

IDENTIFICATION OF THE OPTIMAL HIIT PROTOCOL FOR FATIGUE RESISTANCE IN ADOLESCENT ATHLETES: A RANDOMIZED CONTROLLED TRIAL

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Abstract:

The combat sports athletes developed great gains in both muscular function and fatigue resistance by utilizing high-intensity interval training (HIIT). However, it has not been investigated fully whether different work-to-rest ratios of HIIT show the effectiveness on muscle function in adolescent athletes. The purpose of the study was to compare different work-to-rest ratios by applying different rest times in response to the identical work time during HIIT on muscle function in adolescent taekwondo athletes. Forty-seven adolescent male taekwondo athletes (mean age: 16.7±0.8 years) were randomly assigned to the control group (n=11) vs. three HIIT groups by work-to-rest ratios; (1) 1:2 [30s:60s] (n=12), (2) 1:4 [30s:120s] (n=12), and (3) 1:8 [30s:240s] (n=12). All groups completed 10 experimental sessions over four weeks, while the control group maintained their regular taekwondo training. Muscular functions were measured by assessing isokinetic muscle strength and endurance of the knee extensor and flexor. The participants performed three sets of twenty maximal extension and flexion contractions at 120°s⁻¹ with a 1-min interval between the sets for fatigue resistance. Blood samples were collected to measure free-testosterone, cortisol, creatine kinase, and urea as stress-to-recovery indicators. A positive effect on improving muscle fatigue resistance was observed at the first set of assessments in the HIIT with 1:4 (Δ 10.2%, $p < .05$) and 1:8 groups (Δ 8.6%, $p < .05$). Additionally, the 1:4 group exhibited fatigue resistance improvement in the second set (Δ 7.7%, $p < .01$) without any changes of stress-to-recovery indicators, while the other groups did not show any improvement. The 30s all-out work with 120s rest time, lasting over a brief 4-week period, improved participants' fatigue resistance. A certain amount of rest time between high-intense movements is required to optimize muscle development in adolescent athletes compared with insufficient rest time.

Key words: HIIT, muscle function, stress-to-recovery indicator, optimal protocol, adolescent athlete

Introduction

High-intensity interval training (HIIT) has become increasingly popular in the field of strength and conditioning training because of its time-efficient improvements on athletic performance, specifically muscle function (Engel, Ackermann, Chtourou, & Sperlich, 2018). Previous literature has shown that HIIT increases the rate of large motor unit recruitment through the alteration of motor unit properties (i.e., discharge rate, discharge rate vari-

ability, and recruitment threshold) (Kinnunen, Piitulainen, & Piirainen, 2019). The increase in rate of force development is associated with the development of type II muscle fibers, and it is evidenced that HIIT increases the number of type II muscle fibers in well-trained athletes (Kohn, Essén-Gustavsson, & Myburgh, 2011). It is also postulated that enhancement in athletic performance with HIIT stems from an increase in ATP content and glycogen storage, and/or from a decrease in the

accumulation of hydrogen ions (H^+) and lactate in skeletal muscle (Bishop, Edge, Thomas, & Mercier, 2008). Despite its merit, it is unknown if there is an optimal combination of working and resting time (i.e., work-to-rest ratio) for the muscle function and fatigue resistance described above (Seo, Lee, Jung, Jung, & Song, 2019).

Previous studies have compared various work-to-rest ratios of HIIT in an attempt to find protocols yielding the greatest improvements in muscle function. Lloyd Jones, Morris, and Jakeman (2017) investigated the effect of three different work-to-rest ratios of sprint training on exercise performance in physically active. The study found that 10×6 seconds "all-out" sprints on a cycle ergometer with a 1:8, 1:10, or 1:12 work-to-rest ratio demonstrated similar improvements on peak power, with no differences among the protocols. Moreover, Ouergui et al. (2020) reported that 10×35 m sprint running for four weeks could not improve the neuromuscular performance in adolescent taekwondo athlete and Ojeda-Aravena et al. (2021) did not show any change in physical performance utilizing a 4-week HIIT in taekwondo athletes. However, Seo et al. (2019) demonstrated that the protocol involving a 30s all-out sprint running with a 120s recovery time led to better aerobic and anaerobic capacity developments during the HIIT in adolescent athletes compared with 60s and 240s. The studies highlight that a certain amount of rest time can be a primary driver of generating optimal improvement in physical performance.

