1	The effect of training order on neuromuscular, endocrine and mood response to small sided games and							
2	resistance training sessions over a 24-hour period							
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Abstract

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34 Objectives: This study examined the acute effect of small-sided-game (SSG) and resistance training 35 sequence on neuromuscular, endocrine and mood response over a 24-hour (h) period. 36 37 Design: Repeated measures 38 39 Methods: Fourteen semi-professional soccer players performed SSG-training (4vs4 + goalkeepers; 6x7-40 min, 2-min inter-set recovery) followed by resistance training 2h later (back-squat, Romanian deadlift, 41 barbell-hip-thrust; 4x4 repetitions, 4-min inter-set recovery; 85% 1 rep-max) (SSG+RES), and on a 42 separate week reversed the session order (RES+SSG). Physical demands of SSG's were monitored 43 using global positioning systems (GPS) and ratings of perceived exertion (RPE). Countermovement-44 jump (CMJ; peak power output; jump height) and brief assessment of mood were collected before (pre), 45 during (0h) and after (+24h) both protocols. Salivary testosterone and cortisol concentrations were 46 obtained at the same time-points but with the inclusion of a measure immediately prior to the second 47 training session (+2h). 48 49 Results: GPS outputs and RPE were similar between SSG-training during both protocols. Between-50 protocol comparisons revealed no significant differences at +24h in CMJ performance, mood, and 51 endocrine markers. Testosterone was higher at 0h during RES+SSG in comparison to SSG+RES 52 (moderate-effect; $+21.4\pm26.7$ pg·ml⁻¹; p= 0.010), yet was similar between protocols by +2h. 53 54 Conclusions: The order of SSG and resistance training does not appear to influence the physical 55 demands of SSG's with sufficient recovery between two sessions performed on the same day. Session 56 order did not influence neuromuscular, endocrine or mood responses at +24h, however a favourable 57 testosterone response from the resistance first session may enhance neuromuscular performance in the 58 second session of the day. 59 **Key words:** Fatigue, recovery, concurrent training, training prescription. 60 61 62 63

Introduction

Throughout a competitive season, soccer players are required to develop and maintain multiple physical qualities aligned to successful performance, including strength, power, speed, agility, aerobic capacity, and repeat sprint ability, as well as engaging with technical and tactical training. ¹ As limited training time often separates fixtures, the ability to concurrently develop such physical, technical, and tactical qualities is pertinent to success. ² Accordingly, development of multiple physical qualities is often a focus of training, with multiple sessions, each with a differing training focus, often undertaken on the same day. Indeed, a recent survey of professional soccer practitioners highlighted that the majority of resistance training sessions occurred in the afternoon following field-based training. ³

It is well known that the recruitment of high-threshold motor units is necessary for inducing adaptations associated with strength, speed, agility and power. ⁴ Athletes may be less able to perform the movements required to achieve these adaptations if fatigue and muscle damage are present. Therefore, for positive adaptations to occur in the targeted physical qualities, the training stimulus should be applied in an order and spacing that facilitates recovery to a point where players are able to meet the demands of each training session. ⁵ Recent work in soccer has shown that whilst there is an impairment of neuromuscular function immediately after a small-sided game (SSG) training session, there may be a temporary recovery 2-hours later, before a further impairment after 24-hours. ⁶ Therefore it seems that after 2-hours of passive recovery, the physical performance of a second intense neuromuscular training session may not be impaired. However, Sparkes et al., ⁷ also found that performance of a double training day (SSG's followed by resistance training 2-hours later) resulted in *small* impairments of neuromuscular performance, mood score, and endocrine markers in comparison to a single training session day at +24-hours. Whilst this is important for our understanding of the weekly planning of training, it is currently unclear whether changing the training session order would have any influence on performance of the second session of the day or the fatigue response over a 24-hour period.

Previous studies have examined the order effect of concurrent resistance and endurance training, ^{8, 9, 10} and speed and resistance training, ¹¹ and have shown that manipulating the session order can impact adapations, fatigue and recovery markers. Yet to date, no studies have examined the order effect of SSG and resistance training. This represents an important gap in the literature and our practical understanding of how to best manipulate within-day planning, as it is currently unclear what effect this may have on the either the loss or potentiation of performance experienced in the 24-hours following a double training session. Given that multiple daily training sessions are often performed in soccer,³ an understanding of this effect should be considered when designing and implementing soccer training programmes. Therefore, the aim of this study was to compare the effects of training order on the 24-hour fatigue response following a double training day in soccer players.

