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Effects of cumulative school soccer matches separated by 24-h or 48-h intervals on physical recovery status of U-19 players

Efeitos de partidas consecutivas de futebol escolar com intervalos de 24 e 48 horas no estado de recuperação física de jogadores Sub-19

Vitor Hugo Santos Rezende^{1,4} https://orcid.org/0000-0003-3708-2356 Diego de Alcântara Borba^{2,3} https://orcid.org/0000-0001-7982-3517 Lucas Augusto de Souza¹ https://orcid.org/0000-0002-0591-3875 Suene Franciele Nunes Chaves⁴ https://orcid.org/0000-0002-2670-0334 Maria Hipólito Almeida Pinheiro¹ https://orcid.org/0000-0003-4215-4158 Hugo César Martins Costa⁵ https://orcid.org/0000-0002-0851-4730 Daniel Barbosa Coelho⁶ https://orcid.org/0000-0003-0346-492X Rauno Álvaro de Paula Simola³ https://orcid.org/0000-0001-8576-6934 Carlos Magno Amaral Costa¹ https://orcid.org/0000-0001-9761-9448 https://orcid.org/0000-0003-4150-4961 João Batista Ferreira Júnior¹ https://orcid.org/0000-0002-7541-8212

Abstract – This study aimed to evaluate the effects of cumulative school soccer matches separated by 24-h or 48-h intervals on the recovery status of U-19 players. Thirty-four school athletes $(17.6 \pm 1.1 \text{ years})$ who played in an U-19 school soccer competition (composed of one group with four teams and another group with three teams, followed by semifinals and final) were examined before three matches, which lasted 70 min. Seventeen athletes had a 24-h rest interval between each match (GGG group), while 18 athletes had a 48-h rest interval between the second and third matches (GG48hG group). Total Quality Recovery, countermovement jump, 10-m sprint, and maximum lumbar isometric strength were measured. The internal load of each match was calculated by the product of the session Rating of Perceived Exertion and match time. There was a 22% reduction in Total Quality Recovery (p< 0.001) and 12% in 10-m sprint performance (p< 0.001) before the third match in the GGG group, while the GG48hG group showed no changes for the same variables (p> 0.05). The countermovement jump decreased before the second match in both groups (GGG= 12% and GG48hG= 10%; p< 0.001), with no difference between groups (p> 0.05). In addition, both groups showed no changes in the isometric strength or the internal load match over the games (p > 0.05). Despite not providing complete muscle recovery, a 48-h interval between the second and third matches seems to have minimized the reduction of muscle performance due to consecutive matches.

Key words: Student competition; Muscle recovery; Muscle damage.

Resumo – Este estudo teve como objetivo avaliar os efeitos de partidas consecutivas de futebol escolar com intervalos de 24 e 48 horas no estado de recuperação física de jogadores Sub–19. Foram avaliados 35 homens (17,6±1,1 anos) atletas escolares sub–19. Durante a competição, foram realizados três jogos de 70-min de duração. Dezessete atletas tiveram intervalo de 24h entre cada jogo (grupo GGG). Dezoito atletas tiveram intervalo de 48 h entre o 2º e o 3º jogo (grupo GG48hG). Antes de cada jogo foram medidas a Qualidade Total de Recuperação, altura do salto com contra movimento, velocidade no sprint de 10-m, e força máxima isométrica lombar máxima. A carga interna do jogo foi calculada pelo produto da percepção subjetiva do esforço da sessão e tempo de cada jogo. Houve uma redução de 22% na qualidade total de recuperação (p <0,001) e de 12% no desempenho de sprint de 10 m (p <0,001) antes da terceira partida no grupo GGG, enquanto o grupo GG48hG não apresentou alterações para as mesmas variáveis (p >0,05). O salto com contra movimento diminuiu antes da segunda partida em ambos os grupos (GGG = 12% e GG48hG = 10%; p <0,001), sem diferença entre os grupos (p> 0,05). Em adição, ambos os grupos não apresentaram alterações na forso musicina a carga interna oa (p> 0,05). Em adição, ambos os grupos não apresentaram alterações na forsis sométrica e na carga interna ao longo dos jogos (p> 0,05). Apesar de não proporcionar uma completa recuperação muscular, 48 h de intervalo entre o segundo e o terceiro jogo parece ter minimizado o efeito de jogos consecutivos na redução do desempenho. Palavras-chave: Competição escolar; Recuperação muscular; Dano muscular.

