experimental group in which individual reference ranges are applied). While availability of a reference classification conflicts with the aim of assessing muscle recovery at time points with (pre-test) questionable recovery status, large-scale controlled trials are impractical in an elite sport setting especially within a particular discipline and during preparation for a major event. However, based on the reductions in error rates which have now been demonstrated in different sports, assessing the ecological validity of monitoring muscle recovery based on individualized reference ranges is warranted and should be attempted in an adequately powered application study.

Exercise induced fatigue is multidimensional (Kellmann et al., 2018). Therefore, multivariate analytical tools and interpretation may be expected to be preferable. Of note, the Bayesian approach presented may be generalized to multivariate distributions. However, while this reflects the fact that exercise induced fatigue is a complex construct, the multivariate method lacks an intuitive visualization. Moreover, in a pilot implementation only minor improvements in classificatory performance could be observed (Pitsch, 2017). We have therefore intentionally decided to transfer the univariate approach.

LIMITATIONS

Eventual benefits of monitoring muscle recovery with this method (e.g., mitigated injuries or better performance) could not be analyzed in this study due to the low subject number and lack of control group or period. This aspect warrants to be scrutinized in future studies to verify ecological validity.

The statistical method of individualizing reference ranges may be difficult to understand for the non-statistician. We never the less provide this information to ensure transparency and reproducibility. However, application of the method does not require understanding of the method on the mathematical level. The graphical representation (**Figure 5** and **Supplementary Figure S1**) provide an intuitive plausibility control for the fitting of individual corridors. An excel spreadsheet is provided with the original publication of Hecksteden et al. (2017) and can be used with own data.

Venous blood sampling will be difficult to implement in the context of routine monitoring in high performance sports. While for this study venous blood sampling and standard automated laboratory analyses were conducted mainly to ensure similarity to the methods in the original publication, capillary sampling and mobile devices for analysis may be used for transfer into sports practice.

CONCLUSION

Individual reference ranges for CK and urea seem to offer diagnostic benefits in the monitoring of muscle recovery in elite badminton, in particular when reference ranges are calculated for R and NR states offering an individual two-point calibration of the respective parameter. Visualizing the gradual development of individual "corridors" over successive measurements assists in communicating the interpretation of measured values to coaches and athletes.

ETHICS STATEMENT

This study was carried out in accordance with the declaration of Helsinki. The protocol was approved by

the local Human Research Ethics Committee (Ärztekammer des Saarlandes, approval no. 228/13 and amendments). Due to the small number of world class athletes effective anonymization of data is not possible. Therefore, data can not be published along with the manuscript.

AUTHOR CONTRIBUTIONS

VB conducted the blood sampling and drafted the manuscript. AH provided the notable intellectual input throughout drafting. AH and TM contributed equally as senior authors. HK was the national coach of the German Badminton Association and enabled the collaboration with the badminton players of the German national squad. AF, MK, and MP contributed to the design and funding of the study and to the interpretation of results. All co-authors drafted the manuscript and approved its final version.

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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.2019.00778/full#supplementary-material

FIGURE S1 | CK reference ranges are scaled from 0 to 1250 U/l, in case of spike values the scale was adapted from 0 to 2250 U/l. Urea reference ranges are scaled from 0 to 70 mg/dl. The learning phase of each player consists of 5 R and NR values and durated approximately 6 weeks. During the monitoring phase, data were collected individually on demand of the coaches and thus players have different numbers of values. Player 10 missed measurements during the general learning phase due to illness so that monitoring phase could not be conducted before world championships. For player 14, the learning phase started 4 weeks later than for the other players and durated until shortly before the world championships.

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frontiers in Physiology

ORIGINAL RESEARCH
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Photobiomodulation Therapy Does Not Attenuate Fatigue and Muscle Damage in Judo Athletes: A Randomized, Triple-Blind, Placebo-Controlled Trial

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Fatigue and muscle damage negatively affect performance in lower limb exercises involving the stretch-shortening cycle in judo athletes during competition and training sessions. Photobiomodulation therapy has emerged as an effective non-invasive strategy to attenuate fatigue and muscle damage when applied before different types of exercises. Our objective was to investigate the effects of photobiomodulation therapy on fatigue and muscle damage in judo athletes. Sixteen judo athletes participated in the study (23.1 ± 3.8 years, 77.9 ± 14.9 kg, 173.1 ± 8.9 cm, 17.5 ± 7.3 body fat%, 12.9 ± 5.0 years of practice). Each participant received, in a randomized manner, photobiomodulation in one limb and placebo in the contralateral limb on the same day. Thereafter, subjects performed a stretch-shortening cycle protocol to induce muscle fatigue and damage. Countermovement jump (impulse, peak power, peak velocity, and peak force), echo intensity (*rectus femoris* and *vastus lateralis*), and muscle soreness were assessed at different time points before, during, immediately post, and 24 and 48 h after the protocol. Muscle fatigue was detected due to reductions in countermovement jump impulse (14.7 ± 9.8 and 15.9 ± 15.5%), peak power (12.9 ± 8.5 and 11.9 ± 6.9%), peak velocity (8.6 ± 8.1 and 6.5 ± 6.0%), and peak force (7.0 ± 5.3 and 8.0 ± 6.1%) after the protocol ($p < 0.001$), for placebo and photobiomodulation therapy, respectively. Muscle damage was detected due to reductions in countermovement jump impulse (−6.1 ± 19.2% and −4.5 ± 9.2%, $p < 0.05$), increases in echo intensity (*rectus femoris*, 21.0 ± 11.9 and 20.8 ± 9.0%; and *vastus lateralis*, 22.4 ± 23.2%; and 16.7 ± 23.8%; $p < 0.001$), and *quadriceps* muscle soreness (3.6 ± 1.6 and 3.5 ± 1.7 a.u; $p < 0.011$), 48 h after the protocol, for placebo and photobiomodulation therapy, respectively. No differences were observed between photobiomodulation therapy and placebo at any time points for any variables ($p > 0.05$), indicating no positive effect favoring photobiomodulation therapy. In conclusion, our findings suggest no effect of photobiomodulation therapy applied before exercise to reduce lower limb muscle fatigue and damage during and following a stretch-shortening cycle protocol in judo athletes.

Keywords: combat sports, recovery, performance, low-level laser therapy, stretch shortening cycle, photobiomodulation

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INTRODUCTION

In an official judo competition, athletes perform several matches with short recovery periods between them (approximately 10 to 15 min), which may induce muscle fatigue and damage, reducing athlete performance over the competition. A previous study found decrements of up to ∼8–12% in isometric handgrip strength after the second match during an official judo competition (Kons et al., 2018c). In simulated judo matches, a 3.6% decrease was observed in countermovement jump (CMJ) performance after the second match and increased values of serum creatine kinase (CK) and lactate dehydrogenase (LDH) after the third match (Detanico et al., 2015), supporting that the physical effort expended during judo matches induced fatigue and muscle damage. Fatigue and muscle damage can also be caused by traditional training sessions. It is known that a 2-h judo-specific training session provokes increases in biochemical markers of muscle damage (CK and LDH) and in acute immune response (Umeda et al., 2008). In addition, Detanico et al. (2017a) reported increases in CK, LDH, and muscle soreness, as well as a decrease in lower limb performance (i.e., CMJ) 48 h after a traditional judo training session. However, upper limb performance recovered faster, returning to baseline values after 24 h, which indicates that lower limbs are more affected during judo practice.

High-intensity exercises, especially those involving short recovery time and high eccentric-concentric contractions (e.g., stretch-shortening cycle – SSC) induce immediate and prolonged reductions in muscle function due to fatigue and/or muscle damage (Nicol et al., 1996; Horita et al., 1999). The optimization of SSC seems to be a specific characteristic of judo training, since a previous study found better utilization of SSC in advanced judo athletes and higher CMJ height compared to untrained males (Zaggelidis et al., 2012). Moreover, CMJ performance is positively related to the number of throws in a judo-specific test (Detanico et al., 2012) and technical-tactical parameters in official judo competitions (Kons et al., 2018b). Since the efforts involving SSC generate high mechanical load, producing great stress in muscle structures (Nicol et al., 1996; Horita et al., 1999), strategies that attenuate exercise-induced fatigue would therefore maintain physical performance in combat and training sessions, allowing execution of repeated movement with greater intensities for longer periods. Moreover, reducing fatigue and muscle damage would allow faster recovery intra or between training sessions and between the combat sequences, thus acutely maintaining or chronically increasing physical performance and diminishing the risks of injury (Howatson and Van Someren, 2008).

Evidence has shown that photobiomodulation therapy (PBMT) prior to exercise can be used as a tool to attenuate fatigue in humans following strength and aerobic exercises (Borsa et al., 2013; Leal-Junior et al., 2013; Ferraresi et al., 2016; Rossato et al., 2017; Dellagrana et al., 2018a,b; Lanferdini et al., 2018a,b; Vanin et al., 2018). Moreover, it has recently been reported that PBMT reduced fatigue and facilitated faster recovery in another combat sport (i.e., Brazilian jiu-jitsu) (Araújo et al., 2017; Follmer et al., 2018). In summary, PBMT-related muscle fatigue attenuation mechanisms involve factors such as absorption of photons by chromophores and subsequent transduction of light energy into chemical energy within the cytoplasmic organelles (Reddy, 2004). In addition, increases in permeability and consequent transport by the cytoplasmic membrane have been observed (Klebanov et al., 2001), improving activity of oxidative enzymes associated with the IV complex (Oxidase C Cytochrome) (Silveira et al., 2009; Huang et al., 2011), and increased mitochondrial size and number (Manteifel and Karu, 2005). Considering muscle damage symptoms, several human trials have reported the benefits of applying PBMT prior to exercise to attenuate damage markers, such as delayed onset muscle soreness, strength impairments, and/or echo intensity increases (Baroni et al., 2010a; Antonialli et al., 2014; dos Reis et al., 2014; Fritsch et al., 2016). PBMT acts by reducing fatigue, as abovementioned, leading to improved ATP production and delaying cellular acidosis and its negative effect on cell metabolism (Hayworth et al., 2010; Karu, 2010). A reduction in reactive oxygen species and oxidative stress have also been observed, resulting in an anti-inflammatory action, which is related to muscle damage (Liu et al., 2009).

Athletes require attenuation of fatigue to maintain physical performance for longer periods, supporting high loads during training, high-intensity actions during competition, and reducing the risks of injuries (Abd-Elfattah et al., 2015; Takito et al., 2019). Moreover, they require attenuated muscle damage following training sessions and matches to accelerate recovery, which may result in maintenance of physical performance for the following sessions/matches (Nicol et al., 1996; Horita et al., 1999; Howatson and Van Someren, 2008). Therefore, PBMT may be an effective tool to attenuate judo athletes' exercise-related fatigue and muscle damage in high-intensity efforts, as observed in judo matches and training sessions, maintaining performance and accelerating muscular recovery. Distinct sports can elicit different types of training-related physiological adaptations that are associated with their specificity, which could affect the athletes' responses to PBMT. Although PBMT has been demonstrated to be effective for several sports and exercise modalities (Borsa et al., 2013; Leal-Junior et al., 2013; Ferraresi et al., 2016; Rossato et al., 2017; Dellagrana et al., 2018a,b; Lanferdini et al., 2018a,b; Vanin et al., 2018), no studies have investigated their effects in judo athletes. Thus, the purpose of this study was to investigate the effects of pre-exercise PBMT on fatigue and muscle damage markers (i.e., CMJ performance, muscle echo intensity, and soreness) up to 48 h after exercise in judo athletes. The initial hypothesis was that PBMT would attenuate fatigue-related reductions of CMJ performance, and diminish the increases in echo intensity and muscle soreness following exercise.

MATERIALS AND METHODS

Participants

Athlete selection was performed in the east of Santa Catarina state, Brazil. The following inclusion criteria were adopted: (1) more than 6 years of judo practice; (2) graduation of purple, brown, or black belt athletes; (3) not be in a rapid weight loss or competition period; and (4) not presenting any musculoskeletal injury in the previous 2 years that could limit neuromuscular

testing. Athletes were excluded according to the following criteria: (1) alcohol or medication intake during the data collection period; (2) not properly executing the neuromuscular assessments; (3) not reporting to the laboratory at the correct time of day; and (4) feeling any exacerbation (i.e., injury-related discomfort during the neuromuscular assessment).

Twenty-four judo athletes volunteered, of which sixteen completed all the study procedures. These comprised four purple belts, five brown belts, and seven black belts with the following characteristics: 23.1 ± 3.8 years, 77.9 ± 14.9 kg, 173.1 ± 8.9 cm, 17.5 ± 7.3% of body fat, and time of practice of 12.9 ± 5.0 years. All athletes had already participated in several national and state tournaments and were engaged in regular training (technical–tactical, aerobic, and resistance training) 3–4 times a week. The selected athletes were in the preparatory phase and therefore the athletes were not in a period of rapid weight loss. In addition, the participants were instructed not to intake alcohol or medication (e.g., anti-inflammatory or pain relievers) for at least 48 h before and during the evaluations, and to maintain their normal diet. Eight athletes were excluded due to previous lower limb injury (n = 3), participation in official competitions (n = 3), or a weight loss period (n = 2) during the data collection. Before the assessments, all subjects were informed about the procedures and signed an informed consent form. Ethical approval was obtained from the local Human Research Ethics Committee (30495314000005347), in accordance with the Declaration of Helsinki.

Experimental Design

This study was a randomized, triple-blinded, and placebo-controlled trial. After recruitment, 2 weeks before the experimental protocol, participants were familiarized with the CMJ (3 times/week) during their judo training sessions. The familiarization consisted of five bilateral and five unilateral (each leg) CMJs per day. Thereafter, participants were requested to avoid any lower limb exercises for 48 h before and during the 3 days of the experimental protocol (**Figure 1**).

In the first laboratory visit, body mass, height, and anthropometric measurements were recorded. Baseline measurements began with ultrasonography assessments. Thereafter, participants responded to a visual analog scale of muscle soreness and performed the warm-up and unilateral CMJ records. After baseline assessments, simple randomization was used to determine which lower limb would be treated with PBMT or placebo, to reduce any possible influence of limb preference on muscle fatigue and damage results. Eight participants received PBMT in the preferred limb (self-reported by each athlete) and eight in the non-preferred limb, whereas, the contralateral limb received placebo. In sequence, the plyometric protocol was performed to induce fatigue and muscle damage. After the fifth and after the tenth sets, one unilateral CMJ was performed for each leg. After each set, the rate of perceived exertion (RPE) was recorded, and after the complete protocol, the visual analog scale for fatigue and muscle soreness was applied.

The second and third laboratory visits took place 24 h (±2) and 48 h (±2) after the first visit, respectively. The visual analog scale of muscle soreness, ultrasonography, and CMJ assessments were performed exactly as described on the first visit, in the baseline assessments, before the PBMT application.

