Abstract:
The aim of this study was to analyze the effect of different supplementation conditions on fatigue and performance in flywheel half-squat tests in senior men. Sixteen active males (age: 22.8±4.9 years; body mass index: 23.7±2.4 kg·m⁻²) participated in the intervention during a 4-week period. Four experimental conditions were established using a double-blind design: placebo, caffeine (CAF), beetroot juice (BRJ), and combined BRJ+CAF. To assess the effect of supplementation, participants completed a countermovement jump (CMJ) before (Pre), 30 s after (Post-30s) and 180 s after (Post-180 s) completing a flywheel half-squat exercise protocol (four sets of eight all-out repetitions, with a 3-min inter-set rest, using different inertial loads). Additionally, the mean power output during the flywheel half-squat protocol was recorded. A repeated measures ANOVA showed greater mean power (~1000 W, p<.001) produced in flywheel exercise after the CAF, BRJ and BRJ+CAF consumption compared to the placebo condition. After placebo, CAF and BRJ, CMJ performance at Post-180 s was reduced compared to Pre (p=.003-.087, two-way ANOVA; ES=-0.39/-0.49), although no significant performance reduction (p=.087) was noted after BRJ+CAF. In conclusion, compared to placebo, CAF, BRJ, and BRJ+CAF allow greater total mean power in the flywheel half-squat power test, although without effects on exercise-related fatigue. Additionally, BRJ+CAF improved recovery after a high demanding power-production protocol.

Key words: nutritional ergogenic aids, athletic performance, plyometric exercise, muscle fatigue, resistance training

Introduction
Daily human activities, and specifically sport actions, are characterized by an important strength/power production in both concentric and eccentric movement phases (Bendixen, Lott, Senesac, Mathur, & Vandenborne, 2014). As such, resistance training is considered the key strategy to maximize the individual’s strength/power levels. Flywheel devices have demonstrated to improve muscle growth, maximum dynamic strength and power, during both horizontal and vertical movements (Petře, Wernstål, & Mattsson, 2018). Moreover, flywheel devices allow to reproduce the stretch-shortening cycle of the muscle (Nuñez-Sanchez & Sáez de Villarreal, 2017). Therefore, flywheel devices allow for maximal resistance throughout the whole range of motion both concentric (acceleration) and eccentric (braking) movement phases (Nuñez-Sanchez & Sáez de Villarreal, 2017) during any given muscle action (Petře, et al., 2018). In practical terms, flywheel devices are commonly used by athletes in an attempt to boost performance due to its capability to enhance eccentric load, a relevant factor in the effectiveness of resistance training programs (Maroto-Izquierdo, et al., 2017; Tesch, Fernandez-Gonzalo, & Lundberg, 2017).

Considering the necessity to apply greater magnitudes within resistance training in order to...
get training adaptations at long-term (MacInnis & Gibala, 2017), some nutritional supplements such as caffeine (CAF), creatine, β-alanine, sodium bicarbonate, and beetroot juice (BRJ) incorporated into the athletes’ routines have demonstrated to provide ergogenic effects (Koncic & Tomczyk, 2013). Specifically, acute improvements of concentric and eccentric strength was reported after CAF ingestion (Castillo, Dominguez, Rodriguez-Fernandez, & Raya-Gonzalez, 2019; Grgic, Trexler, Lazinica, & Pedisic, 2018), possibly due to physiological mechanisms, including improved recruitment of motor units and Na⁺-K⁺ pump response (Goldstein, et al., 2010). Similarly, BRJ supplementation demonstrated an increase in peak force and peak power during a maximal voluntary isometric test in mid-thigh muscle (Bender, et al., 2018), possibly due to improvements in the regulation of type II muscle fibers contraction and increased content of Ca²⁺ handling proteins (Stamler & Meissner, 2001). Regarding the power performance related to vertical jump ability, literature has shown acute enhancements of jump performances after consuming CAF, however, no studies have been focused on individual or combined effects of BRJ with CAF (Abian-Vicen, et al., 2014; Tucker, Hargreaves, Clarke, Dale, & Blackwell, 2013; Venier, Grgic, & Mikulic, 2019). Considering the aforementioned relevance of stretch-shortening cycle (SSC) based actions for the sportive performance and the potential benefits of ergogenic supplements, it seems relevant to investigate the likely improvements of individual and combined intakes of CAF and BRJ on the lower limb power performance using exercises which reproduce SSC (i.e. flywheel half-squat and countermovement jump [CMJ]). Also, CAF and BRJ revealed positive physiological effects in different ways; for example, CAF reduced the rating of perceived exertion during exercise (Doherty & Smith, 2005) and BRJ increased the strength muscle contraction (Ferguson, et al., 2015).