Methods

This study profiled two training days, one consisting of SSG training followed by resistance training 2-hours later (SSG+RES), and one consisting of resistance training followed by SSG training 2-hours later (RES+SSG). Each experimental protocol was completed over 24-hours on consecutive weeks. The study took place midway through the 2018-19 competitive season with players being given at least 72-hours rest before involvement.

Data are presented from 14 male semi-professional soccer players (age: 22.1 ± 3.1 years, mass: 79.3 ± 12.2 kg, height: 1.80 ± 0.08 m). All players were healthy, injury free and in the maintenance phase of their season. In a typical microcycle, which consisted of 1 game·week⁻¹, players completed two on field training sessions (1.5-2 h each) and one resistance training session (1 h). Ethical approval was granted by the ethics advisory board of Swansea university. Players were informed of the risks and benefits and provided written informed consent prior to participation.

Countermovement jump (CMJ), mood (BAM+ questionnaire) and saliva (testosterone and cortisol concentrations) were collected before (pre), during (0h) and after (+24h) both protocols. Saliva samples

were also collected immediately prior to the second training session (+2h) during both protocols to assess readiness to undertake the second session of the day. On arrival at the training centre (~17:00 h), pre-measures were collected (saliva, BAM+, and CMJ's). The first training session began at ~17:30 h, and immediately post training (0h), saliva, BAM+, and CMJ's were repeated. After 2-hours of passive rest and immediately before the second training session, players repeated the saliva test, before undertaking the second training session which began at ~20:30 h. The following day (+24h; ~17:00 h), players repeated all measures (saliva, BAM+ and CMJ's). The following week, players repeated the procedure but with the training session order reversed. Immediately after the 0h testing during both protocols, players were provided with water, a banana and a protein bar (Energy: 171 kcal, Fats: 3.7 g, Carbohydrate: 20 g, Sugars: 9.3 g, Protein: 14 g) and were instructed to consume only this during the 2-hour period before the next session.

The SSG format used complemented the player's normal training regimes and was similar to previous literature. ^{6, 12, 13} After a standardized five-min warm up, consisting of dynamic stretching and short sprints, players were split into four teams of five by coaching staff. The teams were organized such that playing positions were balanced (e.g., one goalkeeper, defender, winger, midfielder, and striker). The sport surface was a third-generation artificial grass pitch and players wore their normal soccer boots. Players competed against another team for 6-blocks of 7-min (overall work-time: 42-min) with 2-min between each game allowed for players to drink water and passively rest. Pitch size was 24 m by 29 m and full-sized goals with goalkeepers were used; only data from outfield players was collected. Players were allowed unlimited touches of the ball and the aim was to win each individual SSG repetition.

The content of the lower body resistance training session was selected to include exercises the players were familiar with, whilst also being within the guidelines for strength development. ^{11, 14} Specifically, the session consisted of 4-sets of 4-repetitions of the parallel back squat, Romanian dead lift, and barbell hip thrust, all at 85% of 1-repetition maximum (RM), with 4-min recovery between sets and exercises. Each exercise was preceded by 2-sets of 4-repetitions at 50% and 70% of 1-RM as a warm up. Prior to test involvement, each participant performed a 3-RM testing session of all three exercises,

which occurred exactly 1-week prior to testing. Using the 3-RM data, 1-RM was estimated using a prediction equation. ¹⁵ The session was supervised by an accredited strength and conditioning coach to ensure appropriate technique throughout.

A portable force platform (Type 92866AA, Kistler) was used to measure lower body power via a CMJ (with arms akimbo). Two CMJ's were completed after a standardized warm-up. The vertical ground reaction forces were used to assess peak power output (PPO) from previously reported methods. ¹⁶ This data was converted into relative PPO (W·kg⁻¹) by dividing PPO by the player's body mass. Jump height (JH) was calculated by multiplying the velocity at each sampling point by time (0.005 s). It was then defined as the difference between vertical displacement at take-off and maximal vertical displacement. Test-retest reliability (intraclass correlation coefficient) for PPO, and JH were 0.89 and 0.84, respectively. The coefficient of variation (CV) for PPO and JH were 2.3% and 3.2%, respectively.