Muscle Fatigue and Damage Protocol

To induce fatigue and muscle damage an SSC protocol was adopted, consisting of 10 sets of 10 repetitions of consecutive maximal bilateral CMJs (1-min rest between sets) on a piezoelectric force platform (model 9290AD; Kistler, Quattro Jump, Winterthur, Switzerland), which measures vertical ground reaction force at a sampling rate of 500 Hz. This protocol was recently adopted to investigate PBMT effects on muscle

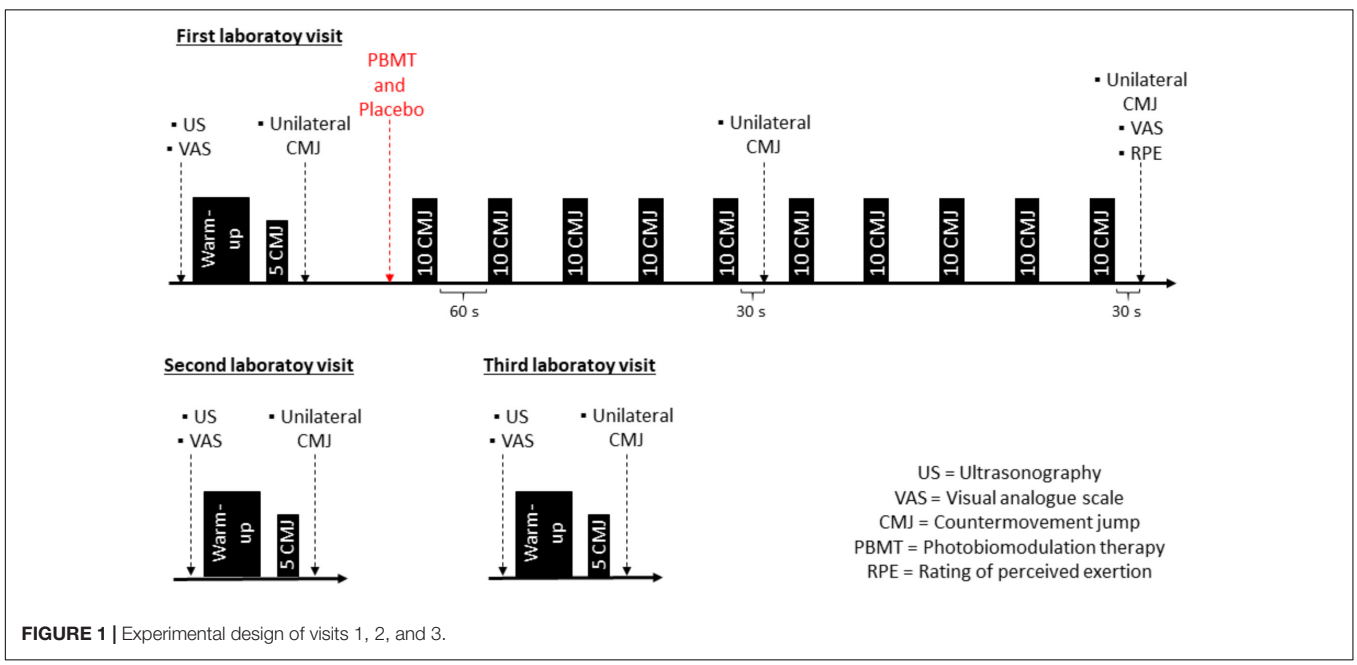

FIGURE 1 | Experimental design of visits 1, 2, and 3.

damage (Fritsch et al., 2016) and a similar protocol (30-s rest between sets) was used to induce fatigue. Participants received standardized instructions, verbal guidance, and encouragement during the protocol and the same researcher controlled all protocols. Participants were required to bend their knees and hips until approximately 90° of flexion in every CMJ. Fatigue was observed during the protocol due to a reduction of $-9.5 \pm 6.6\%$ in mean power output and a rate of perceived exertion (0–10) of 6.4 ± 2.2.

Countermovement Jump Assessment

Neuromuscular status, assessed through CMJ (usually performed bilaterally), has been used as a reliable non-invasive method to assess fatigue and muscle damage effects on performance (Wiewelhove et al., 2015; Claudino et al., 2017). In addition, the bilateral CMJ was correlated with technical-tactical variables (e.g., number of attacks, effectiveness, and effective combat time) obtained in official judo matches and judo-specific test performance (Kons et al., 2018b).

The CMJ was assessed using a strain gauge force platform (AMTI model OR6-6, Watertown, MA, United States), which measures vertical ground reaction force at a sampling rate of 1000 Hz. For the baseline, 24 h, and 48 h measurements participants performed a warm-up, consisting of 5 min on a cycle ergometer, 10 hops, five submaximal bilateral and three submaximal unilateral (each leg) CMJs (30-s rest between jumps). Two minutes later, five consecutive maximal CMJs were performed 30 s before the unilateral CMJs to ensure potentiated performance. To avoid exacerbated recovery, only one unilateral CMJ for each leg was recorded 30 s after the fifth and tenth sets of the plyometric protocol for fatigue monitoring. CMJs always began with the preferred limb, since PBMT was randomized. Half of the participants began with the PBMT treated limb and the other half started with the placebo treated limb.

For a proper technique, the participants started from a static standing on one foot position and were instructed to perform a countermovement (descent phase), followed by a rapid and vigorous extension of the lower limb joints (ascent phase) unilaterally. Their hands remained on their hips and they were instructed to jump as high as possible. Participants were allowed to land with both limbs to avoid exacerbated force on joints of a single limb landing. CMJ impulse (Kirby et al., 2011), peak power output, peak velocity, and peak force in the concentric phase of the jump were used for analysis (Cormie et al., 2008).

Echo-Intensity

Muscular echo intensity, assessed through ultrasonography images, has been used as a reliable non-invasive method to assess the muscle damage level in humans (Chapman et al., 2011; Bottaro et al., 2012). Echo intensity of *rectus femoris* and *vastus lateralis* muscles was assessed using ultrasonography equipment (Logiq S7, GE Healthcare, Milwaukee, WI, United States) along with a linear array probe (50 mm, 5–15 MHz, ML6-15) from the same manufacturer, coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface.

Prior to each assessment, participants remained in the supine position with knees and hips in neutral position and rested for 10 min in order to allow fluid shifts to occur (Lopez et al., 2019). Three transversal images from the *rectus femoris* and *vastus lateralis* were obtained at 50% of the distance between the lateral condyle of the femur and the great trochanter (Korhonen et al., 2009). To ensure that all measurements were performed at the same site, on the first day a waterproof pen was used to mark the exact site where the subsequent assessments should be carried out. All measurements were performed by the same trained researcher.

Posteriorly, all images were analyzed with ImageJ software (National Institutes of Health, United States). A square with an area of 1 cm^2 was positioned at the mid-point of the *rectus femoris* and *vastus lateralis* muscles for the echogenicity calculation (echo intensity). Echo intensity of each muscle was determined using the gray-scale analyses function and expressed in arbitrary units as a value between 0 (black) and 255 (white). The mean value between the three images from each day was used for analysis.

Rate of Perceived Exertion, Rate of Perceived Fatigue, and Muscle Soreness Assessments

A visual analog scale was used to identify the occurrence of muscle soreness related to delayed onset muscle soreness at baseline, immediately after, and 24 and 48 h after the end of the protocol. The scale ranged from 0 to 10, in which 0 was considered the absence of soreness and 10 the presence of unbearable soreness. Participants reported muscle soreness in specific muscle groups of the lower body (knee extensors, knee flexors, and plantar flexors), by indicating the area on an anatomical diagram (Detanico et al., 2017b). The delayed onset muscle soreness after a high intensity effort is considered a marker of muscle damage (Howatson and Van Someren, 2008).

Rate of perceived exertion was recorded on a scale ranging from 0–10 (0 = no exertion at all and 10 = maximal exertion) after every set of the plyometric protocol. In addition, after the complete protocol participants indicated their perception of fatigue during the exercise for each leg using visual analog scales (Kuys et al., 2011), which consisted of a single 100 mm horizontal line with a headline statement at the top. The extreme left of the line indicated no fatigue, and to the extreme right, the statement indicated very fatiguing. Participants were asked to indicate their perception of fatigue with a single vertical line for each leg.

Photobiomodulation/Placebo Treatment

Photobiomodulation therapy or placebo treatments were applied using a Chattanooga Intelect Mobile Laser 2779 system (Chattanooga Group, Guildford, Surrey, United Kingdom). PBMT or placebo were applied on 15 sites on each lower limb: eight sites on the quadriceps (three sites on *vastus lateralis*, three sites on *rectus femoris,* and two sites on *vastus medialis*), four sites on the hamstrings (two on *semitendinosus* and two on *semimembranosus*), two sites on the *gastrocnemius* (one on lateralis and one on mediallis areas), and one site on the *soleus*. Only the researcher responsible for the PBMT

application was aware of the treatment. The other researchers that performed the assessments and participants were blinded to the respective treatments.

The PBMT/placebo treatment lasted about 8 min. While one lower limb received the PBMT, the other received the placebo simultaneously with two probes held stationary with skin contact at a 90° angle with light skin pressure. The placebo probe remained turned off throughout the treatment time. The PBMT general characteristics comprise cluster size = 30.2 cm^2, number of sites = 15, treatment time per site = 32 s, dose per site = 30 J, and total dose = 450 J (Quadriceps = 240 J, Hamstrings = 120 J, and Gastrocnemius = 60 J). Recently a meta-analysis reported effective doses of 60–300 J for large and 20–60 J for small muscle groups (Vanin et al., 2018). Additional PBMT parameters are described in **Table 1**.

Statistical Analysis

All statistical analyses were performed by a blinded researcher, who did not know which treatment groups "1" and "2" received. After analyses, and table and figure production, the groups were revealed and the respective labeling was included. The sample size was calculated (G*POWER software, version 3.1.9.2., Universität Kiel, Germany) for ANOVA repeated measures, within-between interaction (effect size = 0.25, $\alpha = 0.05$, $\beta = 0.80$, number of groups = 2, number of measurements = 3, correlation among repeated measures = 0.5, non-sphericity correction = 1). A minimum of 14 participants per group was determined.

The intraclass correlation coefficient (ICC) and typical error as coefficient of variation (CV) were calculated for the three unilateral CMJ trials and for the echo intensity obtained from the three images for each muscle at baseline to verify their reliability (Hopkins, 2000). The normality of the distribution and homoscedasticity for outcome measures were tested using the Shapiro–Wilk, and Mauchly and Levene criteria, respectively. The Student *t*-test for independent samples was used to compare baseline of the preferred and non-preferred limbs for CMJ variables and echo intensity, as well as to compare the post-SSC protocol, VAS (fatigue), and the fatigue index between PBMT and placebo for CMJ variables. Two-way ANOVA repeated measures was used to analyze: (a) treatment (PBMT × placebo) × time (baseline, middle protocol, post-protocol, 24 h, and 48 h) interaction for unilateral CMJ variables; (b) treatment (PBMT × placebo) × time (baseline, post-protocol, 24 h, and 48 h) interaction for muscle soreness; and (c) treatment (PBMT × placebo) × time (baseline, 24 h, and 48 h) interaction for echo intensity. When Mauchly's test of sphericity was significant and the Greenhouse-Geisser level of violation was >0.75, degrees of freedom were corrected using the Huynh-Feldt adjustment, and when violation was <0.75, the Greenhouse-Geisser correction was used. When a significant *F*-value was achieved, Bonferroni's *post hoc* tests were used to determine the pair-wise differences between the different time points and between groups. An alpha level of 5% was used in all statistical analyses. Effect size was used to quantify the meaningfulness of any differences and was calculated using partial eta squared (η_p^2; trivial, <0.1; small, 0.1–0.29; moderate, 0.3–0.49; or large, ≥0.5) (Hopkins et al., 2009). ANOVA degrees of freedom are reported as df.

TABLE 1 | Photobiomodulation therapy parameters.

Parameters	LASERs (850 nm)	LEDs (670 nm)	LEDs (880 nm)	LEDs (950 nm)
Number of diodes	5	12	8	8
Power output (mW)	100	10	25	15
Spot size (cm^2)	0.06	1.92	1.28	1.28
Power density (mW/cm^2)	1666.6	5.2	1.93	11.71
Frequency	Continuous	Continuous	Continuous	Continuous
Dose (J)	3.2	0.3	0.8	0.5

RESULTS

Baseline Characteristics

Similar values were observed for preferred and non-preferred limb echo intensity (*rectus femoris* and *vastus lateralis*), and CMJ performance (peak force, peak velocity, peak power output, and impulse) (**Table 2**) at the baseline condition. In addition, the same was observed when comparing baseline values between PBMT or placebo treated limbs for all the abovementioned variables (**Figures 1**, **2**), showing homogeneity of data for both legs and both conditions at baseline.

Countermovement Jump

There were no treatment-time interactions for CMJ impulse (df = 2.905; $F = 0.690$; $p = 0.556$; $\eta_p^2 = 0.024$), peak power output (df = 2.206; $F = 0.279$; $p = 0.779$; $\eta_p^2 = 0.009$), peak velocity (df = 2.108; $F = 0.185$; $p = 0.842$; $\eta_p^2 = 0.006$), and peak force (df = 1.631; $F = 0.088$; $p = 0.880$; $\eta_p^2 = 0.003$). While a moderate time effect was observed for impulse (df = 2.905; $F = 15.009$; $p < 0.001$; $\eta_p^2 = 0.349$) and peak power output (df = 2.206; $F = 21.348$; $p < 0.001$; $\eta_p^2 = 0.416$), and small time effect for peak velocity (df = 2.108; $F = 8.456$; $p < 0.001$; $\eta_p^2 = 0.220$) and peak force (df = 2.631; $F = 11.497$; $p < 0.001$; $\eta_p^2 = 0.277$).

Countermovement jump impulse reduced after the fifth set ($p < 0.001$) and at the end of the SSC protocol (immediately post, $p < 0.001$). Thereafter, it increased after 24 and 48 h compared to post ($p < 0.014$), but remained lower than baseline ($p < 0.05$) (**Figure 2A**). CMJ peak power output, peak velocity, and peak force reduced after the fifth set ($p < 0.001$) and at the end of the protocol (immediately post, $p < 0.001$), however, increased after 24 and 48 h ($p < 0.006$), returning to baseline values ($p > 0.430$) (**Figures 2B–D**, respectively). In addition, the fatigue index was similar between PBMT and placebo for CMJ impulse ($p = 0.788$), peak power output ($p = 0.533$), peak velocity ($p = 0.318$), and peak force ($p = 0.560$).

Echo Intensity

There was no treatment-time interaction for echo intensity for either the *rectus femoris* or *vastus lateralis* (df = 2; $F = 1.368$; $p = 0.262$; $\eta_p^2 = 0.044$; and df = 1.947; $F = 0.877$; $p = 0.419$;

TABLE 2 | Mean and standard deviation values (95% confidence interval upper and lower limits) for echo intensity and countermovement jump characteristics at the baseline.

Variables	Preferred limb	Non-preferred limb	*p*-value
EI_{RF} (a.u.)	124.1 ± 14.1 (116.6 – 131.6)	125.9 ± 10.9 (120.1 – 131.7)	0.685
EI_{VL} (a.u.)	118.4 ± 12.6 (111.7 – 125.2)	109.2 ± 14.9 (101.3 – 117.2)	0.069
CMJ_{PF} (N)	1, 438 ± 239 (1, 305 – 1, 570)	1, 415 ± 222 (1, 291 – 1, 538)	0.793
CMJ_{PV} (m/s)	2.18 ± 0.25 (2.05 – 2.32)	2.09 ± 0.17 (2.00 – 2.19)	0.253
CMJ_{PPO} (W)	2, 547 ± 409 (2, 320 – 2, 773)	2, 426 ± 379 (2, 216 – 2, 636)	0.460
CMJ_{IMP} (N·s)	140 ± 24 (126 – 153)	136 ± 23 (123 – 149)	0.659

EI, echo intensity; RF, rectus femoris; VL, vastus lateralis; CMJ, countermovement jump; PPO, peak power output; MPO, mean power output; PF, peak force; PV, peak velocity; IMP, impulse.

η_p^2 = 0.028; respectively), while a large time effect was observed for both (df = 2; $F = 96.911$; $p < 0.001$; $\eta_p^2 = 0.764$; and df = 1.947; $F = 49.155$; $p < 0.001$; $\eta_p^2 = 0.621$; respectively). *Rectus femoris* and *vastus lateralis* echo intensity increased after 24 h ($p < 0.001$) and 48 h ($p < 0.001$), whereas 24 h was similar to 48 h ($p > 0.327$) (**Figures 3A,B**, respectively).

Perception of Fatigue and Muscle Soreness

There was no treatment-time interaction for quadriceps muscle soreness (df = 3; $F = 0.046$; $p = 0.987$; $\eta_p^2 = 0.002$) while a large time effect was detected (df = 3; $F = 44.515$; $p < 0.001$; $\eta_p^2 = 0.597$). Muscle soreness increased after the protocol (immediately post, $p < 0.001$) and increased again after 24 h ($p < 0.001$) and remained large after 48 h ($p < 0.011$) (**Figure 4**).

There was no difference ($p = 0.532$) in the visual analog scale for perception of fatigue between the placebo (62.0 ± 21.4, 95% CI: 50.7–73.4) and PBMT (57.4 ± 20.1, 95% CI: 46.7–68.1).

Data Reliability

Reliability ranged from extremely high (>0.99) to high (>0.75 and <0.90) for the repeated measures of echo intensity and CMJ variables at baseline (**Table 3**).