1 Federal Institute of Sudeste of Minas Gerais. Campus of Rio Pomba. Rio Pomba, MG. Brasil.

2 State University of Minas Gerais. Campus of Ibirité. Ibirité, MG. Brasil 3 State University of Minas Gerais. Campus of Divinópolis. Divinópolis, MG. Brasil.

4 Federal University of Minas Gerais. Belo Horizonte, MG, Brasil 5 Pontifical Catholic University of Minas Gerais. Department of Physical Education. Belo Horizonte, MG. Brasil. 6 Federal University of Ouro Preto. School of Physical Education. Ouro Preto, MG. Brasil.

7 Seção de Educação Física. Colégio Militar de Brasília. Asa Norte. Brasília, DF Brasil

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Corresponding author

João Batista Ferreira Júnior. Instituto Federal do Sudeste de Minas Gerais, Campus Rio Pomba Av. Dr. José Sebastião da Paixão, s/n. 36180-000, Lindo Vale, Rio Pomba (MG), Brasil. E-mail: jbfjunior@gmail.com

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INTRODUCTION

Soccer has been characterized as an intermittent and high-intensity sport, given that the main actions (e.g., jumps, sprints, runs with changes of direction and dribbling) occur in the zone of anaerobic intensity^{1,2}. As a consequence, soccer players may experience decreased physical performance during and after matches, with complete recovery sometimes requiring several days³. Long-term recovery seems to depend on the inflammatory process and muscle tissue repair, which might occur in the skeletal muscle after performing a high intensity training session or competition^{3,4}. A recent meta-analysis has identified that the maximum isometric strength of knee flexion and extension, and the countermovement jump performance remained 4 to 6.9% below baseline values 72-h after a soccer match⁵. Additionally, sprint performance returned to baseline within the aforementioned period⁵.

Long-term recovery demand based on these performance measurements indicates the importance of promoting a balance between physiological stress and physical recovery days after soccer matches or training sessions. Optimizing this process has been one of the most important challenges both for scientific and practical applications, mainly to optimize sporting performance in tournaments and during training⁶⁻⁸. An imbalance between physiological stress and recovery may occur in soccer tournaments such as those with congested matches9-11. Consecutive matches separated by 24-h may be found in professional sports, such as tennis, volleyball playoffs and basketball. Scholar competitions are also frequently accomplished with 24-h intervals between matches¹¹ in a number of sports modalities (e.g., futsal, handball, basketball, soccer, etc.). In such tournaments, consecutive game days (i.e., 24-h between matches) take place, leading to muscle fatigue and suboptimal-recovery⁸. Recent studies have shown that consecutive game days lead to a decrease in the perception of recovery status and jump performance, a reduction of the distance covered at high intensity and in the total distance covered¹⁰⁻¹².

Due to operational and logistical issues, school tournaments do not allow soccer matches to be played weekly or every three or four days, with the most common approach being to hold matches with 24-h between each one. However, an alternative would be the addition of longer intervals (e.g., 48-h), which could contribute to increased muscle recovery. To the best of our knowledge, no study has investigated the effect of a 48-h interval between matches on physical performance of soccer players compared to a 24-h interval. According to Paulsen et al.³, depending on the stress level of the previous training session, a 48-h interval may be sufficient to promote complete muscle recovery. Thus, it was hypothesized that 48-h would minimize the negative effects of consecutive match days on recovery status. The number of U-19 soccer competitions has increased substantially since the International Federation of Association Football established age-restricted soccer tournaments¹³. Additionally, it seems to be common for school sports teams attending school competitions¹¹. Therefore, it becomes important to evaluate U-19 school soccer competitions, since it may be of use for professionals who work in the organization of competitions. Further it could aid managers in the training of athletes, helping trainers and coaches to better understand the factors that affect muscle recovery. Therefore, the aim of the present study was to evaluate the effects of cumulative school soccer matches separated by 24-h or 48-h intervals on the recovery status of U-19 players.