From a perspective of the physical performance, it would be relevant to analyze fatigue derived from the application of exercises common in training periodization like the squat, which is one of the most frequently used resistance exercises for strength training in both athletic and rehabilitation settings (Slater & Hart, 2017), as well as the impact of different supplementation conditions on the exercise-induced fatigue (Mielgo-Ayuso, et al., 2019). While traditional methods of resistance training incorporate the use of isometric external loads (e.g. barbells), one of the most commonly implemented methods is the use of rotational resistance (Moras, et al., 2019). Several studies have compared the differences in post- versus pre-exercise in certain key physical performance measures, namely explosive leg power, as an indirect measurement of exercise-related fatigue (Apostolidis, Mougios, Smilios, Rodosthenous, & Hadjicharalambous, 2019). In this sense, CMJ is considered as the most suitable test for neuromuscular fatigue monitoring (Gathercole, Sporer, Stellingwerff, & Sleivert, 2015). Regarding CAF, while the CMJ performance may be increased after its acute consumption, fatigue was not different between CAF and placebo conditions (Hahn, Jagim, Camic, & Andre, 2018; San Juan, et al., 2019; Santos-Mariano, et al., 2019). However, to the authors’ knowledge, no investigation have analyzed muscle fatigue after a high-demand power test when athletes were supplemented with BRJ or combined CAF and BRJ. Likewise, there is scarce literature about the fatigue effects after the performance of a flywheel half-squat protocol, independent of the supplementation condition. Therefore, it could be interesting to apply ergogenic strategies during training sessions in order to increase the training load without a derived increase in fatigue.

Ultimately, a better understanding of flywheel exercise-related fatigue attending to supplementation conditions could facilitate the development of specific ergogenic strategies to ameliorate athletes’ sports performance. Thus, the aim of this study was to analyze the effect of different supplementation conditions (i.e. CAF, BRJ, and their interaction consumption) on the flywheel half-squat fatigue and performance in senior men. Attending to previous literature, the hypothesis is that no exercise-related fatigue, understood as a decrease in vertical jump performance (i.e. CMJ), will be found under different supplementation conditions.

Methods
Design
All experiments were performed under similar standard laboratory environmental conditions (21-23°C) (Lane, et al., 2014), and at the same time of the day (±0.5 h) for minimizing any effect of circadian rhythm on muscular contraction (Martin, Carpentier, Guissard, van Hoecke, & Duchateau, 1999).

In a double-blind, place-controlled, cross-over study design, the participants were involved in a 2-week familiarization (i.e. four sessions) with the exercise protocol and a nutritional orientation to be carried out throughout the whole study duration. During this period, participants performed the adequate technique of exercises during 90 min in each session with the supervision of a strength and conditioning specialist. In the next four weeks, the participants performed four different experiments, involving one exercise-supplementation session per week every Tuesday. Under double-blind procedures, participants completed four trials: i) control trial, consisting of a placebo of both CAF (sucrose) and BRJ (placebo ECO Saludviva), ii) CAF trial, consisting of CAF (six mg·kg⁻¹) and placebo BRJ
(placebo ECO Saludviva), iii) BRJ trial, consisting of BRJ (140 mL) and placebo CAF, and iv) combined CAF + BRJ trial, involving CAF and BRJ supplementation (six mg·kg⁻¹ CAF and 140 mL BRJ). To avoid the effect of execution order, 25% of the participants randomly took a different experimental condition (CAF, BRJ, BRJ+CAF, or placebo).

For assessing the effect of supplementation condition on fatigue and physical performance, during each of the four experiments the participants completed a CMJ before (Pre), 30 s after (Post-30s) and 180 s after (Post-180 s) a flywheel half-squat exercise protocol (Figure 1). In addition, the mean power output during the flywheel half-squat exercise protocol was recorded.