DISCUSSION

This was the first study to investigate the effects of PBMT on lower limb fatigue and muscle damage of judo athletes. Our initial hypotheses supposed that PBMT applied before exercise would be effective to attenuate fatigue-related reductions in CMJ performance, and thereafter reduce muscle damage symptoms after an SSC protocol. However, our main findings did not support any of the initial hypotheses, suggesting that PBMT was not effective to reduce fatigue and muscle damage in the lower limbs of judo athletes. These results counteract the findings observed in previous studies with non-athlete populations and different exercise modalities that will be described in the discussion.

The SSC protocol adopted in the present study successfully induced fatigue in lower limb muscles, as evidenced by decreased CMJ impulse, peak power, peak velocity, and peak force performance, and higher perception of fatigue. In summary, muscular fatigue can occur through ATP breakdown and accumulation of fatiguing substances (e.g., H^+, Na^{1+}, K^{1+}, Ca^{2+} ions) without enough recovery time for their resynthesizes and removal (respectively) between exercise bouts, reducing contraction performance. It was expected that PBMT would attenuate fatigue through absorption of photons by chromophores and subsequent transduction of light energy into chemical energy within the cytoplasmic organelles (Reddy, 2004); increasing permeability and consequent transport by the cytoplasmic membrane (Klebanov et al., 2001), activity of oxidative enzymes associated with the IV complex (Oxidase C Cytochrome) (Silveira et al., 2009; Huang et al., 2011), and mitochondrial size and number (Manteifel and Karu, 2005). Moreover, the PBMT can increase microcirculation in a nitric oxide synthase–dependent mechanism, increasing the ATP/adenosine diphosphate (ADP) ratio and local arterial blood flow (Larkin et al., 2012). However, both PBMT and placebo treated limbs presented a similar decay through the SSC protocol, which indicates that PBMT had no positive effect to attenuate fatigue.

Our results are in disagreement with previous studies in which PBMT reduced fatigue following high intensity neuromuscular exercise. We could hypothesize that the observed differences may be due to the investigated population since some studies adopted non-athlete participants, which would explain the opposed results (de Souza et al., 2016; Rossato et al., 2016, 2017). However, other studies observed positive effects of PBMT in competitive athletes (e.g., Brazilian Jiu-Jitsu, volleyball, soccer, and cycling) (Ferraresi et al., 2015; Maldonado et al., 2015; Araújo et al., 2017; Follmer et al., 2018; Lanferdini et al., 2018b). Two studies that investigated PBMT effects in Brazilian Jiu-Jitsu athletes reported reduced upper limb fatigue. These positive effects were observed during an isometric elbow flexion (Follmer et al., 2018) or hand-grip strength (Araújo et al., 2017), which indicates that contraction mode, or the assessed limbs may be differently affected by PBMT and could potentially explain the disagreement with our study. For example, some studies adopted isokinetic dynamic contractions for knee extensors/flexors (Baroni et al., 2010b; de Brito Vieira et al., 2014; Rossato et al., 2017) or plantar flexors (de Souza et al., 2016), while others investigated isometric contractions (Rossato et al., 2016; Follmer et al., 2018). Isometric or isokinetic tests allow control of the angular velocity (e.g., 0–180°/s) and involve a single joint, differently

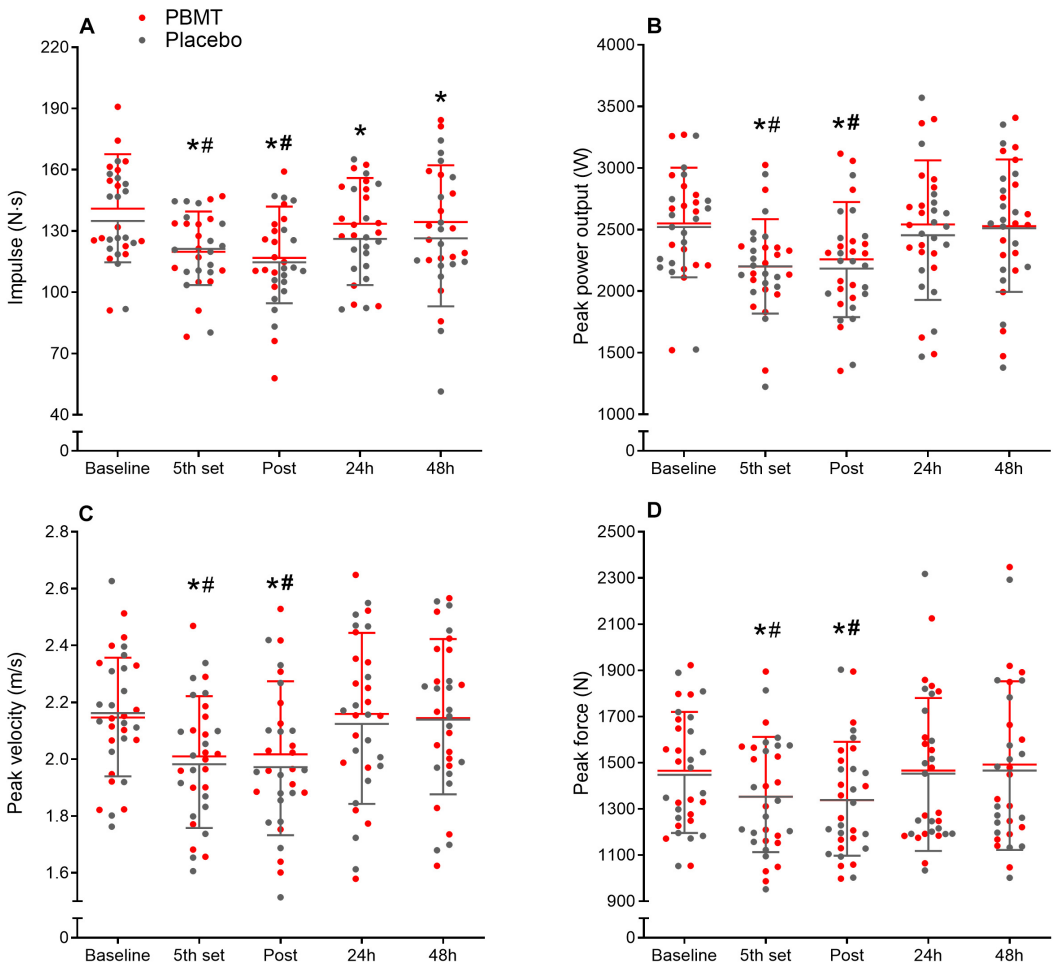

FIGURE 2 | Countermovement jump impulse **(A)**, peak power output **(B)**, peak velocity **(C)**, and peak force **(D)** means and standard deviation at baseline, fifth set, immediately post, and after 24 and 48 h for PBMT (red lines) and placebo (gray lines). *Different compared to baseline; #Different compared to 24 and 48 h.

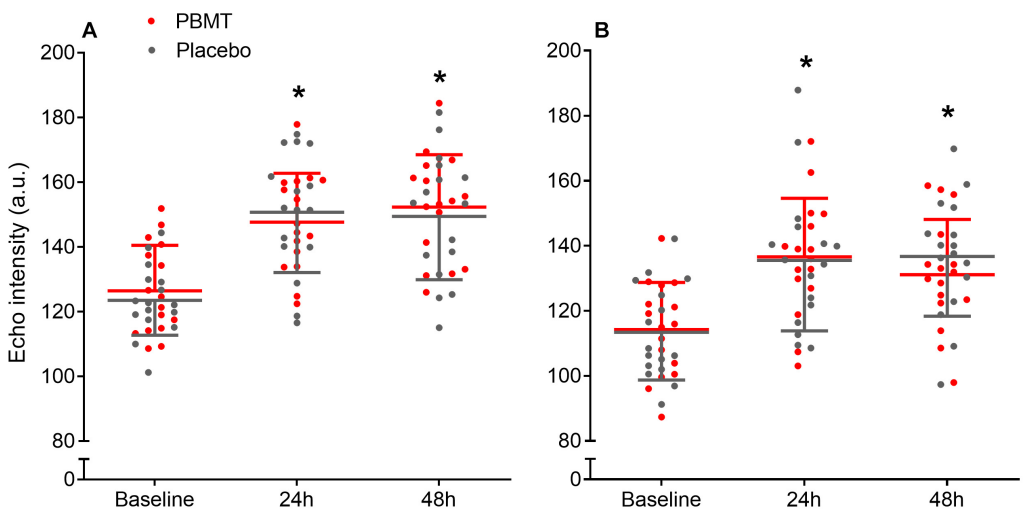

FIGURE 3 | Mean and standard deviation for *Rectus femoris* **(A)** and *Vastus lateralis* **(B)** echo intensity at baseline, and after 24 and 48 h, for PBMT (red lines) and placebo (gray lines). *Different compared to baseline.

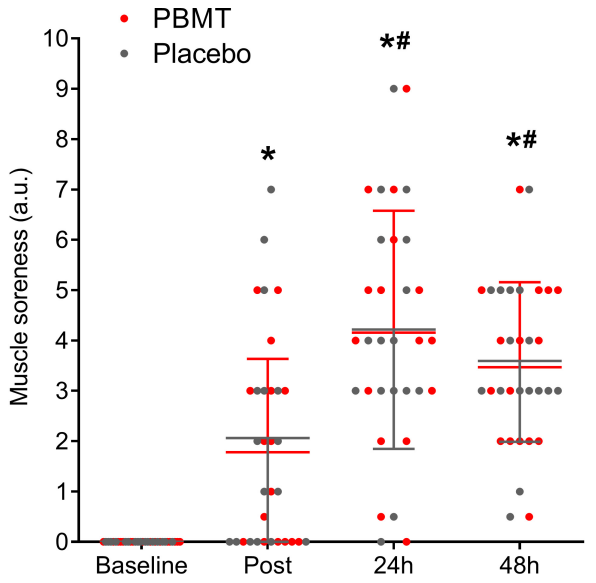

FIGURE 4 | Quadriceps muscle soreness means and standard deviation at baseline, immediately post, and after 24 and 48 h for PBMT (red lines) and placebo (gray lines). *Different compared to baseline; #Different compared to post.

from judo lower limb technique movement, which involves multi-joint explosive SSC contractions (Franchini, 2013). In addition, CMJ and maximum voluntary isometric contraction have demonstrated dissociated time courses of recovery and are possibly mediated by different mechanisms (Kennedy and Drake, 2018). The importance of SSC performance is evidenced by the relationship of CMJ performance with technical-tactical and specific test performance of judo athletes (Kons et al., 2018b). Thus, during competition combats or training sessions, muscle fatigue negatively affects judo athletic performance, and the adopted PBMT parameters seem to provide no benefit to attenuate these reductions.

Following a fatiguing high intensity exercise involving SSC, the presence of a great magnitude of muscle damage is expected, mainly due to the presence of eccentric actions (Nicol et al., 1996; Horita et al., 1999). The SSC protocol was effective in inducing muscle damage, as evidenced by decreased CMJ impulse, as well as increased *rectus femoris* and *vastus lateralis* echo intensity, and muscle soreness after 24 and 48 h. During exercises involving SSC, mechanical damage to muscular structures leads to disorganization of the Z-line, T-tubules, sarcoplasmic membrane and reticulum, myofibrils, and cytoskeletal. In the subsequent hours, alteration in Ca^{2+} homeostasis is observed, as well as induced inhibition of mitochondrial function, adenosine triphosphate depletion, inflammatory response, and proteolytic enzyme activation, increasing damage to muscle tissue (Cheung et al., 2003; Fredsted et al., 2007; Howatson and Van Someren, 2008). Attenuation in muscle damage was expected following the SSC protocol due to lower fatigue (Hayworth et al., 2010; Karu, 2010), which was not observed (as abovementioned). In addition, lower muscle damage was also expected due to a possible reduction in reactive oxygen species and oxidative stress, which result in an anti-inflammatory action, related to muscle damage (Liu et al., 2009).

The presence of muscle damage can decrease contraction performance and consequently reduce force and velocity of contraction (Howatson and Van Someren, 2008), as observed for CMJ impulse. This variable has a large correlation (r^2 = 0.92) with jump height compared to peak power output (r^2 = 0.45) and may better represent athletic performance (Kirby et al., 2011; Kons et al., 2018a). The similar decay in CMJ impulse, without differences between treatments 24 and 48 h after the SSC protocol, is a first indicative of the absence of a PBMT effect. Consequently, it is possible to suggest that PBMT cannot attenuate muscular function and, consequently, physical, technical, and tactical performance reductions following training sessions or combats in judo athletes. Our results are in agreement with a previous study that induced muscle damage with an equal SSC protocol, but assessed neuromuscular function with a knee extensor maximum voluntary isometric contraction in non-athletes (Fritsch et al., 2016). Conversely, other studies that induced muscle damage in knee extensors through isokinetic eccentric contraction and assessed muscular performance with maximum voluntary isometric contraction reported positive effects for muscle recovery when PBMT was applied (Baroni et al., 2010a; Antonialli et al., 2014). Our findings cannot provide information to state if the type of exercise used for inducing muscle damage (plyometric vs. isokinetic vs. constant load), the

TABLE 3 | Baseline data reliability and typical error (95% confidence interval upper and lower limits).

	Preferred limb		Non-preferred limb	
	ICC	CV %	ICC	CV %
EI_{RF}	0.993 (0.985–0.997)	1.04 (0.84–1.45)	0.986 (0.969–0.994)	1.10 (0.88–1.53)
EI_{VL}	0.996 (0.990–0.998)	0.80 (0.64–1.10)	0.998 (0.996–0.999)	0.67 (0.54–0.93)
CMJ_{PF}	0.949 (0.888–0.979)	4.83 (3.87–6.77)	0.978 (0.951–0.991)	2.91 (2.33–4.06)
CMJ_{PVEL}	0.786 (0.581–0.906)	5.69 (4.55–7.98)	0.916 (0.820–0.965)	2.77 (2.22–3.86)
CMJ_{PPO}	0.923 (0.834–0.968)	6.14 (4.90–8.61)	0.976 (0.947–0.990)	3.14 (2.51–4.38)
CMJ_{IMP}	0.905 (0.790–0.962)	6.52 (5.22–9.51)	0.957 (0.900–0.983)	4.24 (3.40–6.15)

EI, echo intensity; RF, rectus femoris; VL, vastus lateralis; CMJ, countermovement jump; PPO, peak power output; MPO, mean power output; PF, peak force; PV, peak velocity; IMP, impulse; ICC, intra-class correlation coefficient; CV, typical error as % of coefficient of variation.

investigated population (athletes and non-athletes), the study design (crossover vs. paired groups), or the different PBMT parameters are responsible for these controversial results.

Echo intensity accessed by ultrasonography presented similar behavior regarding muscle damage. It is suggested that increases in ultrasound echogenicity (i.e., echo intensity) is due to increments in the interstitial space between fibers as a result of connective tissue damage and inflammation, as well as muscle swelling or increase in plasma enzyme levels (Chen et al., 2011, 2013). In our study, both the PBMT and placebo limbs presented similarly increased echo intensity for *rectus femoris* and *vastus lateralis* muscles at 24 and 48 h after the SSC protocol. This is an additional indicative of similar muscle damage between both limbs, showing no positive effect of PBMT. Our initial hypothesis was supported by a previous study (Fritsch et al., 2016) that reported maintenance of echo intensity for the PBMT treated limb while increases were observed only for the placebo limb after an identical study design and SSC protocol. However, our results are contrary to theirs, and although the mechanisms underlying this difference cannot be explained with our methods, this is an indicative that judo athletes and non-athlete physically active people differently respond to PBMT with the intention of muscle damage protection following an SSC protocol. The effectiveness of PBMT to prevent an inflammatory response in judo athletes seems to have no positive response or cannot be detected through echo intensity levels in ultrasonography images.

The muscle damage observed by lower CMJ impulse and higher echo intensity levels is accompanied by a similar increase in muscle soreness 24 and 48 h after the SSC protocol for both PBMT and placebo limbs. Thus, if the muscle damage was attenuated, it was expected that muscle soreness would also reduce. Despite the absence of damage reduction, PBMT can modulate pain through its direct effect on peripheral nerves (Douris et al., 2006), which was not observed in our study. Contrary to our findings, a previous study reported positive results for muscle soreness when PBMT was applied before a knee extensor eccentric protocol performed in isokinetic dynamometry (Fritsch et al., 2016). Fritsch et al. (2016) observed non-statistical differences between PBMT and placebo limbs after a plyometric protocol equal to ours. However, the authors reported that the PBMT limbs presented up to 30% less muscle soreness than placebo limbs, suggesting clinical relevance despite the absence of statistical significance. Conversely, our findings showed trivial percentage differences between PBMT and placebo treatments at 24 and 48 h after the SSC protocol (0 and 2.8%, respectively). For athletes, a minimal analgesic effect would positively affect performance, however, no effect was observed regarding muscle soreness.