METHOD

Participants

Sample size was determined using GPower (version 3.1.2; Franz Faul, Universitat Kiel, Germany), taking into account the counter movement jump from pilot data and the following design specifications: f effect size= 0.25; $\alpha = 0.05$; $(1-\beta) = 0.8$; test family= F test and statistical test= analysis of variance (ANOVA) repeated measures, within-between interaction¹⁴. The total sample size estimated according to these specifications was 28 participants. Initially, 85 male school athletes from six teams aged between 15 and 19 years were randomly selected. Eighteen participants left the study for personal reasons, while 33 were excluded for not completing all the measurement procedures. Thus, in the end, 34 athletes $(17.6 \pm 1.1 \text{ years})$ completed the study. Participants attended the regional stage of a national school tournament, held from August 27 to September 1, 2018, on mornings and afternoons (8 am to 6 pm), in the city Belo Horizonte- MG, Brazil. They were physically active, involved in moderate physical activity (jogging, agility, or endurance) for an average of 3 days a week. Participants and their guardians signed a consent form after being informed of the aims and procedures of the study. The study protocol was previously approved by the Institutional Review Board (CAAE: 65789717.2.0000.5115).

Experimental design

The tournament was composed of a phase group in a Round-Robin format (one group of four teams and another with three teams each), followed by semifinals and finals games. The tournament took place over 6 days. Only games from the phase group, which occurred in the afternoons (2:00 p.m. to 5:30 p.m.), were evaluated. Some teams played on consecutive days, with a 24-h break between matches (GGG), and other teams had a 48-h break between second and third matches (GG48hG). The athletes were divided into two groups: 1) Group GGG - three consecutive matches with an interval of 24-h between each one (n = 16), and 2) Group GG48hG - two consecutive matches and an interval of 48-h between the second and third matches (n= 18). Each match lasted 70-min (two halves of 35-min separated by an interval of 15-min). To evaluate the recovery status, Total Quality of Recovery, countermovement jump performance, 10-m sprint performance, and maximum lumbar isometric strength were measured 60-min before each match in this order. Moreover, the internal load of the matches was recorded. The participants were familiarized with the study procedures and anthropometric parameters (weight and height) were assessed on the day before the first match. Figure 1 shows the experimental design of the study for each group.

Environmental conditions

Wet temperature and dry temperature were measured at 20-min intervals using an analog thermo hygrometer (Incoterm, Porto Alegre, Brazil). As a measure of thermal index, the discomfort index was calculated using the following equation: 0.30 dry temperature + 0.75 wet temperature¹⁵.

Recovery perception

Recovery perception was assessed on the Total Quality of Recovery scale¹⁶ by asking the participants to answer the following question: "How do you feel about your recovery now?"¹⁶. Briefly, this scale presents values from "6" (not recovered), up to "20" (totally recovered).

Countermovement jump

The participants performed the countermovement jump on a contact mat (Multi Sprint, Hidrofit[®], Belo Horizonte, Brazil). They were instructed to place their hands on their hips and jump as high as possible within a self-determined range of motion¹⁷. The jump height was given by the Multi Sprint software.

10-m sprint

A 10-m sprint was carried out using two photocells (Multi Sprint, Hidrofit[®], Belo Horizonte, Brazil) placed at the start (0 m) and finish line (10 m). The participants were instructed to run the 10 m as fast as possible. Running speed was given by the Multi Sprint software.

Maximum lumbar isometric strength

The maximum lumbar isometric strength was assessed using an analog dynamometer (Crown Filizola[®], São Paulo, Brazil) with a capacity of 200 kgf, according to the protocol used by Eichinger et al.¹⁸. The participants positioned themselves on the dynamometer platform, with their knees and torso slightly flexed, and the head following the extension of the trunk with their eyes fixed straight ahead. They were instructed to use maximum strength to extend their trunk. Additionally, two attempts at each test were performed with a 2-min rest interval, with the attempt showing the highest performance being considered for statistical analysis.