At all time-points, 2 ml of saliva was collected by passive drool into sterile containers. Saliva samples were stored at -20°C for seven days until assay. After thawing and centrifugation (2000 rpm x 10-minutes), the saliva samples were analysed in duplicate for testosterone and cortisol concentrations using commercial kits (Salimetrics LLC, USA). The minimum detection limit for the testosterone assay was 6.1 pg.ml with an inter-assay CV of 5.8%. The cortisol assay had a detection limit of 0.12 ng.ml with inter-assay CV of 5.5%. Testosterone to cortisol (T/C) ratio was determined by dividing testosterone by cortisol.

Mood state was assessed using a modified version of the brief assessment of mood questionnaire (BAM+). ¹⁷ This 10-item questionnaire is based on the Profile of Mood State assessment and consists of a scale where players mark on a 100-millimetre scale how they feel at that moment in time. Scale anchors ranged from 'not at all' to 'extremely'. The questions assess the following mood adjectives: anger, confusion, depression, fatigue, tension, alertness, confidence, muscle soreness, motivation and sleep quality. The scores were totalled up by giving the 6 unfavourable questions (anger, confusion,

depression, fatigue, tension and muscle soreness) a positive value, and the 4 favourable questions (alertness, confidence, motivation and sleep quality) a negative value. The original total mood score ranged from -40 - 60, before adding 40 to each score so that the scale ranged from 0 - 100, with 0 indicating the best mood and 100 indicating the worst. ^{6,17} The BAM+ questionnaire has been shown to be an effective tool for monitoring the fatigue and recovery cycles in elite athletes. ¹⁷

The physical demands of the SSG's were assessed both objectively and subjectively. Using Borg's CR10 scale, ¹⁸ players were asked to give an RPE on a scale of 1–10. This was obtained 10-min after the end of the SSG's. RPE has been shown to have high correlations (r= 0.75–0.90) with heart rate-based methods of training load across various team sports. ¹⁹ A limitation of the current study is that heart rate was not directly monitored. Time-motion analysis data was collected via 10 Hz GPS units embedded with 100 Hz tri-axial accelerometers (OptimEye X5, Catapult Innovations, Melbourne, Australia), which have shown to hold an acceptable level of reliability and validity when tracking player movements. ²⁰ Each unit was attached to the upper back of players using a specifically designed vest garment. The data was downloaded and processed automatically using Catapult Sports software (Openfield, Catapult Innovations, Melbourne, Australia). The high-speed running (HSR) threshold was defined as the total distance (m) covered at a velocity ≥5.5 m·s⁻¹ and was set in line with previous work in soccer time-motion analysis. ⁶ Player load [PlayerloadTM] is defined as the sum of gravitational forces on the accelerometer in each individual axial plane (anteroposterior, mediolateral and vertical), and has been reported previously in soccer time-motion analysis. ⁶, ²¹

Results are reported as mean ± SD. Data were collated using Microsoft Excel (Microsoft Corporation, US) where descriptive statistics and graphical interpretations were derived. Statistical analysis was carried out using a Statistical Package for the Social Sciences (version 19; SPSS Inc., Chicago, IL) with the significance level set at p<0.05. Following screening of data for normality and homogeneity of variance, the effects of time and order of training were assessed using a two-way (time-point and protocol) repeated measures analysis of variance test. Where significant F values for time or

interaction between protocols were identified (p<0.05), a post hoc pairwise comparison test with Bonferroni correction was applied to determine where the significant differences occurred. Effect sizes (ES), using Cohen's d, were calculated using a custom-made spreadsheet, with the following thresholds for interpretation: trivial < 0.2, $small \ 0.2 - 0.6$, $moderate \ 0.6 - 1.2$, $large \ 1.2 - 2$. ²² A paired T-test was used to determine if there were any significant differences in the physical demands (GPS and RPE) of the SSG's during both protocols.

Results

Physical metrics for total distance (SSG+RES, 4659 ± 611 m; RES+SSG, 4660 ± 583 m), HSR (SSG+RES, 65 ± 16 m; RES+SSG, 58 ± 13 m), PlayerloadTM (SSG+RES, 470 ± 72 AU; RES+SSG, 465 ± 75 AU) and RPE scores (SSG+RES, 7.3 ± 1.0 AU; RES+SSG, 7.6 ± 1.1 AU) were similar between SSG sessions during both protocols (p>0.05).