Our findings are contradictory to the recent literature regarding PBMT effects on muscle fatigue (Vanin et al., 2018) and damage (Fritsch et al., 2016). The different markers of muscle fatigue (CMJ variables and visual analog scale for fatigue) and muscle damage (CMJ impulse, echo intensity, and muscle soreness) showed similar behavior following the SSC protocol, which differed from Fritsch et al. (2016) who showed dissociated results for echo intensity and strength production. The mechanisms underlying these differences cannot be explained by our methods, thus further studies should focus on understanding how and why different populations (e.g., according to training level, age, and illness), different muscle groups (e.g., lower or upper limbs), methods of fatigue and damage induction (e.g., jumping, running, and eccentric based protocols), assessment of fatigue and damage markers (e.g., CMJ, and dynamic or isometric peak torque or rate of force development), and different PBMT parameters are differently affected by PBMT.

Our study has both strengths and limitations. The use of a randomized, triple-blind, placebo-controlled trial design, where each evaluator performed the same tests throughout data collection, gave our study internal validity and reduced evaluator bias. The sample size allowed enough power ($\beta > 0.8$) to avoid any type II statistical error. In addition, the use of PBMT and placebo applied in the contralateral limb evaluated on the same day avoided within-subject between-day performance variability and a repeated bout effect that would hide possible positive effects. However, our design did not include biochemical muscle damage blood marker monitoring (e.g., CK). In addition, a wide variety of PBMT parameters have been used to reduce muscle fatigue and damage; therefore, it is unclear if this result would be the same using different parameters to those adopted in our study. Thus, future studies should focus on understanding the ideal PBMT parameters for athletes in order to attenuate fatigue and improve recovery, avoiding sports-related disorders (e.g., overtraining and muscle injuries).

CONCLUSION

Our findings suggest no effects of PBMT applied before exercise to reduce lower limb muscle fatigue assessed by CMJ impulse, peak power output, peak velocity, peak force, and perception of fatigue during an SSC protocol (10 sets of 10 CMJ) for judo athletes. Additionally, no effects of PBMT to attenuate muscle damage markers (i.e., CMJ impulse, echo intensity, and muscle soreness) were observed following the damaging protocol adopted. Thus, judo athletes did not benefit from PBMT (with the respective parameters) applied before exercise to attenuate fatigue and muscle damage symptoms during and following our SSC protocol.

ETHICS STATEMENT

Ethical approval was obtained from the local Human Research Ethics Committee of the Federal University of Santa Catarina.

AUTHOR CONTRIBUTIONS

All authors conceived the study design, participated in the interpretation of data, drafted the manuscript, and read and approved the final version of the manuscript. LO, RK, RS, and JS carried out the data collection. LO carried out all the statistical analyses.

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frontiers in Physiology

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Foam Rolling as a Recovery Tool Following Eccentric Exercise: Potential Mechanisms Underpinning Changes in Jump Performance

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Purpose: Recovery from exercise-induced muscle damage (EIMD) is paramount in sports performance. Foam rolling (FR) has been suggested to improve acute performance; however, the ability to facilitate recovery from eccentric (ECC) exercise remains unclear.

Methods: Eleven males undertook 6 × 25 ECC knee extensions to induce muscular damage. Immediately, 24, 48, and 72 h post-training countermovement jump (CMJ), maximal voluntary isometric contraction (MVIC), pressure-pain threshold (PPT), knee flexion range of motion (ROM), and mid-thigh circumference (MTC) were assessed. Neurophysiological measures included voluntary activation (VA), peak twitch torque (PTT), time to peak twitch (PTT_{time}), and rate of twitch torque development (RTD). Participants then spent 15 min FR prior to each time point or control (CON). Repeated measures analysis of variance (ANOVA) and standardized effect sizes (Hedges' *g*) ± 95% confidence intervals (95% CI) were used to compare FR and CON.

Results: CMJ was greater for FR compared to CON (P = 0.030) at 72 h (8.6%, P = 0.004) with moderate effects observed at 48 and 72 h (g = 0.54–0.66). PPT was greater with FR (P = 0.018) at 48 h only (23.7%, P = 0.013), with moderate to large effects noted at all-time points (g = 0.55–0.98). No significant differences were reported for MVIC (P = 0.777, −5.1 to 4.2%), ROM (P = 0.432, 1.6–3.5%), VA (P = 0.050, 3.6–26.2%), PTT (P = 0.302, −3.9 to 9.9%), PTT_{time} (P = 0.702, −24.4 to 23.5%), RTD (P = 0.864, −16.0 to −1.0%), or MTC (P = 0.409, −0.5 to −0.1%) between conditions.

Conclusion: FR appears to improve jump performance in the later stages of recovery following ECC exercise. This may be in part due to improved pain tolerance; however, mechanical and neurophysiological are not modulated with FR.

Keywords: self-massage, myofascial release, resistance training, power, delayed onset muscle soreness, muscle damage

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Abbreviations: CMJ, countermovement jump; CON, control; DOMS, delayed onset muscle soreness; ECC, eccentric; EIMD, exercise-induced muscle damage; FR, foam rolling; MTC, mid-thigh circumference; MVIC, maximal voluntary isometric contraction; PPT, pressure-pain threshold; PTT, peak twitch torque; PTT_{time}, time-to-peak twitch torque; ROM, range of motion; RTD, rate of twitch torque development; VA, voluntary activation.

INTRODUCTION

Following training and competition, reductions in neuromuscular performance occur at least in part, due to acute fatigue and longer-lasting EIMD. EIMD is common following intense exercise, especially when repeated ECC contractions are performed. Consequently, ECC exercise can impair neuromuscular function for prolonged periods of time due to muscle soreness and pain, structural perturbations, and inflammation (Kouzaki et al., 2016). In sport, the spatiality of training sessions and/or competition are often more frequent than the ideal recovery period, leading to sub-optimal performance, burnout, and injury (Lehmann et al., 1999; Kellman, 2010). Therefore, several, often concurrent techniques are employed in an attempt to facilitate recovery. For example, contrast- or cryo-therapy, stretching, massage, light exercise, and FR are commonly used, however, the supporting evidence and the understanding of potential underlying mechanisms are largely inconclusive (Luttrell and Halliwill, 2015).

It is well established that fatigue occurs via a combination of central (neural) and peripheral (muscular) mechanisms (Enoka and Duchateu, 2016). For instance, a reduction in central drive to the muscle (i.e., VA) has been shown following sustained isometric tasks (Gandevia et al., 1996; Taylor and Gandevia, 2008) and in particular, the days following ECC exercise (Behm et al., 2001; Prasartwuth et al., 2005). Other evoked contractile properties (e.g., PTT and RTD) may also be compromised under fatigue. Furthermore, neuromuscular performance can also be impaired by various physiological processes at the muscular level including metabolic perturbations and mechanical stress (Allen et al., 2008; McKenna et al., 2008). In the days following repeated ECC contractions, pro-inflammatory responses induce swelling and increase pain sensitivity, likely due to structural damage of the myofibrils, cellular matrix, and connective tissue (Proske and Allen, 2005; Kanda et al., 2013). Thus, a reduction in neuromuscular function may severely impact performance and increase the likelihood of injury, especially following muscle damaging ECC exercise.

In recent years FR, a form of self-massage, has gained popularity in sports science settings. However, despite continued scientific enquiry the effectiveness of FR to improve functional performance and recovery, and the underlying mechanisms that may be responsible remain somewhat unclear. Previous literature has sought to investigate the various mechanical, tissue, perceptual, and functional responses when FR is employed (Cheatham et al., 2015; Schroeder and Best, 2015; Behara and Jacobson, 2017; Schroeder et al., 2018). Specifically, an acute decrease in tissue stiffness (Krause et al., 2017) improved joint ROM (MacDonald et al., 2013, 2014; Cheatham et al., 2015; Schroeder and Best, 2015; Kalichman and David, 2017; Su et al., 2017; Smith et al., 2018), reduced soreness (MacDonald et al., 2013; Beardsley and Skarabot, 2015; Paz et al., 2017), and reduced perceptions of pain and fatigue (Rey et al., 2017; Richman et al., 2018) have been reported. However, the effects of FR on maximal strength and power expression are mixed (MacDonald et al., 2013; Halperin et al., 2014; Jones et al., 2015; Richman et al., 2018; Smith et al., 2018). In particular, several studies have investigated the effects of FR following exercise in the lower limbs (MacDonald et al., 2013; Behara and Jacobson, 2017; Cavanaugh et al., 2017a) with authors reporting improvements in either ROM, jump height, power, sprint performance, or change of direction (MacDonald et al., 2014; Cheatham et al., 2015; Pearcey et al., 2015; Schroeder and Best, 2015; Freiwald et al., 2016; D'Amico and Gillis, 2017; Rey et al., 2017; Richman et al., 2018). Recent evidence has also suggested that FR may benefit functional outcomes during the recovery period (Fleckenstein et al., 2017) despite no changes in tissue properties (Schroeder et al., 2018); however, specific ECC exercise studies are limited (Pearcey et al., 2015; Romero-Moraleda et al., 2017). Additionally, it is unclear if neurophysiological mechanisms (i.e., VA or PTT) contribute to the performance improvements (i.e., jump performance) often observed following FR.

The aim of this study was to investigate the effects of acute FR on the functional, mechanical, and subjective outcomes, and neurophysiological mechanisms following a single bout of ECC exercise. Specifically, we aimed to quantify these responses during the fatigue and recovery period, up to 72 h post-exercise. Based on the previous evidence, we hypothesized that: (1) the recovery of performance variables (MVIC and/or CMJ) will be facilitated with a FR intervention and (2) improved neural, mechanical, and subjective outcomes will accompany an improvement in performance. The results are expected to provide evidence regarding the efficacy of FR as a tool to improve functional recovery and elucidate the potential underpinning neurophysiological mechanisms responsible. These findings will be particularly important for athletes who have consecutive bouts of training and competition resulting in muscle damage with minimal inter-session recovery periods.

MATERIALS AND METHODS

Experimental Approach to the Problem

Following an initial familiarization session, each participant was involved in two identical ECC protocols with 3 weeks between sessions in a randomized, counter-balanced cross-over design. The two experimental conditions consisted of the ECC exercise followed by either: (1) quiet sitting for 15 min rest following exercise and before each testing point; CON or (2) completed 15 min FR immediately post-training and before each testing point and at 24, 48, and 72 h at the same time of day for each participant across both conditions. The order of testing was as follows: MTC, ROM, CMJ, and then MVIC followed by electrical stimulation.

Participants

Eleven healthy young males (age: 24.0 $\pm$ 0.7 years, height: 180.0 $\pm$ 7.0 cm, body mass: 82.0 $\pm$ 7.0 kg) with at least 2 years of regular ($\geq$2 days per week) general resistance training experience and no report of lower extremity injuries within the last 6 months volunteered for this study. Participants were asked to abstain from food and caffeine 3 h prior to testing, and physical activity and alcohol 24 h prior to testing and during recovery. Participants were informed of the study requirements and written consent was

obtained prior to testing. This study was approved by the Charles Sturt University Human Research Ethics Committee.

Eccentric Exercise Protocol

The protocol designed to elicit muscle damage involved the participant seated upright on an isokinetic dynamometer (HUMAC NORM, CSMi Medical Solutions, MA, United States) with the knee and hip positioned at 90° of flexion. The participant was secured with a harness and the leg secured to the lever arm with a strap placed at the ankle 1 cm above the lateral malleolus. The axis of rotation of the dynamometer was aligned with the lateral epicondyle of the right femur. During all contractions the participant placed the arms across the chest. The protocol involved 150 ECC contractions segmented into 6 sets of 25 minimally resisted knee extensions and maximally resisted ECC flexion of the right knee (30° s^{-1} extension and 120° s^{-1} flexion). Each set was separated by 60 s of passive recovery. Strong verbal encouragement was provided to the participant throughout each set to ensure maximal effort. The ECC protocol was centered on eliciting DOMS in the quadriceps (agonist), however, due to the biomechanical movement employed, resultant effects on the antagonist and synergist muscles were also likely.

Foam Rolling

The FR intervention specifically targeted five lower extremity areas (3 min per area) of the right leg as previously described by Pearcey et al. (2015). The participant consistently placed as much body mass as bearable onto the foam roller (HART Sport Foam Roller, 30 cm × 15 cm, Virginia, QLD, Australia) and was instructed to roll their body weight along the roller as evenly as possible at a rate of one rolling motion per second. The description and order of the areas targeted include: (i) quadriceps: the participant commenced in a prone position with one leg over the other. The roller moved from the anterior superior iliac spine to the patellar tendon with the participant using elbows to guide the movement, (ii) adductors: the participant commenced in a prone position with the hip positioned at 90° and externally rotated. The roller moved from the proximal portion of the adductor group (inferior to the inguinal area) to the medial condyle with a consistent shifting of body weight, (iii) iliotibial band: the participant commenced in a side lying position with the placement of the free leg anterior to the supported leg and rolled back and forth from the greater trochanter to the lateral condyle with the free foot controlling movement, (iv) gluteals: the participant commenced with one foot crossed over the opposite knee in a figure-four configuration while supporting body weight on the one hand. Utilizing the support hand, the participant rolled from the posterior portion of the iliac crest to the gluteal fold, and (v) hamstrings: the participant commenced with one foot crossed over the other and body weight supported by the hands, posterior to the body and the participant rolled from the gluteal fold to the popliteal fossa. Standardization of the positioning for each participant was provided during the familiarization and monitored throughout the intervention by the research team.

Mid-Thigh Circumference

Mid-thigh circumference was assessed with a steel tape measure (MURATEC-KDS, F10-02, Kyoto, Japan) with the participant in the anatomical position. Girth measurements were recorded from the right thigh perpendicular to the long axis of the thigh, midway between the trochanterion and tibiale laterale. Results were recorded to the nearest millimeter and the mean was recorded from three consecutive measurements.

Pressure-Pain Threshold

Pressure-pain threshold was assessed over the right rectus femoris. The participant was seated upright on a physiotherapy table with the hip and knee at 90° of flexion and popliteal fossa flush with the edge of the padded table. Following identification of the muscle belly of the rectus femoris, an algometer (Wagner Instruments, FDIX-RS232 Force One, Greenwich, CT, United States) was placed over the belly of the muscle with a downward pressure gradation of 1 kg cm^2 s^{-1} until the participant acknowledged the initial point of shift in sensation from "pressure" to "pain."

Range of Motion

Knee flexion ROM of the right leg was assessed utilizing a modified Ely's test and mechanical goniometer (JAMAR, Jackson, MI, United States) (Peeler and Anderson, 2008). Previous research suggests that Ely's test demonstrates moderate reliability (Peeler and Anderson, 2008). The participant was placed in a prone position on the physiotherapy table and the axis of rotation of the goniometer was fixed to the tibiale laterale. The stationary arm was fixed to the trochanterion and the movement arm was rotated against the lateral malleolus. The movement of the limb through its ROM was controlled by the investigator's even pressure placed against the participant's ankle at a rate of approximately 5° s^{-1} and measurement was taken when the participant acknowledged the elicitation of pain (Pyne et al., 2012). The procedure was completed three times with the greatest ROM recorded.

Countermovement Jump

The participant then completed a 5 min cycling warm-up at 60 rpm with a 2-kp resistance (Monark 828E, Monark Exercise AB, Varberg, Sweden). Following the warm-up, CMJ height was assessed. The participant completed five CMJs on a 100 × 80 cm contact mat (AXON Jump T, Kinematics Sports Test System, Version 2.01, Buenos Aires, Argentina) separated by 60 s recovery. Each jump consisted of the participant standing straight, feet shoulder-width apart with hands fixed on hips; the body then dropped to a self-selected depth and immediately followed by the highest jump possible. CMJ height was calculated using the flight time. The mean jump height was calculated from the five CMJs recorded.