Match internal load

The internal load of each match was calculated using the product between the session Rating of Perceived Exertion and the total match time in minutes¹⁹. Ten minutes after each match²⁰, the participants were instructed to carry out a global assessment of the effort made in the match based on the CR-10 Rating of Perceived Exertion scale, answering the following question: "How intense was the game?"¹⁹. Briefly, numbers from 0 (rest) to 10 (maximum effort) were used to quantify the effort. The participants reported the number verbally without any contact between them¹⁹. Moreover, monotony was calculated using the ratio between the mean and standard deviation of the internal load for each match¹⁹.

Statistics

The Shapiro Wilk test was used to verify data distribution. Descriptive characteristics are presented as means and standard deviations, unless otherwise noted. Dependent variables (countermovement jump, 10-m sprint, muscle strength, and Total Quality of Recovery) and environmental conditions (dry and wet temperatures, and discomfort index) were analyzed using percent change from baseline. These variables were analyzed using a mixed model ANOVA (2 [GGG,GG48hG] x 3 [match 1, 2, and 3]) with repeated measures. In the case of significant differences, a Tukey post-hoc test was applied. The physical characteristics and baseline dependent variables were evaluated using an independent t-test. In addition, Eta squared (η^2) for group by match interaction was calculated and interpreted as a small effect (< 0.01), small-to-medium effect (0.01 – 0.10), and medium to large (0.10 – 0.25)²¹. Results were considered significant at p< 0.05.



Figure 1. Experimental design of the study. GGG group, 24-h rest interval between each match. GG48hG group, 48-h rest interval between the second and the third matches. (*) Assessment of Total Quality Recovery, countermovement jump performance, 10-m sprint performance, and maximum lumbar isometric strength. (#) Assessment of session Rating of Perceived Exertion.

RESULTS

There was no difference between groups by age, height, body mass and BMI (Table 1). Also, no differences were found between groups for countermovement jump performance, maximum isometric strength, and Total Quality Recovery before the first match (Table 1). The 10-m sprint performance before the first match was higher in the GGG group when compared to the GG48hG group (p= 0.01, Table 1).

Table 2 shows the environmental variables. There was no interaction between group and match factors for the dry temperature (F= 4.5, p= 0.093), wet temperature (F= 3.6, p= 0.13), and discomfort index (F= 4.0, p= 0.11). There was also no group or match effect for the dry temperature (F= 1.36, p= 0.36 and F= 0.11, p= 0.89; respectively), wet temperature (F= 0.61, p= 0.51 and F= 0.07, p= 0.93; respectively), and discomfort index (F= 1.0, p= 0.42 and F= 0.08, p= 0.92; respectively).

There was no interaction between group and match factors for the variation of performance in the countermovement jump (F= 1.28, p= 0.29, η^2 = 0.019), and no differences were observed between groups (F= 2.29, p= 0.14). However, there was an effect for the match factor (F= 11.08, p< 0.001). Both groups showed a reduction in countermovement jump performance in the second match (p= 0.001, Figure 2A).

There was an interaction between group and match factors for the variation of performance in the 10-m sprint (F= 6.37, p= 0.003, η^2 = 0.068) (Figure 2B). A reduction in performance of the GGG group in the third match compared to the first and second matches (p< 0.001) was observed, while the GG48hG group showed no change in performance in the 10-m sprint (p> 0.05). In addition, the GGG group showed a greater reduction in the 10-m sprint in the third match when compared to the GG48hG group (p= 0.007). The variation in the maximum isometric strength test showed no interaction between group and match factors (F= 0.09, p= 0.92, η^2 = 0.001), the effect of the group (F= 0.014, p= 0.91) or the match (F= 2.33, p= 0.11) (Figure 2C).

There was an interaction between the group and match factors in the Total Quality Recovery variation (F= 5.79, p= 0.005, η^2 = 0.081) (Figure 2D). A decrease in the third game in the GGG group (p< 0.01) was observed, while the GG48hG group showed no change (p> 0.05). Additionally, the GGG group presented a greater decrease in Total Quality Recovery in the third match compared to the GG48hG group (p= 0.004).