Participants

Sixteen active males (age: 22.8±4.9 years; height: 177±1.0 cm; body mass: 74.4±9.6 kg; body mass index [BMI]: 23.7±2.4 kg·m⁻²) volunteered to participate in this study. The inclusion criteria entailed being physically active with at least one year of resistance training experience, with a frequency of 3.0±1.0 times per week and reported no use of medication or nutritional supplements during the previous three months and during the experimental period. Exclusion criteria were having consumed narcotic and/or psychotropic agents, drugs or stimulants during the test or supplementation period, and being diagnosed with any cardiovascular, metabolic, neurologic, pulmonary, or orthopedic disorder that could limit performance in different tests. Prior to participation, volunteers were informed of the procedures, benefits and risks involved before providing written informed consent and assent. This investigation was performed in accordance with the Declaration of Helsinki, approved by the Ethics Committee (code: Ui-PI008), and met the ethical standards in Sport and Exercise Science Research (Harriss & Atkinson, 2015).

Nutritional intervention protocol

Diet control

Informative session was carried out with the participants in order to give them some nutritional guidelines to ensure that 48 hours before each test session they followed a similar diet consisting of 10% protein, 60% carbohydrates, and 30% lipids. In addition, 24 hours before each experimental session, the participants were instructed to avoid alcohol and those foods and drinks rich in CAF such as tea, coffee, mate, cola drinks, chocolate, energizing drinks, and chocolate drinks. Likewise, because the use of oral antiseptics can prevent increased blood NO₂- levels after the intake of NO₃⁻ by their bactericidal effect on the bacteria in the mouth, participants were asked to refrain from brushing their teeth or using a mouthwash, chewing gum or sweets that could contain a bactericidal substance such as chlorhexidine or xylitol 24 hours prior to the experimental sessions (Hoon, Fornusek, Chapman, & Johnson, 2015). Additionally, the participants were asked to avoid foods with high NO₂- content (i.e. beetroot, celery, turnip, spinach, lettuce, leak, cabbage, parsley, endives). Participants were provided with a list of vegetables they should avoid the day before the study outset. Finally, the last meal was to be eaten by the participants 180 min before each experiment.

Beetroot juice (BRJ) intake. Participants received 140 mL of either nitrate-rich BRJ (Beet It Sport®, James White Drinks, Ipswich, UK) or nitrate-depleted placebo. During two of the four experimental trials the participants received 140 mL of BRJ (two x 70 mL) or placebo (two x 70 mL) two and a half hours prior to the onset of testing, supplied in two unlabeled, 100-ml, brown glass bottle. The placebo was done following the instructions carried out by Garnacho-Castaño et al. (2018). As the blood NO₂⁻ peak occurs two-three hours post-ingestion (Webb, et al., 2008), the lower limb physical performance assessment and exercise protocol started two and a half hours after the BRJ supplementation.

Caffeine (CAF) intake. The CAF beverage (six mg·kg⁻¹) or placebo (sucrose) was dissolved in a 250 mL red bottle, shaken, and chilled in a refrigerator for two hours prior to the participant consumption in the laboratory. The CAF supplement was administered to the participants 35 min before starting the warm-up, to begin the physical performance assessment and exercise protocol alongside peak CAF concentration (Castillo, et al., 2019). Participants were instructed to consume the beverage within five minutes (Hahn, et al., 2018).

Figure 1. Temporal sequence of the supplementation and testing protocol. CMJ: countermovement jump.
Half-squat power test

Participants performed a half-squat power test using a flywheel device (K-Box 4, Exxentric®, Stockholm, Sweden). The flywheel device is considered valid and reliable to assess power output values in inexperienced athletes after two familiarization sessions (Bollinger, et al., 2020). Participants completed four sets of eight all-out repetitions with a rest interval fixed at 3-min, and each set was performed using different inertial loads (i.e. 0.025, 0.050, 0.075, and 0.100 kg·m⁻²). Considering the possible fatigue effects, the participants were randomly divided into either the ascending order (starting with the lighter inertial load of 0.025 kg·m⁻²) or descending order (starting with the higher inertial load of 0.100 kg·m⁻²) group. The protocol of the half-squat power test was described by Castillo et al. (2019). Before starting the half-squat power test, a standardized 10-min warm-up was performed, including cycling, joint mobility and dynamic stretching, as well as a submaximal set of eight repetitions of the half-squat flywheel exercise with an inertial load of 0.050 kg·m⁻². During each repetition, total mean power (W) was recorded by means of an optical receiver (SmartCoach, Europe AB, Stockholm, Sweden) coupled to the flywheel device. The obtained information was then processed using specialized software (SmartCoach Power Encoder, Europe AB, Stockholm, Sweden). In this sense, the data analysis for all variables was performed using the mean of the six repetitions for each set due to the two first ones were used to accelerate the device (Sabido, Hernández-Davó, & Pereyra-Gerber, 2018).