There was a significant time effect on mood score (F= 4.117, p= 0.028). During the SSG+RES protocol, mood score was significantly increased at 0h (see table 1), before returning to near pre-values at +24h. Mood score did not significantly change from pre-values during RES+SSG (p>0.05). There was no interaction effect between protocols (F= 1.460; p= 0.251). For JH, analysis revealed that there was a significant effect of time (F= 10.986; p= 0.000). During RES+SSG, JH was significantly reduced at 0h (see table 1), before returning to near pre-values again at +24h. Analysis revealed there was no significant interaction effects between protocols (F= 4.122; p= 0.052). For PPO, there was a significant effect of both time (F= 5.877; p= 0.008), and interaction between protocols (F= 5.695; p= 0.009). Post hoc analyses revealed that during RES+SSG, PPO was significantly impaired at 0h, before returning to near pre-values at +24h (see table 1). PPO remained similar to pre-values during SSG+RES. Further analyses revealed significantly reduced PPO at 0h during RES+SSG in comparison to SSG+RES, however these differences were similar at +24h (see figure 1 and table 1).

*** TABLE 1***

Analysis revealed that there was a significant time effect on testosterone (F = 5.471, p = 0.003), whereby during both protocols, concentrations remained similar to pre-values at all time-points with the exception of +2h (see table 2). There was a significant interaction between protocols for testosterone (F= 5.196, p= 0.004), where further analysis revealed that there was a greater elevation in testosterone at 0h during RES+SSG in comparison to SSG+RES (see figure 1 and table 2). Both protocols had a significant time effect on cortisol (F=11.665; p=0.000) and the T/C ratio (F=15.333; p=0.000). Further analyses revealed that during both protocols, cortisol concentrations remained similar to pre-values at all time-points with the exception of +2h (see table 2). There were no significant interaction effects between protocols for both cortisol (F = 0.814; p = 0.494) and the T/C ratio (F = 0.877; p = 0.462).

239 *****TABLE 2** ***

241 *****FIGURE 1** ***

Discussion

To our knowledge, this is the first study to examine the influence of manipulating the order of SSG and resistance training on acute neuromuscular, endocrine and mood responses over a 24-hour period. The primary study findings was that while comparisons between the two training days revealed significant differences in PPO, testosterone, and cortisol on the same day, there were no significant differences between protocols after a 24-hour recovery period. A secondary finding was that the order of resistance and SSG training did not appear to affect the objective or subjective physical demands of the SSG's.

The current study found that the GPS and RPE outputs of the SSG's were similar between protocols, suggesting that physical performance and intensity of SSG's is not dampened when preceded by a resistance training session earlier in the day. Therefore, it seems likely that in well-trained athletes, the +2h time-point represents a time-frame prior to the initiation of inflammatory process but after metabolic recovery, during which the athlete can undertake additional training. ^{6,23,24} This supports previous work

which reported performance of a speed training protocol was maintained 2-hours after a resistance training session in academy rugby union players. ¹¹

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Both measures of neuromuscular function (PPO and JH) decreased immediately (0h) after the resistance session during RES+SSG but not the SSG session during SSG+RES (see table 1 and figure 1). It may seem curious that the SSG's did not significantly impair both jump variables immediately in this study, however the *small* decreases in JH were similar to previous work with exactly the same SSG protocol in professional soccer players. ⁶ Whilst peripheral fatigue may result from simultaneous failure at a number of sites, for a specific task such as a CMJ, a particular site may be primarily responsible for a loss in muscle force production, a concept referred to as task dependency fatigue. ²⁵ Due to the exercise selection in the current study, specifically the back squat, it could be that the targeted musculature shares similar movement patterns to a CMJ, therefore accumulated more task dependant fatigue than the SSG session, which was primarily running, cutting, tackling and kicking. Secondly, it is well known that repetitive high-force activities are a primary source of peripheral fatigue, therefore it is possible that the greater intensity of the muscle contractions in the resistance training session (85% 1-RM) resulted in greater neuromuscular fatigue than the SSG's. However, by +24h, there were no significant differences between protocols, suggesting that the order of SSG and resistance training does not influence the neuromuscular response at 24-hours post. Immediately after the first session during both protocols, testosterone, cortisol and the T/C ratio did not significantly change from pre-values. However, one interesting finding is that comparisons between protocols showed that the changes in testosterone were *moderately* and significantly higher at 0h after the resistance session in comparison to the SSG session (see table 2 and figure 1). This supports previous literature suggesting that performance of a resistance training session may alleviate the normal circadian declines in testosterone throughout the day. ²⁶ Given that previous work has observed this effect of

morning strength training on afternoon performance, ²⁶ it is interesting that we may see this pattern in

the current study considering the time that the sessions were performed (17:30 and 20:30 hours).