Isometric Voluntary Torque

Maximal voluntary isometric contraction was assessed using the right knee extensors conducted with the same participant set up as the ECC exercise protocol. The participant

completed three MVICs of the right knee extensors (90° of flexion) for 5 s duration (2 s ramp up, 3 s maximal effort), with 60 s recovery between each contraction to avoid the effects of fatigue. The best of the three trials was recorded as the MVIC.

Evoked Responses

An additional three MVICs were superimposed with a constant current electrical stimulus when a steady plateau in peak torque was achieved. A potentiated twitch was also evoked 3–5 s after the contraction when the muscle was at rest. Electrical stimuli to knee extensors were delivered using 1.5 cm lead electrodes (Nicolet, Cardinal Health, Madison, WI, United States) placed over the femoral nerve on the thigh 1.5 cm inferior to the inguinal fold. The current was delivered via a stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, United Kingdom) using single square-wave pulse with a width of 200 μs, linked to a terminal block and a signal acquisition system (PXI1024; National Instruments, Austin, TX, United States). The electrical current was increased incrementally until a plateau in the PTT was achieved, and then increased by a further 10% to ensure supra-maximal stimulation. VA levels were calculated using the twitch interpolation technique and the formula $1 - \left(\frac{\text{superimposed\_twitch}}{\text{potentiated\_twitch}}\right) \times 100$ (Todd et al., 2003). The maximal twitch was determined as the difference in peak voluntary torque in the 50 ms prior to the delivery of the stimulus and the peak evoked torque value from stimulation. The RTD was calculated as the time elapsed to reach PTT.

Mean torque–time curves from the potentiated evoked resting twitch determined: (1) peak potentiated twitch torque (PTT; highest evoked torque obtained); (2) time to peak potentiated twitch torque (PTT_{time}; time between the onset of the potentiated twitch and the PTT); and (3) RTD. These procedures were performed using MatLabTM Software (R2009b 7.9.0.529, The Mathworks Inc., Natick, MA, United States).

Statistical Analysis

Differences in the mean changes between the interventions (FR and CON) were determined for each outcome variable using a two-way repeated measures of analysis of variance (ANOVA). Where significance was detected a *post hoc* paired samples *t*-test was conducted to examine differences between conditions at each individual time point. Significance was set at $P < 0.05$. Additionally, effect sizes were calculated using Hedge's *g* and expressed using the following criteria: trivial <0.2, small 0.2–0.49, moderate 0.5–0.79, and large >0.8. Only results with a moderate or large effect were reported. Precision of mean differences was expressed with the 95% confidence interval (95% CI), which defines the range representing the uncertainty in the true value of the (unknown) population mean. All effect size calculations were performed in Excel (version 2013; Microsoft Corporation, Redmond, WA, United States) and ANOVAs were performed using SPSS (version 25; IBM Statistics). To display the 95% confidence interval of the effect sizes, results are displayed graphically as the mean, upper and lower 95% confidence limits.

RESULTS

The values for each outcome measure are displayed in **Table 1** and the effect sizes in **Figure 1**.

Neuromuscular Variables

A repeated measures ANOVA demonstrated a significant interaction ($P = 0.030$) and main effect of time ($P = 0.034$) for CMJ height between FR and CON. *Post hoc* analyses revealed that the recovery of CMJ height was greater for FR at 72 h ($P = 0.004$), compared to CON (**Figure 2A** and **Table 1**). Effect size analysis suggests a moderate effect for CMJ with FR 48 ($g = 0.66$) and 72 h ($g = 0.54$) compared to CON, respectively (**Figure 1**). No significant interaction was observed for MVIC ($P = 0.777$) between FR and CON (**Table 1**). Additionally, effect sizes were mostly trivial to small for FR on MVIC in comparison to CON across all time points ($g = -0.13$ to 0.28) (**Figures 1**, **2B**).

Mechanical Variables

A repeated measures ANOVA demonstrated a significant interaction ($P = 0.018$) and main effect of time ($P = 0.002$) for PPT between FR and CON. *Post hoc* analyses revealed that PPT was greater for FR at 48 h ($P = 0.013$) compared to CON (**Table 1**). Effect size analysis demonstrated a moderate effect for PPT with FR immediately post-training ($g = 0.58$) at 24 ($g = 0.55$), 48 ($g = 0.98$), and 72 h ($g = 0.60$) when compared to CON; however, these results did not reach statistical significance (**Figures 1**, **3A** and **Table 1**).

No significant interaction was observed for ROM ($P = 0.881$) between FR and CON (**Figures 1**, **3B**). No interaction was observed for MTC ($P = 0.940$) between FR and CON (**Figures 1**, **3C**).

Neural Variables

No significant interactions were observed for VA, PTT, PTT_{time} or RTD (**Table 1**). Additionally, there were no substantial effects of FR for VA (**Figure 4A**), PTT_{time} (**Figure 4B**) and PTT (**Figure 4C**) and RTD (**Figure 4D**) at most if not all time points, however a large effect size was observed for VA ($g = 0.97$) at 72 h.

DISCUSSION

Muscle damaging ECC exercise can impair performance for several days or longer. Due to the known debilitating effects on performance the aim of this study was to investigate if FR can improve functional recovery and in addition identify the potential underlying mechanisms that may contribute to this response. Specifically, we investigated neuromuscular (MVIC and CMJ), neural (VA, RTD, PTT, PTT_{time}) and mechanical (ROM, MTC, PPT) outcomes in the lower limbs. The results showed significant improvements in CMJ at 72 h, with small to moderate effects observed at post-training and 48 h. Pain tolerance also increased at 48 h, with effects also observed at post-training, 24 and 72 h, respectively. No clear significant differences were observed during the recovery period for all other variables. Collectively, the results suggest

TABLE 1 | The effect of the exercise protocol on each outcome variable: CMJ, MVIC, VA, PTT, PTT_{time}, RTD, MTC, PPT, and Ely's test for ROM for each condition (FR or CON) across all time points.

Variable	Time	CON (mean ± SD)	FR (mean ± SD)	CON Δ	FR Δ	Effect size (*g*, 95% CI)	Interaction	Condition	Time
CMJ	Pre	29.5 ± 4.6	29.4 ± 5.0						
(cm)	Post	26.2 ± 4.1	27.8 ± 4.1	−3.4	−1.6	*0.39* (−0.12, 0.91)	$F_{4,40}$ = 2.994, P = 0.030*	$F_{1,10}$ = 4.640, P = 0.057	$F_{4,40}$ = 4.618, P = 0.034*
	24	27.2 ± 4.6	28.2 ± 4.8	−2.3	−1.1	0.26 (−0.22, 0.73)			
	48	27.7 ± 4.8	30.8 ± 5.3	−1.8	1.5	0.66 (0.07, 1.25)#			
	72	28.4 ± 4.4	30.7 ± 5.0	−1.1	1.4	0.54 (0.12, 0.96)#			
MVIC	Pre	139 ± 31	135 ± 33						
(Nm)	Post	115 ± 34	122 ± 38	−23.8	−14.2	0.28 (−0.44, 0.99)	$F_{4,40}$ = 0.443, P = 0.777	$F_{1,10}$ = 0.029, P = 0.869	$F_{4,40}$ = 3.872, P = 0.009*
	24	117 ± 42	113 ± 40	−22.0	−22.8	−0.02 (−0.77, 0.73)			
	48	129 ± 35	121 ± 42	−9.8	−14.4	−0.13 (−0,78, 0.51)			
	72	130 ± 36	130 ± 41	−8.7	−5.50	0.09 (−0.62, 0.80)			
VA	Pre	82.8 ± 17	74.3 ± 19						
(%)	Post	83.3 ± 14	72.7 ± 25	0.5	−1.6	−0.11 (−0.94, 0.72)	$F_{4,36}$ = 2.627, P = 0.050	$F_{1,9}$ = 0.730, P = 0.415	$F_{4,36}$ = 1.572, P = 0.203
	24	71.9 ± 21	54.0 ± 46	−10.9	−20.3	−0.32 (−1.57, 0.92)			
	48	77.2 ± 19	71.2 ± 17	−5.6	−3.1	0.14 (−0.92, 1.20)			
	72	65.7 ± 27	77.0 ± 15	−17.1	2.7	0.97 (−0.12, 2.06)#			
PTT	Pre	64.2 ± 21	63.8 ± 17						
(Nm)	Post	51.6 ± 21	54.1 ± 15	−12.6	−9.6	0.16 (−0.34, 0.66)	$F_{4,40}$ = 1.259, P = 0.302	$F_{1,10}$ = 0.087, P = 0.773	$F_{4,40}$ = 4.292, P = 0.031*
	24	56.8 ± 15	55.6 ± 15	−7.4	−8.2	−0.05 (−0.62, 0.53)			
	48	65.1 ± 11	62.0 ± 13	0.9	−1.8	−0.17 (−0.84, 0.50)			
	72	55.1 ± 23	62.9 ± 11	−9.1	−0.8	0.45 (−0.26, 1.16)			
PTT_{time}	Pre	174 ± 76	147.4 ± 43						
(ms)	Post	160 ± 51	137 ± 72	−14.7	−10.6	0.07 (−1.01, 1.14)	$F_{4,40}$ = 0.547, P = 0.702	$F_{1,10}$ = 1.788, P = 0.211	$F_{4,40}$ = 0.884, P = 0.482
	24	179 ± 76	163 ± 74	4.3	16.1	0.17 (−0.76, 1.11)			
	48	186 ± 73	151 ± 58	12.0	4.1	−0.12 (−1.36, 1.11)			
	72	137 ± 62	149 ± 54	−37.4	1.4	0.64 (−0.29, 1.59)#			
RTD	Pre	460 ± 277	494 ± 241						
($Nm.s^{-1}$)	Post	381 ± 201	484 ± 235	−79.0	−9.6	0.29 (−0.55, 1.14)	$F_{4,40}$ = 0.319, P = 0.864	$F_{1,10}$ = 1.605, P = 0.234	$F_{4,40}$ = 0.512, P = 0.727
	24	386 ± 229	428 ± 255	−74.7	−65.9	0.04 (−0.67, 0.74)			
	48	406 ± 164	479 ± 216	−54.1	−14.8	0.18 (−0.74, 1.09)			
	72	453 ± 229	460 ± 143	−7.1	−33.6	−0.12 (−0.71, 0.47)			
PPT	Pre	9.0 ± 2.0	7.9 ± 2.1						
($kg{\cdot}cm^2$)	Post	7.7 ± 2.5	6.8 ± 2.5	−1.3	−1.1	0.58 (−0.14, 1.31)#	$F_{4,40}$ = 3.372, P = 0.018*	$F_{1,10}$ = 0.026, P = 0.875	$F_{4,40}$ = 5.153, P = 0.002*
	24	6.8 ± 2.1	6.9 ± 2.8	−2.2	−1.0	0.55 (−0.18, 1.28)#			
	48	6.2 ± 2.1	7.5 ± 2.7	−2.8	−0.4	0.98 (−0.26, 2.21)#			
	72	8.0 ± 2.4	8.1 ± 2.7	−1.0	0.2	0.60 (−0.37, 1.57)#			
ROM	Pre	140 ± 7	141 ± 8						
(°)	Post	136 ± 12	141 ± 19	−3.4	−0.3	0.25 (−0.21, 0.71)	$F_{4,40}$ = 0.881, P = 0.432	$F_{1,10}$ = 6.744, P = 0.027*	$F_{4,40}$ = 1.869, P = 0.197
	24	136 ± 13	140 ± 11	−3.5	−1.4	0.22 (−0.17, 0.60)			
	48	137 ± 16	142 ± 10	−2.5	1.2	0.34 (−0.37, 1.05)			
	72	139 ± 15	146 ± 14	−0.5	4.5	0.42 (0.01, 0.84)			
MTC	Pre	54.8 ± 2.7	54.9 ± 3.0						
(cm)	Post	55.6 ± 2.6	55.6 ± 2.9	0.8	0.7	−0.04 (−0.19, 0.11)	$F_{4,40}$ = 0.940, P = 0.409	$F_{1,10}$ = 0.013, P = 0.911	$F_{4,40}$ = 19.802, P < 0.001*
	24	55.4 ± 2.7	55.5 ± 2.8	0.6	0.5	−0.02 (−0.20, 0.15)			
	48	55.4 ± 2.7	55.2 ± 2.9	0.6	0.2	−0.11 (−0.19, −0.03)			
	72	55.0 ± 2.8	54.9 ± 2.9	0.2	0.0	−0.08 (−0.17, 0.00)			

**Indicates significant difference between groups ($P < 0.05$), while # indicates and effect (Hedge's g). All values are presented as Mean ± SD.*

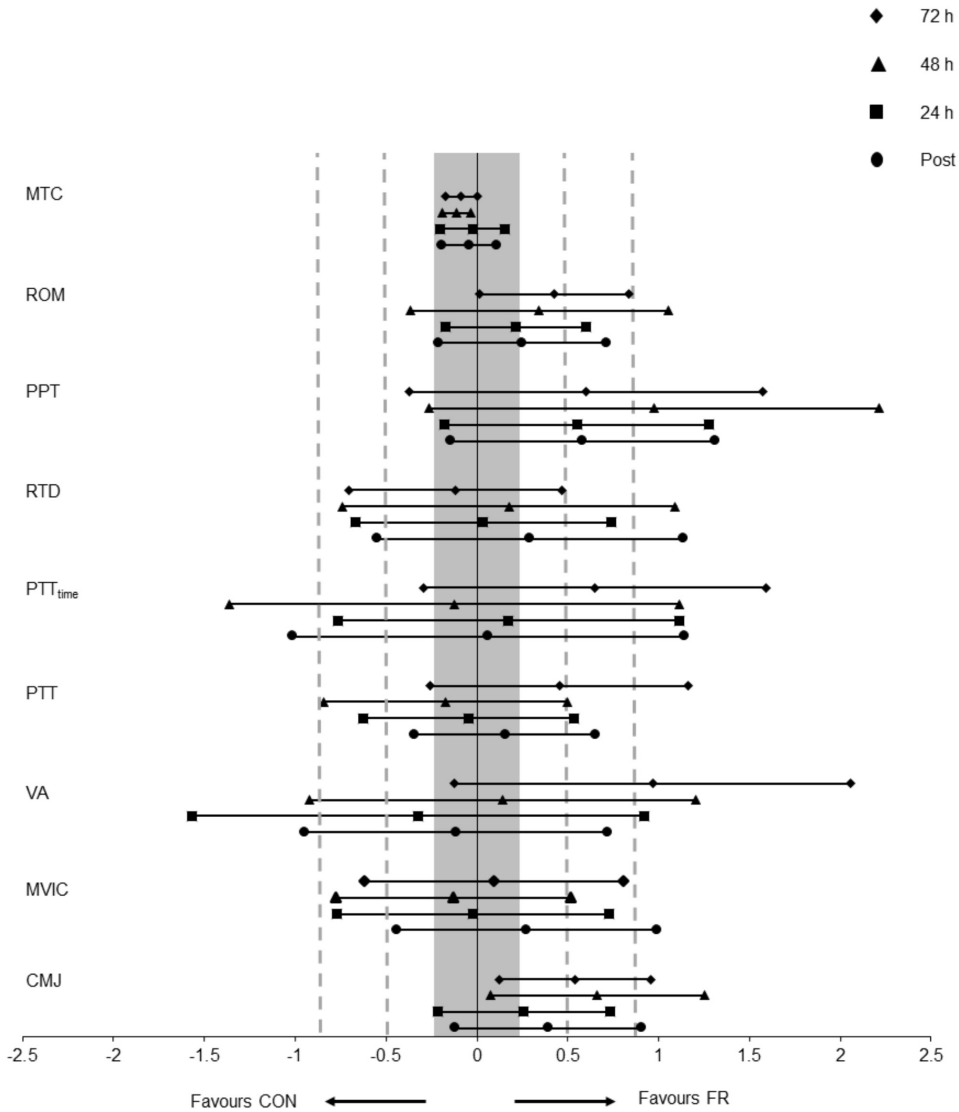

FIGURE 1 | Displays the mean effect size (Hedges's g) and 95% CI for each outcome variable; CMJ, MVIC, VA, PTT, PTT$_{time}$, RTD, MTC, PPT, and Ely's test for ROM for each condition (FR or CON) across all time points.

that FR improves jump performance during recovery which may be at least partly mediated by and increased quadriceps pain tolerance, despite no improvement in maximal isometric force. FR may be an advantageous tool to aide recovery following muscle damaging ECC exercise, however it appears unlikely that neurophysiological mechanisms contribute to performance improvements.