CMJ fatigue assessment

In order to assess potential effects of supplementation protocols on fatigue, the CMJ height was assessed before (Pre), 30 s after (Post-30s) and 180 s after (Post-180s) the flywheel half-squat power test protocol. For the CMJ test, the participants were instructed to perform a downward movement followed by a complete explosive extension of the lower limbs, maintaining their hands on the hip (Sáez de Villarreal, Suarez-Arrones, Requena, Haff, & Ferrete, 2015). The CMJ test is considered as the most suitable test for neuromuscular fatigue monitoring (Gathercole, et al., 2015). Jump height was quantified being recorded using an iPad Air 2 (Frame Rate 120 fps, Apple Inc, Cupertino, CA, USA). The take-off and landing frames from the video were determined using My Jump 2 (Haynes, Bishop, Antrobus, & Brazier, 2019) and flight height was then calculated (Bosco, Luhtanen, & Komi, 1983).

Statistical analysis

Results are presented as mean (M) ± standard deviation (SD). Normal distribution and homogeneity of variances was tested using the Kolmogorov-Smirnov and Levene tests, and statistical parametric techniques were conducted. The repeated measures analysis of variance (ANOVA) with the Bonferroni post-hoc test was used to compare the total mean power among supplementation conditions. The effects of supplementation on CMJ performance were assessed through a two-way repeated-measures ANOVA test for condition (placebo, CAF, BRJ, and BRJ+CAF) and time (Pre, Post-30s, and Post-180s). The percentages of changes (Δ%) among the Pre, Post-30s, and Post-180s in CMJ performance in each supplementation condition was calculated using the following formula:

\[ \Delta\% = \frac{(\text{Pre} - \text{Post})}{\text{Pre}} \times 100 \]

Effect sizes (ES) were calculated using Cohen’s ES to quantify the magnitude of the difference among-group power performances (Cohen, 1988). ES >0.8, between 0.8 and 0.5, between 0.5 and 0.2, and <0.2 were considered large, moderate, small and trivial, respectively (Cohen, 1988). Pearson’s product-moment correlation coefficient (r) was used to examine the relationship between the total mean power and percentage of change in CMJ performance. The following scale of magnitudes was used to interpret the magnitude of the correlation coefficients: <0.1, trivial; 0.1–0.3, small; 0.3–0.5, moderate; 0.5–0.7, large; 0.7–0.9, very large; >0.9, nearly perfect (Hopkins, Marshall, Batterham, & Hanin, 2009). The data analysis was carried out using the Statistical Package for Social Sciences (SPSS 25.0; SPSS Inc., Chicago, IL, USA). Statistical significance was set at p<.05.

Results

In the half-squat power test, greater total mean power was observed after the CAF (5,654.53±1,106.79 W; p<.01), BRJ (5,418.50±1,203.77 W; p<.01) and BRJ+CAF (5,746.08±1,216.58 W; p<.01) supplementation in comparison to the placebo condition (4,526.93±1,077.18 W).

Figure 2 depicts the CMJ performances at Pre, Post-30s, and Post-180s the flywheel half-squat power test protocol in each supplementation condition. No significant differences in CMJ height performance were found between placebo, CAF, BRJ and BRJ+CAF groups at any time point.

The percentages of change among Pre, Post-30s and Post-180s the flywheel half-squat power test protocol in each supplementation condition are presented in Table 1. A significant overall interac-
tion (p<.05) was found between the supplementation condition and time in CMJ performance. A lower CMJ performance (p<.001; ES=-1.15/-1.63) was found at Post-30s compared to Pre in each supplementation condition. Also, a lower CMJ performance (p=.003-0.087; ES=-0.39/-0.49) was found at Post-180s compared to Pre after the placebo, CAF and BRJ supplementation, although not after the BRJ+CAF supplementation (p=.087). In addition, a higher CMJ height performance (p=.000-.001; ES=0.80/1.04) was found at Post-180s compared to Post-30s in each supplementation condition.