There was an interaction between group and match factors for the session Rating of Perceived Exertion (F= 5.22, p= 0.008, η^2 = 0.072) (Table 3). The GGG group had a greater value in the second match compared to first (p= 0.021) and third matches (p= 0.013), while the GG48hG group did not show any change throughout the matches (p> 0.05). Moreover, the GGG group reported a higher session Rating of Perceived Exertion in the second match compared to the GG48hG group (p= 0.003). The match time and the internal match load did not show any interaction between the group and match factors (F= 1.26 and p= 0.29; F= 2.69, p= 0.075, η^2 = 0.038; respectively) (Table 3), group effect (F= 0.13 and p= 0.72; F= 0.59 and p= 0.44, respectively) or effect of the match (F= 0.35 and p= 0.71; F= 0.94 and p= 0.39, respectively). Finally, the monotony of the first to the third match was 3.8 AU in the GGG group and 3.3 AU in the GG48hG group.

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Figure 2. (A) Percent change in the countermovement jump performance before each soccer match; (B) Percent change in the 10-m sprint performance before each soccer match; (C) Percent change in the maximal lumbar isometric strength before each soccer match; (D) Percent change in the Total Quality Recovery before each soccer match. GGG group, 24-h rest interval between each match. GG48hG group, 48-h rest interval between the second and third matches. (*) p < 0.05, lower than the first match. (†) p < 0.05, lower than the Second match. (#) p < 0.05, lower than the GG48hG group.

Table 1. Physical characteristics, baseline performance and perceived recovery of the participants of each experimental group.

Variables	Gro	n voluo	
	GGG (n= 16)	GG48hG (n= 18)	h-vaine
Age (Years)	17.9 ± 0.9	17.4 ± 1.1	0.18
Height (cm)	179.3 ± 4.5	177.4 ± 6.4	0.32
Body mass (kg)	69.0 ± 6.3	68.8 ± 6.5	0.94
BMI (kgm-2)	21.3 ± 1.7	21.9 ± 1.7	0.63
Countermovement jump (cm)	33.43 ± 4.06	32.29 ± 5.19	0.49
10-m sprint (s)	1.625 ± 0.213	1.778 ± 0.084*	0.01
Maximal lumbar isometric strength (kgf)	115.2 ± 25.3	130.5 ± 23.1	0.08
Total Quality Recovery (AU)	17 ± 2	18 ± 2	0.34

GGG group, 24-h rest interval between each match. GG48hG group, 48-h rest interval between the second and the third matches. BMI = Body Mass Index; AU = Arbitrary units. * p< 0.05, different from the GGG group.

 Table 2. Mean ± SD of dry temperature, wet temperature, and discomfort index of each experimental group during a U-19 school soccer competition.

Variables	Groups	Matches			
variables		1 st	2 nd	3 rd	
Dry temperature (°C)	GGG	23.0 ± 0.7	29.5 ± 3.5	26 ± 2.8	
	GG48hG	27.8 ± 0.5	23.0 ± 0.7	26 ± 2.8	
Wet temperature (°C)	GGG	16.9 ± 0.5	20.4 ± 2.7	18.8 ± 1.4	
	GG48hG	19.8 ± 0.3	16.9 ± 0.5	18.8 ± 1.4	
Discomfort index (°C)	GGG	19.6 ± 0.6	24.1 ± 3.0	21.9 ± 1.9	
	GG48hG	23.1 ± 0.4	19.6 ± 0.6	21.9 ± 1.9	

group, 24-h rest interval between each match. GG48hG group, 48-h rest interval between the second and third matches.

 Table 3. Mean ± SD of the session Rating of Perceived Exertion, playing time, and internal match load of each experimental group during an U-19 school soccer competition.