Performance in the CMJ was greater at 72 h for FR compared to CON, with a moderate effect also observed at 48 and 72 h, respectively. Interestingly, this observation in CMJ performance was not consistent with changes in MVIC torque, however, is in line with previous studies reporting neuromuscular outcomes (MacDonald et al., 2013, 2014; Halperin et al., 2014; Jones et al., 2015; Richman et al., 2018). Evidence from other studies has demonstrated that MVIC performance is unaltered by FR (MacDonald et al., 2013; Halperin et al., 2014) and thus, suggests that FR is at least unlikely to impair the development of acute maximal strength. However, power development may be of greater importance in functional and performance tasks than maximal strength. Our findings support Pearcey et al. (2015) who suggests that FR is unlikely to benefit a single joint isometric task but rather have feasibility for multi-joint dynamic movements requiring acceleration of the body in a single plane. The reasons for this are at this stage speculative. However, our CMJ results are also in in line with the results demonstrated by MacDonald et al. (2014), who reported an increase in CMJ at 48 h following a high-volume back squat protocol, and are similar to other massage interventions (Mancinelli et al., 2006; Willems et al., 2009). Therefore, acute FR may offer task specific performance improvements. Specifically, the attenuation of power loss appears

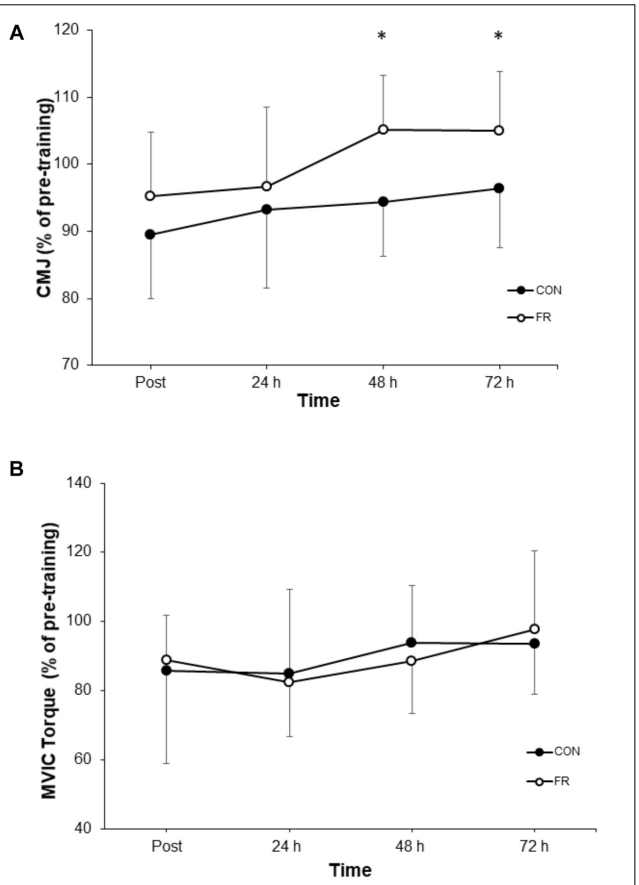

FIGURE 2 | Shows the percentage change (±SD) from pre-training for **(A)** CMJ, and **(B)** MVIC between FR and CON. *Indicates significant difference between groups ($P < 0.05$).

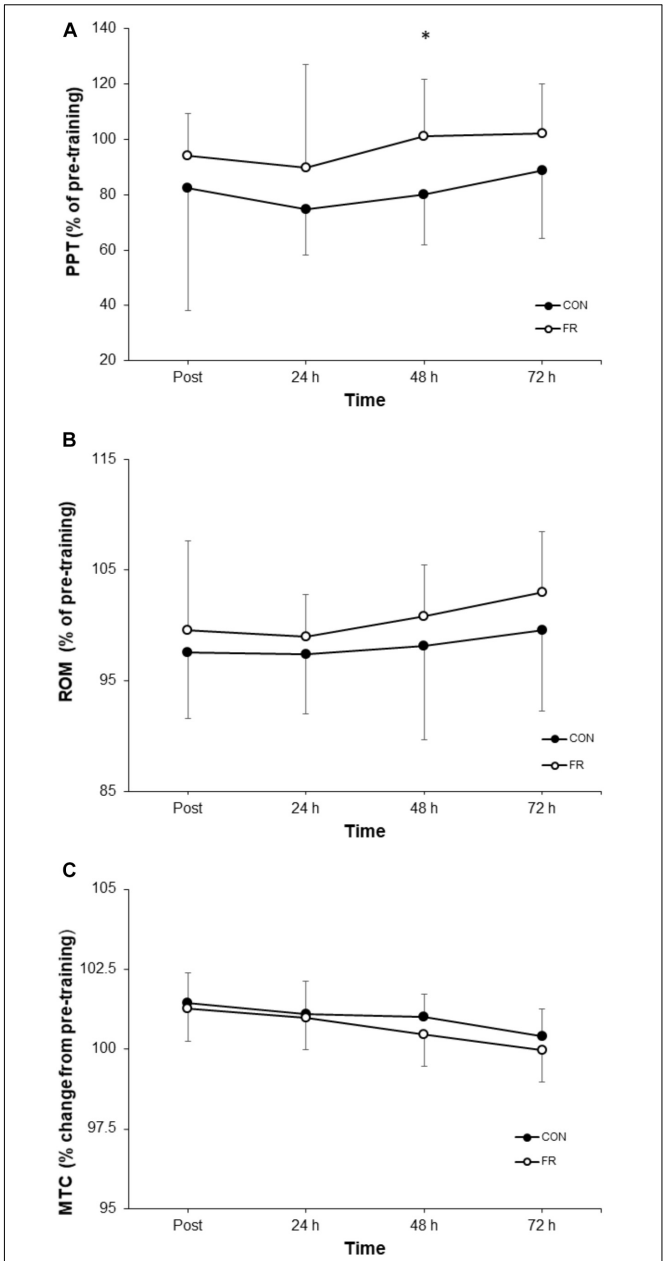

FIGURE 3 | Shows the percentage change (±SD) from pre-training for **(A)** PPT, **(B)** ROM and **(C)** MTC between FR and CON. *Indicates significant difference between groups ($P < 0.05$).

to be the most likely during recovery from damaging ECC exercise although the factors contributing to this response are yet to be fully elucidated.

The results of this study showed an increase in pain tolerance at 48 h for the FR condition, with moderate effects also observed at post-training, 24, 48 and 72 h. Several investigations have demonstrated an improved pain tolerance in the lower limbs with FR (Saxton and Donnelly, 1996; MacDonald et al., 2014; Pearcey et al., 2015). However, the physiological mechanisms responsible remain unclear. One possibility is that massage and FR increase blood flow directly to the area (Crane et al., 2012; Hofitel et al., 2016), thus acutely aiding the removal of metabolic by-products. In the latter stages of recovery, repeated exposure to manual pressure (i.e., FR) to the agonist, synergist and antagonist musculature may modulate monosynaptic group Ia muscle spindle afferent firing in response to stretch, or, alternatively downregulate pain sensitive afferent feedback caused by inflammation (Beardsley and Skarabot, 2015). Thus, it can be theorized that this may have potentially improved stretch reflex contractility and hence the power development observed in this study, however this is speculative at this stage. Another possibility is that acute FR causes a widespread modulatory response to pain. In particular, two studies, Aboodarda et al. (2015) and Cavanaugh et al. (2017b) both showed that contralateral FR improved pain tolerance in the opposite limb. Thus, our current findings and those of Aboodarda et al. (2015) and Cavanaugh et al. (2017b) suggest that neural mechanisms may, at least in part, contribute and may involve a temporary downregulation of pain sensitive afferent pathways. Further, muscle soreness is delayed following ECC contractions, despite muscle function being impaired immediately following exercise,

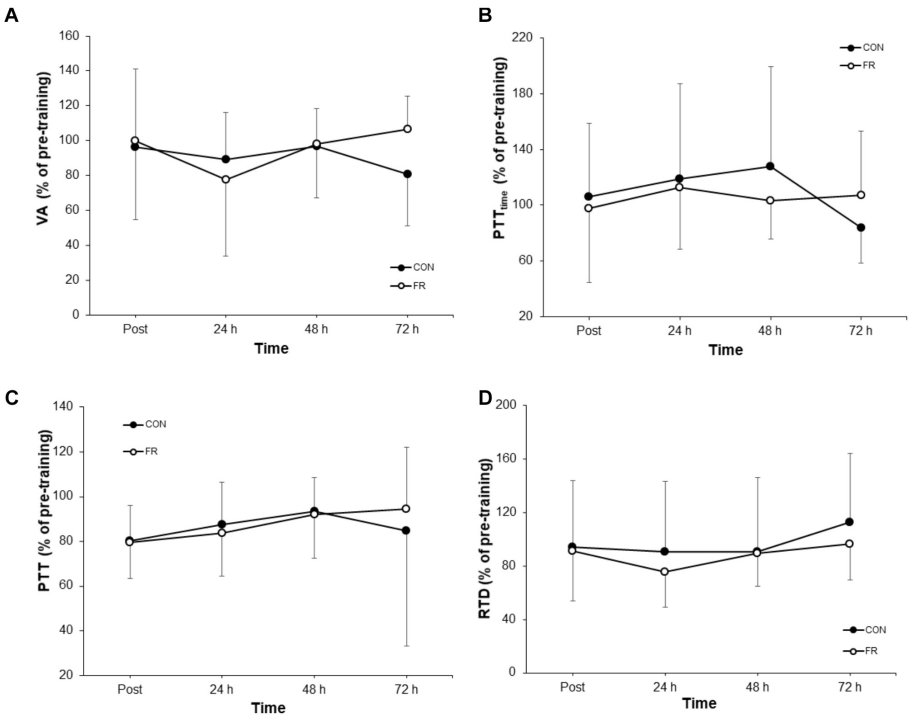

FIGURE 4 | Shows the percentage change (±SD) from pre-training for **(A)** VA, **(B)** PTT_{time}, **(C)** PTT and **(D)** RTD between FR and CON.

and thus the change in pain tolerance cannot completely explain changes/reductions in neuromuscular performance (Byrne et al., 2004). Moreover, although fatigue and pain sensitive afferent feedback has been shown to acutely reduce torque of the antagonist musculature in a flexor/extensor relationship (Kennedy et al., 2013), an immediate reduction in agonistic VA was not demonstrated in our study (i.e., quadriceps). Therefore, the relationship between poorer pain threshold and performance remains somewhat unclear. As suggested by MacDonald et al. (2014), the improvement in pain tolerance and ROM during the recovery period may be due to the facilitation of connective tissue repair. However, a clear decrease in MTC, indicative of a reduction in swelling, was not demonstrated which also renders this interpretation difficult. Thus, the mechanical and perceptual improvements observed in this study are unlikely to be explained by the suggestions of MacDonald et al. (2014).

This study did not show any significant changes in any evoked responses. Although, moderate to large effects were observed for VA and PTT_{time} at 72 h the meaning of this effect at a single time point is unclear. Following exercise, VA is thought to be affected by both central and peripheral factors (Gandevia et al., 1995). Interestingly, early ECC investigations have showed mixed results regarding VA changes in the days following exercise. For example, Gibala et al. (1995) and Saxton and Donnelly (1996) showed no change in VA despite more recent studies demonstrating the ability of fatiguing exercise to reduce VA of the quadriceps musculature (Kennedy et al., 2015; Goodall et al., 2018). Conversely, a reduction in VA has been demonstrated in the days following muscle damaging exercise causing DOMS in the elbow flexors (Behm et al., 2001). However, the results of this study suggests that FR does not improve neural activation/drive are therefore, is unlikely to explain the improvement in CMJ. Furthermore, the proposed neurophysiological changes proceeding FR proposed by other authors (Beardsley and Skarabot, 2015; Aboodarda et al., 2018), suggests that any central changes may be due to autonomic process rather than the capability to voluntarily activate the musculature. Additionally, the evoked responses obtained in this study (VA, PTT and PTT_{time}) are reflective of efferent pathways and no not account for potential sensory changes that may have occurred following FR. Thus, we suggest that future studies investigate acute changes in afferent pathways such as the H-reflex response which may be more sensitive to changes caused by the innervation of muscle spindles following acute FR interventions as has recently been conducted by Young et al. (2018). Alternatively, the decrease in pain threshold may cause a downregulation of group III/IV pain sensitive afferent firing. Although group III/IV afferents have been shown to decrease VA (Kennedy et al., 2013, 2015), changes are short lived. Moreover, it is not clear if the changes observed in blood flow occlusion studies where the acute increase in metabolite concentration causes sustained group III/IV afferent firing, is consistent with the pain related afferent feedback observed during the recovery (24–72 h) following muscle damaging exercise.

Although the results of this study investigated the effect of FR on recovery following ECC exercise we acknowledge several factors that may require consideration. For example, although

ECC is known to cause EIMD, biochemical markers (i.e., creatine kinase) were not measured in this study. Additionally, although a repeated bout effect may also exist, protecting against EIMD from a secondary bout of ECC exercise, the randomized and counterbalanced order of the conditions, and prolonged time between conditions likely controlled for such effect. Lastly, we acknowledge that the ECC contractions performed in the leg extensors may not entirely represent the nature of muscle damage following multi-joint exercise and thus, should be considered in future research.

Collectively, the results of this investigation provide some support for the use of FR to improve jump performance, with minimal effects on other measures of recovery following muscle damaging ECC exercise. Despite no clear evidence for a neural contribution, the improvements in jump performance may at least in part, by facilitated by an increase in pain tolerance. Furthermore, the lack of improvement in maximal force suggests task specific, rather than broad functional performance improvements may be expected. These findings are likely to hold important implications in applied sports settings where lengthening muscle contractions cause muscle damage, especially when training and competition schedules do not allow for sufficient recovery.

ETHICS STATEMENT

This study was approved by the Charles Sturt University Human Research Ethics Committee.

AUTHOR CONTRIBUTIONS

ED was responsible for overseeing the project including data collection, statistical analyses and manuscript preparation. CL was contributed to the data analysis and manuscript preparation. CW, SB, and MS contributed to the study design, discussion of results and manuscript preparation.

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Kinetics of Muscle Damage Biomarkers at Moments Subsequent to a Fight in Brazilian Jiu-Jitsu Practice by Disabled Athletes

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Purpose: Evidence indicates that muscle injury caused by exercise can lead to functional, biochemical, and clinical damage. These outcomes encompass an intrinsic potential to understand the real magnitude of interpretation of classic signs in sport environments and to monitor athletes, contributing to specific actions. However, little or no research has explored the general behavior of the variables presented in response to paradesportivo Brazilian jiu-jitsu. The objective of this study was to investigate the physiological behavior through clinical, functional, and metabolic outcomes in the moments following a simulated fight.

Methods: Six disabled athletes, male Brazilian jiu-jitsu practitioners (34–44 years old), were included. The participants had their outcomes analyzed individually and the variables studied were correlated. It is noteworthy that participants I and II are professional athletes with world titles. The ethics committee involving human beings of the Federal University of Mato Grosso (register no. 2.997.241) accepted the study. The participants attended the collection site four times, with a 24-h interval between sessions, characterizing the following moments: pre-exertion, and post-exertion, 24, 48, and 72 h after the simulated fight. Data collected were muscle pain, perception of recovery, muscle strength, and blood samples for creatine kinase (CK) and lactate dehydrogenase (LDH) analysis. The variables described were measured at all collection moments. The data were presented in individual raw values of each participant, with Spearman correlation analysis to verify the relationship between variables and moments.

Results: The outcomes demonstrated that the CK and LDH activity was higher of high-performance parathletes (I and II) and the reported muscle pain was lower. The fight did not influence maximal isometric strength levels in either participant. In addition, regarding delayed effects, the participants reported peak pain, CK, LDH, and decreased perception

of recovery within 24 h. However, it was found that, at 72 h, all values had recovered, close to baseline levels.