Table 2 shows the correlations between total mean power during the flywheel half-squat power test protocol and the percentages of changes in CMJ jump height at Pre, Post-30s and Post-180s in each supplementation condition. No significant (p>.05; r=-0.402-0.296) correlations were found.

**Table 1. Changes in countermovement jump height after the flywheel half-squat protocol under different supplementation conditions**

|        | Pre–Post-30s | Pre–Post-180s | Post-30s–Post-180s |
|--------|--------------|---------------|-------------------|
|        | Δ% (mean±SD) | p; ES          | Δ% (mean±SD)      | p; ES            | Δ% (mean±SD) | p; ES            |
| Placebo| -17.31±7.11  | 0.000; -1.15  | -5.76±6.07        | 0.003; -0.39     | 14.69±11.81   | 0.000; 0.81     |
| CAF    | -18.51±10.06 | 0.000; -1.63  | -5.14±6.89        | 0.029; -0.47     | 17.30±8.86    | 0.000; 1.04     |
| BRJ    | -15.10±8.85  | 0.000; -1.37  | -5.23±5.76        | 0.006; -0.49     | 12.34±9.25    | 0.000; 0.80     |
| CAF+BRJ| -16.65±7.43  | 0.000; -1.25  | -5.33±9.35        | 0.087; -0.41     | 14.10±12.72   | 0.001; 0.86     |

Note. SD: standard deviation; p: level of significance; CAF: caffeine; BRJ: beetroot juice; Δ%: percentages of changes; ES: effect sizes.

**Table 2. Correlations (p; r, interpretation) for total mean power with the percentages of changes (Δ%) at Pre, Post-30s and Post-180s in countermovement jump (CMJ) performance in each supplementation condition**

| CMJ performance (Δ%) | Pre–Post-30s | Pre–Post-180s | Post-30s–Post-180s |
|----------------------|--------------|---------------|-------------------|
|                      | p; r         | p; r          | p; r              |
| Placebo              | p=0.275; r=-0.291, small | p=0.874; r=0.042, trivial | p=0.265; r=0.296, small |
| CAF                  | p=0.751; r=0.086, trivial | p=0.933; r=0.023, trivial | p=0.553; r=0.160, small |
| BRJ                  | p=0.123; r=0.402, moderate | p=0.717; r=0.098, trivial | p=0.380; r=0.222, large |
| CAF+BRJ              | p=0.464; r=0.197, small | p=0.661; r=0.119, small | p=0.409; r=0.222, small |

Note. p=level of significance; CAF: caffeine; BRJ: beetroot juice.

**Discussion and conclusions**

The aim of this study was to analyze the effect of the CAF, BRJ and their interaction consumption on fatigue and performance during flywheel half-squat tests. The main novel results of this investigation showed a higher total mean power after CAF, BRJ and BRJ+CAF compared to the placebo condition. Moreover, BRJ+CAF seems to accelerate the recovery of power-production capability after a fatiguing protocol, with CMJ performance at Post-180s maintained when compared to Pre.

Our results showed that all supplementation conditions failed to improve the CMJ performance compared to the baseline values (placebo). In this sense, Tucker et al. (2013) showed similar results as our study with amateur athletes, observing no effect of CAF on vertical jump test results. Conversely, other studies (Abian, et al., 2015; Venier, et al., 2019) have shown higher vertical jump performances after consuming CAF. For example, Venier et al. (2019) observed an acute enhancement of 3.7% and 3.3% in a vertical-jump height in the squat jump and CMJ, respectively, in resistance-trained men after the consumption of CAF. Also, Abian-Vicen et al. (2014) reported that an energy drink based on CAF significantly increased jump height during the...
CMJ (38.3±4.4 vs 37.5±4.4 cm) in elite badminton players. These differences could be explained by the initial physical level of the participants, because high-trained athletes use to be more sensitive to the CAF supplementation (Grgic, et al., 2018). Another possible explanation may be related to the CAF dose administered since the previous studies showing no effect of CAF supplementation in CMJ height performance used a lower dose compared to our study (three mg·kg⁻¹ vs. six mg·kg⁻¹). On the other hand, no investigation has so far focused on determining the effects of individual BRJ consumption or the one combined with other ergogenic aid on vertical jump performance. Therefore, in the current study no significant differences in CMJ performance was found when, prior to the CMJ trials, an all-out flywheel protocol was performed. Future studies may be developed to understand the acute effects of supplementation conditions on vertical jump performance and on the on-field training/match loads in participants with different competitive levels attending to sex.