Considering the evidence that changes in testosterone concentrations can moderate or support the

performance capacity of the neuromuscular system through various short-term mechanisms (e.g. second messenger signalling, lipid/protein pathways, neural activity, behaviour, cognition, motor system function, muscle properties and energy metabolism),²⁷ altering this rate of decline may potentially create an environment later in the day when the ability to generate strength, speed and power is enhanced. ¹¹, ^{26,28} By +24h, testosterone had returned to near pre-values in both protocols (table 2 and figure 1).

Conclusion

In summary, session order did not significantly influence neuromuscular, endocrine or mood responses at +24h, however a favourable testosterone response from the resistance first session could potentially enhance neuromuscular performance in the second session of the day. Additionally, the order of SSG and resistance training sessions does not appear to influence the perceived effort or physical demands of SSG's, when sufficient recovery is given between two sessions performed on the same day.

Practical implications

- Those responsible for designing concurrent training programs should consider allowing sufficient recovery (i.e ≥2 hours) between sessions when programming multiple daily training sessions.
- The order of small-sided games and resistance training does not appear to influence fatigue and recovery markers on the following training day (+24h).
- Prescribing a resistance training session earlier in the training day could alleviate the circadian
 decline in testosterone production, which could contribute to a maintenance in performance of
 a second training session later in the day.

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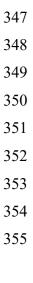
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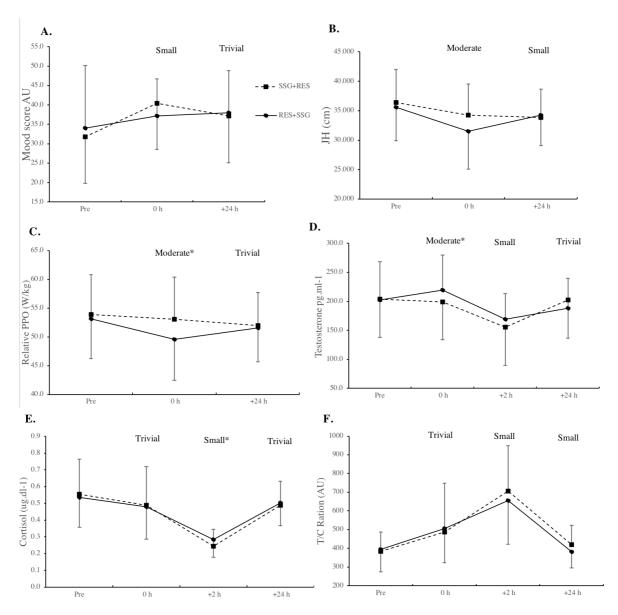
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Figure Legends

- Figures 1 A-F. Mean±SD mood (A), jump height (JH) (B), relative peak power output (PPO) (C),
- testosterone (D), cortisol (E) and testosterone to cortisol ratio (T/C) (F) responses to each protocol
- 341 (SSG+RES vs RES+SSG). Effect sizes are shown above the figure for the between protocol differences
- between each time point and pre-values. Asterisk (*) indicates a significant difference between
- 343 protocols.

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Figures 1 A-F. Mean±SD mood (A), jump height (JH) (B), relative peak power output (PPO) (C), testosterone (D), cortisol (E) and testosterone to cortisol ratio (T/C) (F) responses to each protocol

(SSG+RES vs RES+SSG). Effect sizes are shown above the figure for the between protocol differences
 between each time-point and pre-values. Asterisk (*) indicates a significant difference between trials.
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Table 1. Mean (\pm SD) fatigue marker changes between time-points. Statistical inferences (p values and effect sizes) are shown for both the within and between protocol differences (SSG+RES vs RES+SSG).