Conclusion: The presented outcomes provide parameters and suggest a safe scenario based on the intensity and volume commonly adopted in this sports parade modality where the level of effort recommended during combat does not seem to cause deleterious damage.

Keywords: creatine kinase, martial arts, sports medicine, physical therapy specialty, inflammation, musculoskeletal physiological phenomena

INTRODUCTION

Martial arts are related to a complex set of corporal strategies that include physical and mental aspects. Brazilian Jiu-jitsu, represented by intermittent movements of high intensity interspersed by brief periods of less intensity, characterizes one form of martial art (Andreato et al., 2017). With regard to the parasport, Brazilian jiu-jitsu has been demonstrated to be an inclusive sport. As for inclusive sport, several studies (Tweedy and Vanlandewijck, 2011; Tweedy et al., 2014; Ravensbergen et al., 2016; Stoter et al., 2017) classify parathletes according to the different levels of deficiencies presented.

It is known that inclusion in sports tends to provide positive repercussions on anthropometric, physiological, social, and psychological measures (Tweedy and Vanlandewijck, 2011; Tweedy et al., 2014; Ravensbergen et al., 2016; Stoter et al., 2017). However, athletic performance is related to levels of overload, which, when not properly administered and periodized, can lead to damage to the body systems (Damas et al., 2016). These losses, considered as exercise-induced muscle damage, include loss of the capacity to generate force, reduction in range of motion, muscular pain, and edema (Buchheit et al., 2009; Chen et al., 2013; Belli et al., 2018).

When considered, clinical and functional parameters are able to diagnose the general recovery condition of the subject. In this sense, previous studies (Cesnaitiene et al., 2015; Machado et al., 2018; Papassotiriou and Nifli, 2018) have shown that these variables also have a positive correlation with athletic performance and adequate monitoring allows the use of strategies that improve recovery and sports performance and minimize exposure to the occurrence of musculoskeletal injuries.

To analyze the deleterious effects mentioned above, based on the inexistence of studies that address the outcomes mentioned in paradesports Brazilian jiu-jitsu, it is considered pertinent to investigate the physiological responses under these conditions. Moreover, the results found could contribute to the understanding of the real magnitude of the interpretation of classic signs in a sports environment, contributing to guidance on specific intervention actions.

To our knowledge, this is the first study to propose the investigation of the kinetics of muscle damage in Brazilian jiu-jitsu paradesports. We believe that the disability, characterized for example by the lack of a limb, may require higher metabolic demands and result in a greater level of muscular damage, compared to non-disabled athletes. Therefore, the objective of the present study was to investigate the immediate and delayed physiological responses triggered by a fight, in Brazilian Jiu-Jitsu parathletes with different levels of physical conditioning.

MATERIALS AND METHODS

Participants

Six male Brazilian jiu-jitsu parathletes participated in the study. It is noteworthy that participants I and II are professional athletes with world titles. The eligibility criteria adopted included the practice of paradesportivo Brazilian jiu-jitsu for a period of more than 6 months. The sample size was characterized by a convenience scenario, attributed to the logistical difficulty in grouping a high number of participants with the inclusion characteristics adopted in the study.

For define the sample size, an *a priori* knowledge was used, based on the findings of Andreato et al. (2015). The chosen variable referred to the values of creatine kinase in subsequent moments to the simulated fight of Brazilian jiu-jitsu. For that, two-tailed hypothesis test was used, with significance level of 5 and 80% of power and possible sample loss of 15%, the stipulated sample size would correspond to eight volunteers.

All participants attended the same training center, and no musculoskeletal injuries were reported during the procedures. The anonymity of the participants was guaranteed. Masking of the participants, investigator, and evaluator was performed regarding the results, the hypotheses, and analyzed outcomes.

In addition, to be included, participants were required to report the absence of anemia, inflammation, diabetes, cardiovascular disease, and musculoskeletal injuries within 6 months prior to data collection. Furthermore, they were advised to refrain from anti-inflammatory drugs, analgesics, alcoholic beverages, and tobacco and not to perform any exercise not proposed by the study.

All subjects followed a similar diet and did not receive special supplements. Thus, at each collection session, all guidelines on a controlled diet were reinforced and participants were asked about possible diet adversity. In this sense, the diet control of the participants was performed only subjectively by verbal orientations.

For description of the functional classifications and types of injuries, the participants had physical motor disability (66.4%) and visual disability (33.6%), represented by functional

classification S6 (amputations of the leg) and S12 (partial visual disability), respectively.

The anthropometric characteristics of the participants are presented in **Table 1**.

Ethics Statement and Clinical Trial Registry

The participants were informed about the procedures and objectives of the study and, after agreeing, signed a consent form. The consent obtained from the participants was both informed and written. The Ethics Committee in Research of the Federal University of Mato Grosso previously approved all procedures (Araguaia campus, sob seem number: 2.997.241).

Study Design

This is an observational study. Data collection was carried out in June 2018 at the usual training center of the participants (Grace Barra Academy) located in the municipality of Barra do Garças, MT, Brazil, and data analyses were conducted at the Federal University of Mato Grosso, Araguaia Campus. All procedures were performed under standard conditions (temperature: 28 ± 1°C, relative humidity: 84%).

The procedures took place on four consecutive days, always in the same period, in order to avoid influences of the circadian cycle. At first, all participants were submitted to an anthropometric evaluation (**Table 1**) using a scale (Tanita BC554, Iron Man/InnerScaner, Tanita, Illinois, USA) and a stadiometer (Sanny, American Medical do Brasil, São Paulo, Brazil). On the first day, a simulated fight was performed as well as evaluation of the metabolic, clinical, and functional parameters in the pre- and post-fight moments. On the subsequent days (24, 48, and 72 h after the fight), all parameters were reevaluated in order to measure outcomes related to the delayed effect. An overview of the study is presented in **Figure 1**.

The order of the fights was defined by prior randomization. A warm-up was performed with Brazilian jiu-jitsu movements of light intensity, characterized by low heart rate and low strength requirement, for 5 min. The simulated fight protocol occurred in accordance with the rules of the International Brazilian Jiu-Jitsu Federation (IBJJF), excluding any type of finalization (International Brazilian Jiu-jitsu Federation (IBJJF), 2018). In these cases, the parathletes were separated and directed to return to the fight immediately. Thus, maximum effort was advocated as well as a similar activity for all participants. The parathletes fought with non-disabled athletes, not included in the study, who were previously trained and guided on standardized fighting behavior, with all participants, in order to minimize the influence of possible bias. The choice of opponents was based on similar graduation similar body mass.

Procedures

Blood Sampling and Analysis

Creatine kinase (CK) and lactate dehydrogenase (LDH) activity were verified by blood collection from the antecubital vein, collected using a syringe (62 μl) by a qualified professional. The blood sample, evaluated in serum, was analyzed by the ELISA method and an Advia 1650 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA) in a specialized laboratory.

TABLE 1 | Anthropometric characteristics of participants.

	Age (years)	Stature (m)	Body mass (kg)	Training time (years)	Training time per week (h)	Competitive level	Belts
Parathlete I	43	1.63	96.5	22	18	Professional	Black belt
Parathlete II	36	1.70	82.3	7	9	Professional	Purple belt
Parathlete III	41	1.65	90.0	1	7	Amateur	White belt
Parathlete IV	34	1.76	89.0	1.2	6	Amateur	Blue belt
Parathlete V	13	1.46	35.5	0.9	5	Amateur	Blue belt
Parathlete VI	40	1.63	84.0	0.7	6	Amateur	Blue belt

Parathletes I and II were world champions in their respective categories.

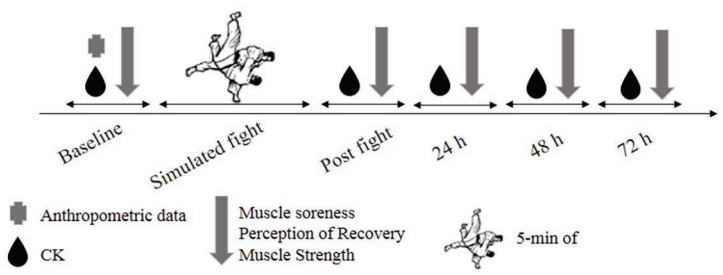

FIGURE 1 | Design study. CK, creatine kinase; 5-min of, five minutes of simulated fighting.

Muscle Soreness

Participants were instructed to assess muscle discomfort (induced by the simulated fight) using a Visual Analogue Scale (VAS) ranging from 0 "no pain" to 10 "extreme pain" (Machado et al., 2017).

Perception of Recovery

The perception of recovery was obtained using a 10-point Likert Scale, where 1 indicates the feeling "not recovered" and 10 indicates "fully recovered". The participant was asked the following standardized question: If you had to perform the fight again at this time, how recovered do you feel? (Lopes et al., 2018).

Maximum Voluntary Isometric Contraction

Measurement of the Hand Grip Strength (HGS) was performed using an analog hydraulic dynamometer, brand JAMAR® (Asimow Engineering®, USA), with an accuracy of 0.5 kg/f and a maximum capacity of 100 kg/f. The position suggested by the American Society of Hand Therapists (ASHT) was used to perform the test (Franchini et al., 2011), which occurred with the participant in a sitting position, with hips and knees at 90° flexion, shoulder at adduction, elbow flexed at 90°, and wrist and forearm in a neutral position, resting on a table. The participant was instructed to use the greatest possible strength, and the peak value was subsequently recorded.

Statistical Analysis

The raw data of each participant were presented for each investigated variable.

Spearman's correlation was used between the analyzed outcomes (pain, recovery, strength, and muscle damage) and were compared between the post-fight, and the other moments were evaluated (baseline, 24, 48, and 72 h). The variables were considered as independent–designated as $\boldsymbol{X}$, at the post-fight moment, while the data obtained at the other moments were treated as dependent variables designated as Y_{baseline}, $Y_{24\text{h}}$, $Y_{48\text{h}}$, and $Y_{72\text{h}}$. In cases of correlation, linear regression was performed to demonstrate the markers temporal evolution.

In order to present hypotheses referring to the sample characteristics, a non-parametric method was used, using the following formula:

$$r_S = 1 - 6\sum_{i=1}^{n} \frac{d_i^2}{n\left(n^2-1\right)}.$$

The expression above take into account pairs of variables and the difference between the two ranks of $\{X_i, Y_i\}$.

Thus, from the calculated r_S values, the following hypothesis tests were performed to investigate correlation between variables in the population (Kraemer, 1973; Kruskal, 1978).

Hypothesis Test 1

- **Null Hypothesis $\mathbf{H_0}$**: $\rho = 0$. There is no correlation between the population variables X and Y;
- **Alternative Hypothesis $\mathbf{H_1}$**: $\rho \neq 0$. There is correlation between the population variables X and Y.

Linear Regression

The linear regression problem consists into determining a in the matrix equation

$$Y = Xa + e,$$

where $\boldsymbol{Y} \equiv$ response vector, $\boldsymbol{X} \equiv$ design matrix, $\boldsymbol{a} \equiv$ vector of regression parameters, and $\boldsymbol{e} \equiv$ error vector.

The assumed model in the linear adjustment in the used parameters was the second-degree polynomial, by the following equation:

$$Y = a_1 + a_2 X + a_3 X^2$$

Above, the adjustment parameters in the fit are $\{a_1, a_2, a_3\}$.

The *least-squares method* is able to determine the set of parameters $\{a_1, a_2, a_3\}$ through the matrix equation (Spearman, 1904). The distribution of residuals in the linear regression is of interest because it allows to evaluate if the variance in the adjustment approaches the minimum for the selected linear estimator. If this distribution is normal, assumptions of the Gauss-Markov theorem are satisfied and the parameters estimation will be the best linear unbiased possible (Scheffe, 1999). A highly efficient test to evaluate if the distribution is normal – available in the Maple System (Char et al., 1983) used in this analysis – is the Shapiro-Wilk. These test hypotheses are.

Hypothesis Test 2

- **$\mathbf{H_0}$**: The residuals of the fit follow a normal distribution.
- **$\mathbf{H_1}$**: The residuals of the fit do not follow a normal distribution.

In this proposal, the Chi-Square goodness of fit test (Char et al., 1983) meets the proposed needs and was applied in combination with the Shapiro-Wilk test. The results of both took into account the level of significance $\alpha = 0.05$. The goodness of fit test hypotheses are presented below.

Hypothesis Test 3

- **$\mathbf{H_0}$**: The calculated observables from the data fitting do not differ from the actual observables.
- **$\mathbf{H_1}$**: The calculated observables from the data fitting differ from the actual observables.

RESULTS

The anthropometric characteristics of the participants are presented in **Table 1**. **Figures 2–4** present the values of the analyzed endpoints of pain, recovery, creatine kinase, lactate dehydrogenase, and strength, respectively.

The peak markers of muscle damage, CK, and LDH occurred between 24 and 48 h after the simulated fight. With regard to CK values, the baseline moment observed were above the reference value (196 IU/L), suggesting that the participants were already in a possible state of muscle injury.

Muscle pain peaked in 24 h. However, the participants reported a reduced pain score, similar to baseline, at 72 h post exercise.

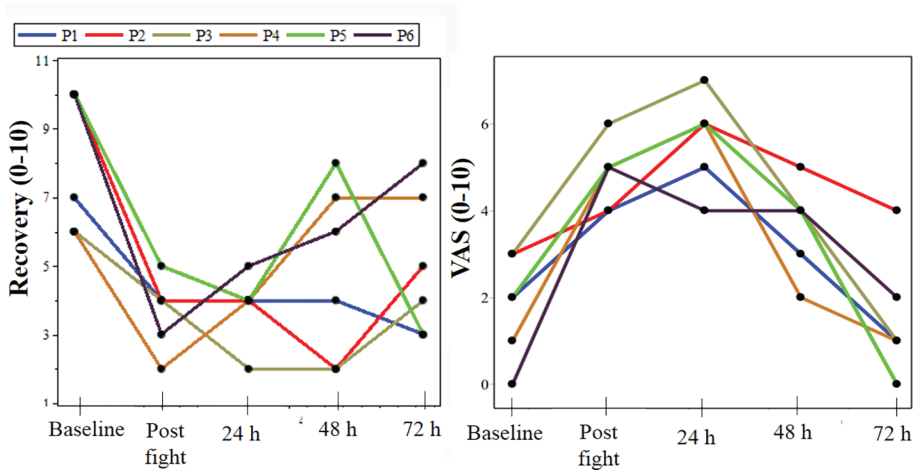

FIGURE 2 | Clinical outcomes, represented by pain and perception of recovery. h, hours; P, parathlete; parathletes I and II were world champions in their respective categories; VAS, analogic visual scale; U/L, units per liter.

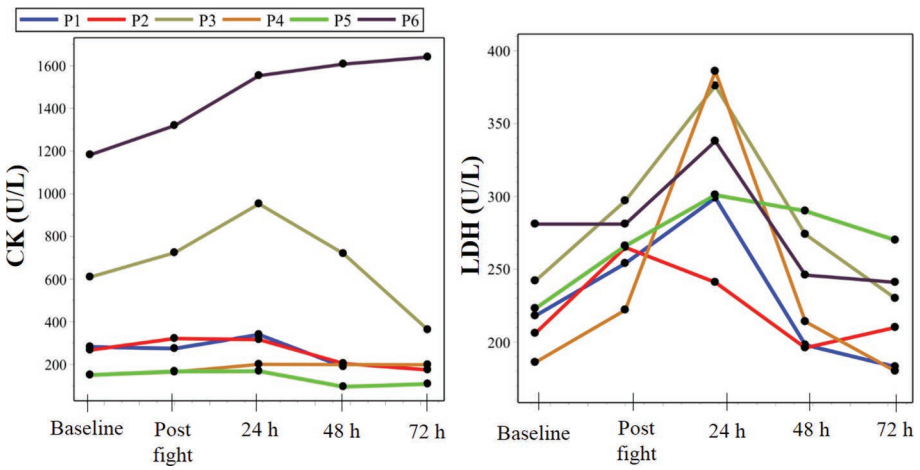

FIGURE 3 | Biochemical markers, represented by creatine kinase (CK) and lactate dehydrogenase (LDH). CK, creatina quinase; LDH, lactate dehydrogenase; P, parathlete. Parathletes I and II were world champions in their respective categories.