Variables	Groups –	Matches		
Variaules		1 st	2 nd	3 rd
Session Rating of Perceived Exertion (AU)	GGG	6.5 ± 2.2†	8.3 ± 1.5	6.4 ± 2.8†
	GG48hG	6.3 ± 1.7	6.1 ± 1.8#	6.9 ± 2.4
Playing time (min)	GGG	65,4 ± 17,1	67,6 ± 12,6	70,9 ± 11,3
	GG48hG	69,6 ± 12,7	70,9 ± 12,7	67,7 ± 17,5
Internal match load (AU)	GGG	442 ± 195	563 ± 144	449 ± 213
	GG48hG	441 ± 152	426 ± 143	480 ± 233

GGG group, 24-h rest interval between each match. GG48hG group, 48-h rest interval between the second and the third matches. AU = Arbitrary units. † p< 0.05, different from the 2nd match. # p< 0.05, different from the GGG group.

DISCUSSION

This cross-sectional study sought to assess the effects of cumulative school soccer matches separated by 24-h and 48-h intervals on recovery status of U-19 players. The initial study hypothesis was partially confirmed, since the 48-h interval between the second and third matches avoided a reduction in the 10-m sprint performance and maintained the recovery perception at similar levels of baseline. Notably, however, a 48-h interval between the second and third games did not provide recovery of the countermovement jump performance. Additionally, the observed effect sizes were small to medium for all variables, except for the maximum lumbar isometric strength, which showed a small effect size.

Considering the high intensity of several actions occurring during soccer matches (e.g., sprints with change of direction, jumps, and kicks)^{1,2,22}, structural changes in sarcomeres take place, triggering an inflammatory response after matches²³⁻²⁵. As a consequence of these processes, decreased physical performance can occur^{3,4}. Thus, several parameters have been used to assess the state of physical recovery, such as physical performance (e.g., isometric contractions, countermovement jump, and sprints), and psychometric scales (e.g., recovery state and muscle pain). Three physical performance tests were currently applied: maximum lumbar isometric strength, countermovement jump and 10-m sprint.

Countermovement jump performance decreased after the first match in both GGG and GG48hG groups, which has also been observed in previous studies²³⁻²⁶. However, both groups maintained a reduced countermovement jump height following the second match, suggesting that a 48-h interval between the second and third matches was not enough to recover this type of performance. This result agrees with previous studies which showed that countermovement jump performance remained reduced 48-h after a soccer match in professional players^{24,27}. Ten meter sprint performance was not reduced 24-h after the first match, which is similar to previous studies that reported a complete recovery in sprint performance within 24-h after a soccer game^{5,26,28}. Notably, the accumulation of matches imposed on the GGG group athletes resulted in an increased time in the 10-m sprint from the second to the third match. On the other hand, no difference was observed for the 10-m sprint time of GG48hG group after the second match. Other studies have shown that a 48-h recovery period is sufficient for sprint performance to return to baseline values in professional soccer players^{25,27}.

Based on previous studies, it was expected a reduction in maximum isometric strength after the first match^{26,27,29}. Interestingly, 24-h seems to have been enough time to recover this variable. A previous study also reported no reduction in the maximum isokinetic strength of the knee extensor and flexor muscles 24-h after an official soccer match in elite players ²⁵. According to a recent meta-analysis⁵, 22 studies have evaluated the recovery of muscle strength after a soccer match by performing tests involving knee extension and flexion. Thus, the isometric strength test used in the present study (i.e., lumbar isometric dynamometry) may have low specificity to assess the physiological stress caused by soccer matches, making it insensitive to changes in muscle strength. Therefore, further analysis of strength recovery by means of dynamic or isometric uni- or multi-joint tests involving the hip and knee joints is recommended⁵.

Recent systematic reviews have shown that subjective recovery parameters (e.g., soreness, fatigue and perception of recovery) are more sensitive for assessing the physiological stress imposed by training sessions or soccer games in comparison to muscle performance parameters (e.g., strength, jumps, etc.)^{5,30}. The cumulative matches caused a reduction in perception of recovery of the athletes from the GGG group. A diminished perception of recovery after a school soccer match was found by Chaves et al.¹¹, and 24-h was not sufficient to return this variable to baseline values. Another study reported similar results with professional soccer players²⁷. On the other hand, Total Quality Recovery was not altered throughout the games in the GG48hG group, which was also observed in elite soccer athletes²⁷. The current results also showed a similar time-frame for recovery between Total Quality Recovery and 10-m sprint performance. An analog response between subjective recovery measures and objective performance measures (e.g., muscle strength) has been reported, as well as biochemical recovery markers (e.g., creatine kinase)⁵. Therefore, it can be hypothesized that 48-h between the second and the third matches produced an improvement in the athletes' physiological state, leading to greater perceived physical readiness.