Many studies have analyzed the acute effects of ergogenic supplements on athletes’ physical performance recovery after different physical performance tests (Mielgo-Ayuso, et al., 2019). However, this study is the first that analyzed the vertical jump performance as an indirect measure of exercise-related fatigue under four experimental conditions (i.e. CAF, BRJ, BRJ+CAF, or placebo). Our results showed a lower vertical jump height immediately after the flywheel half-squat power test protocol compared to pre-exercise (around six cm), independent of the supplementation protocol. Nevertheless, San Juan et al. (2019) observed an improvement of 2.4 cm in jump height in the CMJ test after the ingestion of caffeine (six mg·kg⁻¹) in Olympic-level boxers after an anaerobic test (i.e. Wingate). Moreover, Hahn et al. (2018) did not indicate significant variations in power indices assessed through vertical jump in recreationally-active males; however, CAF ingestion was beneficial to reduce perceived fatigue during acute anaerobic exercise (i.e. anaerobic sprint running test). Contrary to the two previous cited studies, players supplemented with CAF (six mg·kg⁻¹) decreased CMJ height (1.1 cm) after two simulated soccer-game protocols on a treadmill (Apostolidis, et al., 2019). These contradictory results could be explained by the nature and characteristics of the neuromuscular tests or by the increase in performance in the condition prior to fatigue, seeing the attenuated effect of the administration of CAF in the post assessment. On the other hand, we observed higher CMJ performance (p=.000-.001; ES=0.80/1.04) at Post-180s with respect to Post-30s in each supplementation condition, providing interesting information to coaches in order to apply the subsequent adequate training loads within training session considering the acute effects of a half-squat protocol using flywheel devices.

In addition, our results indicated that when participants were supplemented with CAF, BRJ or BRJ+CAF, they produced the greater total mean power in the flywheel half-squat power test in comparison to the placebo condition. However, this fact did not imply higher exercise-related fatigue (i.e. percentages of change in CMJ) under different supplementation conditions versus the placebo condition. Therefore, athletes were able to produce higher mean power in a flywheel device without accumulating higher fatigue, measured as performance in CMJ 180 s after the power exercise. This was supported by the lack of association (p>.05; r=-0.402-0.296) between the percentage of change in CMJ and the total mean power in each experimental condition. In this sense, athletes achieved higher intensity during the flywheel half-squat power test protocol under the CAF, BRJ, and BRJ+CAF conditions compared to placebo, without accumulating higher fatigue, potentially leading toward greater adaptations at long-term (Maclin & Gibala, 2017). However, high-intensity training may be associated with decreased performance in athletes under poor training programs (i.e. where training principles are violated) (Collete, Kellmann, Ferrauti, Meyer, & Pfeiffer, 2018). Additionally, a novel finding of this study was the faster recovery of vertical jump height at Post-180s after BRJ+CAF, suggesting that combined supplementation might accelerate athlete’s recovery.

This study is not exempted of potential limitations. Firstly, we investigated the lower body musculature only. If current findings are transferable to the upper-body muscles needs to be further investigated. Secondly, our reduced sample size may limit results generalization, particularly to females. Thirdly, we did not assess test-retest reliability of the half-squat test. However, previous research provided good levels. Finally, we only assessed bilateral-vertical jump performance, while most of human body activities are typically unilateral and multi-directional. Future research may investigate the acute effects of supplementation on performance and exercise-related fatigue, including measures of inter-limb neuromuscular asymmetries, which are an important risk factor for sport injuries.

In conclusion, compared to placebo, CAF, BRJ, and BRJ+CAF allow greater total mean power in a flywheel half-squat exercise protocol, without higher exercise-related fatigue. Additionally, CAF+BRJ seems to accelerate recovery after a demanding exercise protocol, since no significant differences in CMJ performances were observed at Post-180s compared to pre-exercise.
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Correspondence to:
Rodrigo Ramirez-Campillo, Ph.D.
Human Performance Laboratory, Department of Physical Activity Sciences
University of Los Lagos, Osorno, Chile
E-mail: r.ramirez@ulagos.cl