The perception of recovery reduced after the fight, with values that remained low until the 72 h moment. Significant

With respect to strength, there were no significant losses in muscle function between the baseline condition and the moments following the simulated fight.

Table 2 shows the Spearman correlation values obtained from the sample and the limit values for acceptance or rejection at the level of significance $\alpha = 0.05$.

The analyses showed a correlation between the variables ($\rho = 0$), CK, LDH, and strength. In the case of LDH indicators, only the observable $Y_{baseline}$ showed no evidence of a significant relationship with the independent variable X (post). Although there is statistical evidence in favor of the null hypothesis in the case of the CK indicator between $\boldsymbol{Y}_{72h}$ and $\boldsymbol{X}$, the others point in favor of the alternative hypothesis.

Results of the linear regression using the polynomial model are presented below in **Figure 5**. In these two graphs, the Parathlete II data available in **Table 2** were used. The corresponding graphical representations for the other parathletes are equivalent and so are not shown. Keeping the set of independent variables as $\boldsymbol{X}$, the horizontal axis of the graphs represents the instants at which the indicators were obtained; and these have a delay of 24 h. However, the vertical axes represent the observables of a given marker, assigned as $\boldsymbol{Y}$. The respective linear regressions analyses are presented in **Tables 3, 4**.

DISCUSSION

The present study aimed to explore the immediate and delayed physiological responses triggered by combat in Brazilian

jiu-jitsu parathletes. The main findings show that the CK and LDH activity in high-performance parathletes was superior and the athletes reported lower muscle pain. The fight did not influence isometric muscular strength levels. Regarding the delayed effects, it was verified peak pain, CK, LDH, and decreased perception of recovery in 24 h. However, within 72 h after the fight, all values had recovered, close to baseline levels.

On the other hand, it has been observed that, in individuals with sedentary deficiency, these values are higher (Hruby and Hu, 2015; Andrea et al., 2018). For this, sport is preventive management since obesity is directly related to high levels of comorbidity, chronic pathologies, psychosocial disorders, and mortality (Coswig et al., 2013; Karjoo, 2018; Kim et al., 2019).

With regard to the training time of the participants, a much higher experience time was observed in the professional parathletes. It is believed that this fact could substantiate the evidence of chronic injury in muscle tissue, in response to inadequate periodization over the years, which would explain the marked CK levels height even in basal conditions in these athletes, since they have reported not adopting an adequate periodization program. In this regard, the study of Adams and Kirkby (2001) addressed the several consequences in body systems due to overtraining, which include high concentrations of CK in individuals who accumulate many hours of training inadequately. After the simulated fight, CK increased in all participants, in the moment 24 h. This fact supports the initial hypothesis that the effort implemented during the practice of Brazilian jiu-jitsu is enough to alter the homeostasis of the systems in the short term. Similar outcomes related to CK behavior after Brazilian jiu-jitsu matches have demonstrated similar results to the present study (Andreato et al., 2015; Detanico et al., 2015; Branco et al., 2016; Fonseca et al., 2016). In addition, it was observed that the recovery of baseline concentration levels occurred at 72 h post-fight. These data also corroborate other studies (Andreato et al., 2015; Branco et al., 2016; Fonseca et al., 2016) in demonstrating that the intensity and volume adopted in this sport modality, when respected, are not sufficient to cause deleterious effects in the long term.

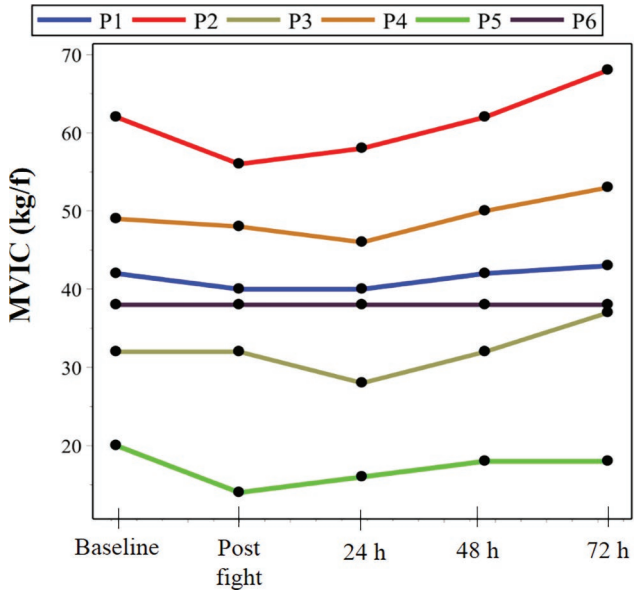

FIGURE 4 | Functional outcomes, represented by isometric strength analysis. MVIC, maximum voluntary isometric contraction; P, parathlete. Parathletes I and II were world champions in their respective categories.

In contrast, some studies (Warren et al., 1999; Morton et al., 2005; Detanico et al., 2015; Prendergasta et al., 2016) state that the actual mechanisms involved in CK alterations are unclear and question whether CK levels reliably assess the accuracy of muscle damage. Furthermore, these studies suggest that the measurement of maximum voluntary isometric contraction is a more relevant parameter (Stanley et al., 2012; Erkan et al., 2015). In this regard, the outcomes related to maximal isometric strength, recorded in the present study, did not demonstrate a statistically significant difference between moments. This finding, associated with the findings of CK activity, supports the ideas presented on the fact that Brazilian jiu-jitsu practice does not cause a significant decline in long-term homeostasis of the body system.

In addition, regarding the isometric muscle strength-related outcomes, the highest values found in high-performance athletes present similar results to other studies which compared the strength level between high-performance athletes and amateurs (Aboodarda et al., 2018) and are observed due to the superior time of physical training. In agreement, a study that investigated Brazilian jiu-jitsu athletes found similar outcomes for strength,

TABLE 2 | Spearman correlation between the studied markers.

M	Baseline ($Y_{baseline}$)			24 h (Y_{24h})			48 h (Y_{48h})			72 h (Y_{72h})		
	r_s	r_c	Res	r_s	r_c	Res	r_s	r_c	Res	r_s	r_c	Res
Pain	0.043	0.866	A	0.850	1.000	A	0.050	1.000	A	0.250	1.000	A
Recovery	0.629	0.866	A	0.350	1.000	A	0.325	1.000	A	0.575	1.000	A
CK	0.929	0.866	R	0.886	0.866	R	0.886	0.866	R	0.600	0.866	A
LDH	0.886	0.866	R	0.086	0.866	A	0.600	0.866	A	0.771	0.866	A
MVIC	1.000	0.866	R	1.000	0.866	R	1.000	0.866	R	1.000	0.866	R

All the coefficients are calculated in relation to the postfight, considered as an independent variable (X) in this analysis. The other observables are considered dependent (Y). The values used in the calculations were extracted from ***Tables 3, 4*** *for the set of parathletes I, II, III, IV, V, and VI. The null hypothesis is rejected when p is less than 0.05, which corresponds to the inequality $r_s > r_c$. M, marker; r_c, critical coefficient; Res, result; A, accepted; R, rejected.*

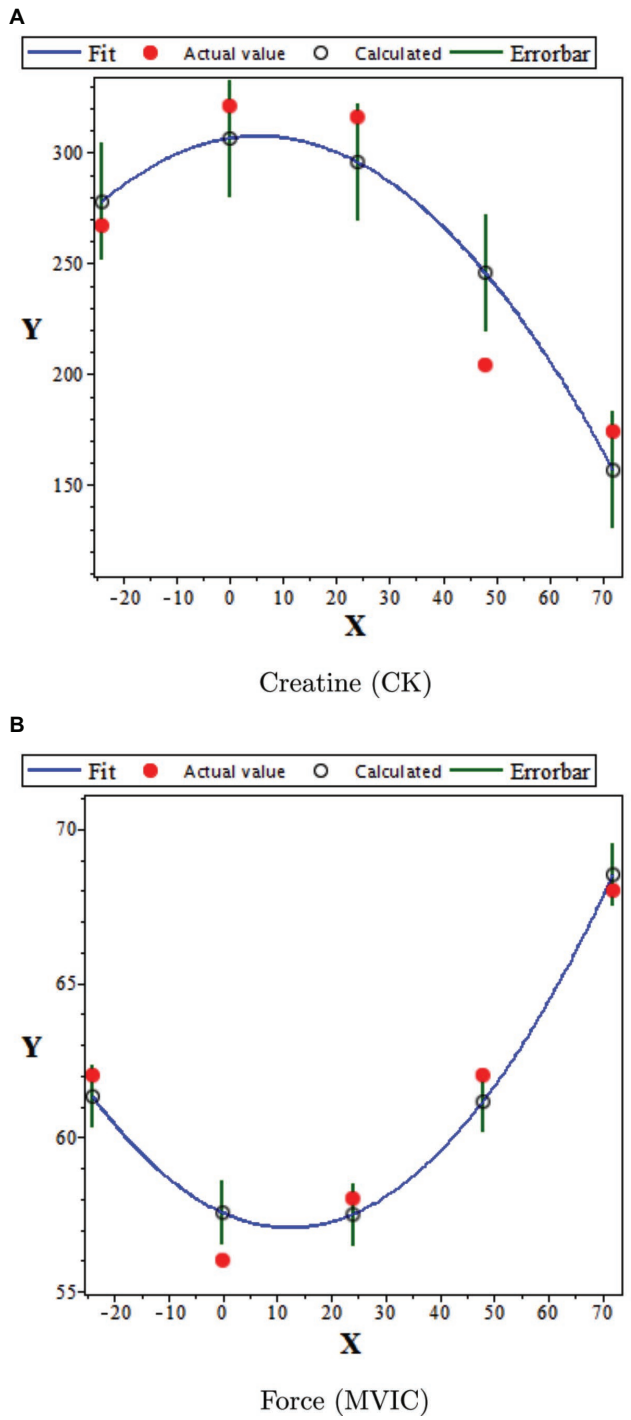

FIGURE 5 | Linear regression for creatine and maximal voluntary isometric contractions. Typical polynomial fit for the time evolution of measurements for creatine **(A)** and maximal voluntary isometric contractions **(B)**. This graph employs the data available in **Table 4** for parathlete II. The model for the linear regression is $Y = a_1 + a_2X + a_3X^2$. Here, the dependent observable Y is the measure of CK or MVIC and the dependent variable X is the time in hours. The error bars correspond to the standard deviation of the residuals (RMSE). The graphical representations for the other parathletes are equivalent to these.

TABLE 3 | Normality tests and goodness-of-fit for the linear regression of the marker CK (**Y**) as a function ofwe (**X**).

Parathlete	Shapiro-Wilk		Goodness-of-fit		
	p	Result	χ^2	*p*	Result
I	0.930	Accepted	11.340	0.023	Rejected
II	0.944	Accepted	8.957	0.062	Accepted
III	0.904	Accepted	9.226	0.056	Accepted
IV	0.266	Accepted	400.554	0.000	Rejected
V	0.422	Accepted	0.281	0.991	Accepted
VI	0.865	Accepted	9.956	0.041	Rejected

TABLE 4 | Normality tests and goodness-of-fit for the linear regression for MVIC (**Y**) marker as a function of time (**X**).

Parathlete	Shapiro-Wilk		Goodness-of-fit		
	p	Result	χ^2	*p*	Result
I	0.238	Accepted	0.025	1.000	Accepted
II	0.238	Accepted	0.070	0.999	Accepted
III	0.803	Accepted	0.255	0.992	Accepted
IV	0.311	Accepted	0.059	1.000	Accepted
V	0.663	Accepted	0.627	0.960	Accepted
VI	0.533	Accepted	0.520	0.720	Accepted

with higher levels in professional athletes when compared to amateurs. These findings are essential to assist trainers and athletes in understanding the metabolic demands in jiu-jitsu, acting as a parameter to monitor adaptation and performance during periodized training (Silva et al., 2015).

The perception of recovery has been described as an important tool to evaluate responses to exercise in the adaptive process (Machado et al., 2017), where improvement in recovery perception seems to be directly related to the subsequent performance in sports practice. In this respect, Andreato et al. (2017) suggest that there is a contribution of the psychological mechanism and that high-performance athletes tend to perform better when they believe in the importance of recovery. In addition, higher performance and athletic experience seem to impact less sensitivity to pain and recovery.

With regard to the observed correlation outcomes, only the CK and MVIC indicators justify attribution over the alternative hypothesis –i.e., there is a correlation between the training (moment post-fight) and the posterior moments. On this regard, it is reiterated the fact that correlations quantify the association between the studied markers as a result of the training performed. In contrast, no significant correlations were found between pain, recovery, and handgrip × CK and LDH in post, 24, 48, and 72 h-post. This information is relevant for the association of the discussed markers and serves as a parameter for appropriate periodization prescription, since they relate to the levels of clinical, metabolic, and functional recovery of the subjects in question.

Moreover, the graphical analysis obtained by the linear regression shows a consonance between the calculated values and those observed for the MVIC, as shown in **Figure 2**. In

fact, for all the athletes, the calculated data sample can be considered as corresponding to those observed. The same is not apparent in relation to the temporal dependence of CK measures, since the goodness of fit was not acceptable for athletes I, IV, and VI. Still, for the complexity of a study like this, agreement of half the sample suggests that the polynomial adjustment of the second degree can be proposed as a functional model for the temporal evolution of both indicators, as verified in the exposed data.

Regarding the isometric palmar grip strength outcomes, Andreato et al. (2011) performed the same analysis with Brazilian jiu-jitsu athletes. The outcomes showed mean values of 43.7 ± 4.8 kgf. This result is similar to those reported in the present study. Such similarity demonstrates that the incapacity caused by amputation or visual impairment does not characterize limiting factors that compromise the strength of the upper limbs, highly recruited to perform this sporting modality. In addition, these findings are encouraging and should serve as a motivation for practitioners of Brazilian Jiu-Jitsu paradesports, since strength characterizes basic physical ability, being responsible for the good functionality of the subject.

In summary, the presented results provide us with support to infer that the whole strategy used in this analysis serves the research purpose. In this sense, the data demonstrate important correlations between clinical, metabolic, and functional parameters in response to the Brazilian jiu-jitsu paradesportivo practice. In addition, the proposed model for the CK and MVIC indicators presents a logical temporal reasoning supported by reference scientific literature that indicates reliable outcomes and encourages the improvement and use of this type of analysis in the human physiology research field.

To the knowledge of the authors, this is the first study to verify clinical, functional, and muscle damage marker outcomes in response to Brazilian jiu-jitsu paradesportivo practice. This may be due to the possibility of large variations between the types of disabilities, which may influence the heterogeneity of the assessed group associated with the logistical difficulty mentioned above in gathering a high number of individuals with the same type of disability. In addition, this difficulty characterizes a limitation. However, it is necessary to consider the theme and to understand the kinetic behavior in response to the practice of this sport modality, to assist the scientific and clinical community in the management of specific actions that aid athletic performance and lower the incidence of injuries in this particular population.

The current study presents strengths. First, it was elaborated with high methodological quality. Second, the procedures were carried out in a field setting, identical to that used in competitive combat. In addition, the presented outcomes are unprecedented and constitute intrinsic potential under new perspectives and parameters related to the parasport. It is pertinent that future studies address the analysis of other biochemical markers such as hormonal rate and cytokines as well as the application of specific recovery techniques based on the observed physiological responses in order to measure possible differences in physiological parameters after the fight.

CONCLUSION

The findings of this study demonstrated the results of biochemical markers related to muscle damage, after a fight, in professional and amateur Brazilian jiu-jitsu parathletes. For this, peak values of the analyzed variables were recorded in 24 h. However, at 72 h, the values returned to levels close to baseline. It was also observed that there were no deleterious effects on muscle function after the fight. The presented outcomes provide parameters and suggest a safe scenario based on the intensity and volume adopted in this parasport modality.

ETHICS STATEMENT

Participants signed a free and informed consent form, agreeing to participate in it. In addition, the study was approved by the ethics and research committee involving human subjects of the Federal University of Mato Grosso (UFMT).

AUTHOR CONTRIBUTIONS

JL designed the study, conducted the analyses, and wrote the manuscript. PA, AA, and LG assisted in the acquisition, analysis, and interpretation of data, and reviewed and edited the article. CA and AN made substantial contributions including conception and design of the study, and a critical revision of the article. All authors read and approved the final manuscript.

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