Internal match load has been used to assess the physiological stress induced by training sessions and competitions. Both GGG and GG48hG groups showed no change in the internal match load over the matches, suggesting that the physiological stress of the matches was not affected by 24-h or 48-h intervals. However, caution is necessary regarding this assumption, since variables related to external load (e.g., distance covered) or objective measures of internal load (e.g., heart rate, substrate availability, blood lactate levels, etc.) were not ascertained. A recent study evaluated four consecutive matches with 24-h interval between each one, with a greater internal load for the fourth match being observed ¹¹. The authors attributed the increase in internal match load to the importance of the match, since it was a semifinal, and the most important games may represent greater stress stimuli and, consequently, greater internal load. Although the competition examined in the present study has a format similar to that investigated by Chaves et al.¹¹, only group phase matches were assessed, which may have influenced the results observed in internal match loading. Additionally, it is important to highlight that both GGG and GG48hG groups showed a monotony above 2 AU (GGG= 3.8 UA, and GG48hG= 3.3 UA). Thus, consecutive soccer matches separated by 24-h or 48-h intervals do not seem to be adequate, as monotony above 2 AU can cause a decrease in muscle performance, and increased incidence of infectious diseases and injuries¹⁹.

The present study has a number of strengths and limitations. External validity of our findings and novelty of the results are strengths. To the best of our knowledge, this is the first study that examined whether a 48-h recovery period between two matches in a school soccer tournament among U-19-year-old players affects muscle recovery. On the other hand, no variable related to game performance (e.g., distance covered and heart rate, etc.) or lower limb strength was measured. Muscle enzymes (e.g., creatine kinase) and biochemical inflammatory markers were also not evaluated, which would have allowed for an assessment of muscle stress and the inflammatory process induced by matches. Evaluating the aforementioned parameters in future research would be important to compare the results of the present study with the hypotheses raised.

CONCLUSION

In summary, the present research is consistent with the literature supporting a longer interval for recovery of U-19 soccer athletes as valuable. Our results showed that a 48-h interval between the second and third matches led to the maintenance of performance in the 10-m sprint and a recovery perception similar to baseline levels. Therefore, a 48-h interval between two matches in an U-19 school soccer tournament seems to minimize the deleterious effects on physical performance caused by playing matches on consecutive days. These findings can be useful for professionals who schedule tournaments in order to make the competition calendar more flexible, as well as for conditioning trainers and coaches to better understand the factors that affect muscle recovery and, consequently, search for methods that can speed up athlete recovery. Future studies are needed to investigate the effect of different tournament calendars (e.g., 72-h interval between matches, or 48-h interval between first and second matches) on physical recovery. The effect of tournaments with congested matches in other sports (i.e., volleyball, handball, basketball) should also be assessed.

COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval

Ethical approval was obtained from the local Human Research Ethics Committee – Federal Institute of Sudeste of Minas Gerais and the protocol (no. 65789717.2.0000.5115) was written in accordance with the standards set by the Declaration of Helsinki.

Conflict of interest statement

The authors have no conflict of interests to declare.

Author Contributions

Conceived and designed the experiments: VHSR, HCMC, JBFJ. Performed the experiments: VHSR, LAS, SFNC, MHAP, JBFJ. Analyzed the data: VHSR, DAB, LAS, MHAP, JBFJ. Contributed reagents/materials/analysis tools: HCMC, DBC, RAPS, CMAC, ABG, JBFJ. Wrote the paper: DAB, LAS, SFNC, MHAP, HCMC, DBC, RAPS, CMAC, ABG.

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