		Time-point							
Variable		Pre – 0h	p value	d	Pre – 24h	p value	d		
Mood Score (AU)	SSG+RES	8.6 ± 9.1	0.011	0.72 (M)	5.3 ± 11.1	0.291	0.44 (S)		
Wood Scole (AU)	RES+SSG	3.2 ± 11.4	0.930	0.24(S)	4.0 ± 8.5	0.316	0.29(S)		
	Protocol difference	-5.3 ± 11.2	0.098	0.52 (S)	-1.4 ± 14.8	0.738	0.14 (T)		
	SSG+RES	-2.2 ± 3.1	0.061	0.4 (S)	-2.6 ± 4.9	0.210	0.49 (S)		
JH (cm)	RES+SSG	-4.1 ± 2.6	0.000	0.67 (M)	-1.3 ± 2.0	0.075	0.25 (S)		
	Protocol difference	-1.9 ± 3.3	0.052	0.68 (M)	1.2 ± 5.4	0.408	0.33 (S)		
	SSG+RES	-0.84 ± 2.75	0.836	0.12 (T)	-1.95 ± 3.81	0.233	0.31 (S)		
CMJ Relative PPO (W·Kg ⁻¹)	RES+SSG	-3.53 ± 2.48	0.000	0.50 (S)	-1.56 ± 2.30	0.075	0.25 (S)		
, <i>2</i> /	Protocol difference	-2.69 ± 3.30	0.009	1.03 (M)	-0.37 ± 4.19	0.747	0.12 (T)		

SSG+RES, Small-sided games followed by resistance training, RES+SSG, resistance training followed by small-sided games

SD, standard deviation; SSG, small-sided game; RES, resistance training; AU, arbitrary units; ES, effect size.

Effect sizes (ES, d); T, trivial; S, small; M, moderate.

Table 2. Mean (\pm SD) endocrine marker changes between time-points. Statistical inferences (p values and effect sizes) are shown for both the within and between protocol differences (SSG+RES vs RES+SSG).

	Time-point										
Variable		Pre – 0h	p value	d	Pre – 2h	p value	d	Pre – 24h	p value	d	
Testosterone (pg.ml ⁻¹)	SSG+RES RES+SSG Protocol difference	-4.4 ± 32.5 17.0 ± 25.3 21.4 ± 26.7	1.000 0.157 0.010	0.07 (T) 0.27 (S) 0.73 (M)	-48.0 ± 35.9 -33.2 ± 34.3 14.9 ± 27.6	0.001 0.019 0.065	0.89(M) 0.59 (S) 0.42 (S)	-1.3 ± 71.8 -14.0 ± 62.0 -12.7 ± 32.4	1.000 1.000 0.166	0.02 (T) 0.24 (S) 0.19 (T)	
Cortisol (ug.dl ⁻¹)	SSG+RES RES+SSG Protocol difference	-0.066 ± 0.279 -0.057 ± 0.217 0.009 ± 0.175	1.000 1.000 0.845	0.30 (S) 0.31 (S) 0.04 (T)	-0.310 ± 0.192 -0.251 ± 0.178 0.059 ± 0.100	0.000 0.001 0.052	1.89 (L) 1.72 (L) 0.32 (S)	-0.065 ± 0.208 -0.033 ± 0.173 0.032 ± 0.104	1.000 1.000 0.264	0.36 (S) 0.21 (S) 0.17 (T)	
T/C Ratio (AU)	SSG+RES RES+SSG Protocol difference	102.6 ± 216.9 112.9 ± 115.0 10.4 ± 170.5	0.602 0.017 0.823	0.52 (S) 0.73 (M) 0.06 (T)	322.1 ± 237.7 261.8 ± 232.4 -60.4 ± 212.8	0.001 0.006 0.308	1.73 (L) 1.41 (L) 0.26 (S)	35.7 ± 117.7 -11.0 ± 98.6 -46.6 ± 109.2	1.000 1.000 0.134	0.35 (S) 0.10 (T) 0.43 (S)	

SSG+RES, Small-sided games followed by resistance training, RES+SSG, resistance training followed by small-sided games

SD, standard deviation; SSG, small-sided game; RES, resistance training; AU, arbitrary units; ES, effect size.

Effect sizes (ES, d); T, trivial; S, small; M, moderate; L, large.