Neural and muscular mechanisms are responsible for muscle fatigue, impairing exercise performance as evidenced by reductions in speed and power output (Girard, Mendez-Villanueva, & Bishop, 2011). HIIT-induced neuromuscular adaptations may increase fatigue tolerance (or resistance), thereby increasing the ability to endure the extreme physiological and/or psychological stress associated with this training (Carroll, Taylor, & Gandevia, 2017; Reilly, Drust, & Clarke, 2008). Consequently, HIIT increases central fatigue tolerance and develops exercise performance through improvements in the neuromuscular system. As a result, the capacity to endure the extreme physiological and/or psychological stress is improved through being affected during the continued high-intensity movements (O'Leary, Collett, Howells, & Morris, 2017). However, the relationship between fatigue resistance and various HIIT protocols has not been studied previously. taekwondo competition is an official Olympic sport that consists of intermittent attacks and defenses executed by high-intensity movements such as kicks and punches. Particularly, muscle fatigue resistance is an important variable affecting the competition success in taekwondo athletes. Examining the muscle fatigue

resistance adaptations in response to various HIIT protocols can reveal an optimal HIIT strategy for adolescent taekwondo athletes.

Optimal balance between training load and recovery is crucial for enhancing maximal athletic performance (Siegl, et al., 2017). Stress-to-recovery indicators are valuable surrogate serum markers for evaluating the physiological response associated with training-induced fatigue because of their high accuracy and precision (Hecksteden, et al., 2016). These surrogate markers can also help to minimize the risk of overtraining syndrome or injury.

HIIT has been shown to positively impact athletic performance in various sports athletes; however, there is a lack of research that has examined the effects of different HIIT protocols during in-season training on muscle function in adolescent taekwondo athletes. We hypothesized that although muscle function and fatigue resistance would improve in all HIIT groups, the 30s of sprint running with 120s of active recovery time, compared with 60s and 240s of recovery time, would provide greater improvements in muscle function after the intervention (Seo, et al., 2019). The aim of the present study was to compare the effects of different work-to-rest ratios on muscle function and fatigue resistance in adolescent taekwondo athletes by applying different recovery times in response to the identical exercise time during HIIT.

Methods

Participants

In the present study, 55 adolescent male taekwondo athletes, aged 15-18 years (mean age 16.7 ± 0.8 years), were recruited from South Korea. They had been regularly performing both strength and conditioning programs and skill and technique training at least five times a week (15 to 21 hours/week) for a minimum of three years. The inclusion criteria for the present study were the following: (1) no history of medical conditions, medications use, and disease states, and (2) no injuries experienced in the last six months. All participants were affiliated with the Korea Taekwondo Association. A power analysis using G*Power program 3.1.9.2 (Düsseldorf, Germany) was used to determine the sample size required to detect within- and between-factor differences for a repeated-measures ANOVA. With an estimated power of 0.80 and alpha of 0.05, a total sample of 40 was required to detect a medium effect size (effect size of $f = .33$). Written informed consent was obtained from the participants, their legal guardians, and coaches after the study procedure, withdrawal process, potential risks, and benefits had carefully been explained to them. The present study was carried out following The Code of Ethics

of the World Medical Association (Declaration of Helsinki) and approved by the Institutional Review Board at Kyung Hee University (KHSIRB-17-39).

Randomized controlled trial (RCT)

Fifty-five adolescent athletes initially participated for the present study and were randomly assigned to the following four groups (Figure 1): (1) 1:2 (30s:60s) group (n=14), (2) 1:4 (30s:120s) group (n=14), (3) 1:8 (30s:240s) group (n=14), and (4) the control group (CON, n=13). However, eight participants dropped out during the intervention period due to personal reasons and injuries that were not due to the applied HIIT program. Note that four participants were excluded due to the quadriceps or ankle strains during the simulated taekwondo sparring session. Eventually, forty-seven participants completed the present study.

Training intervention

A certified strength and conditioning specialist (CSCS) from the National Strength and Conditioning Association conducted the HIIT program. Table 1 details the HIIT program. The HIIT

program was implemented in ten sessions over four weeks: two times in the 1st and the 3rd week, and three times in the 2nd and the 4th week. All participants in the 1:2, 1:4 and 1:8 work:rest ratio groups performed either six for week 1 and 2 or eight repetitions for week 3 and 4 of 30s all-out sprint exercise, and rested between repetitions for 60s, 120s, and 240s, respectively. Participants had their heart rates recorded during training sessions (Polar RS400, Polar Electro Oy, Kempele, Finland) while performing a sprint running on the track. Heart rate monitors were set to record heart rate at five-second intervals. The HIIT intensity was aiming at achieving 90% or higher than individual HR_{max}, interspersed with rest periods of walking (active recovery). The CSCS verbally encouraged each participant to sprint at an “all-out” effort. In addition, each participant completed a taekwondo technique and skill training program along with their regular in-season training program. The taekwondo technique and skill training, led by a coaching staff, included 20 sessions over four weeks and was executed at the light to moderate intensity for 2.0 to 2.5 hours.

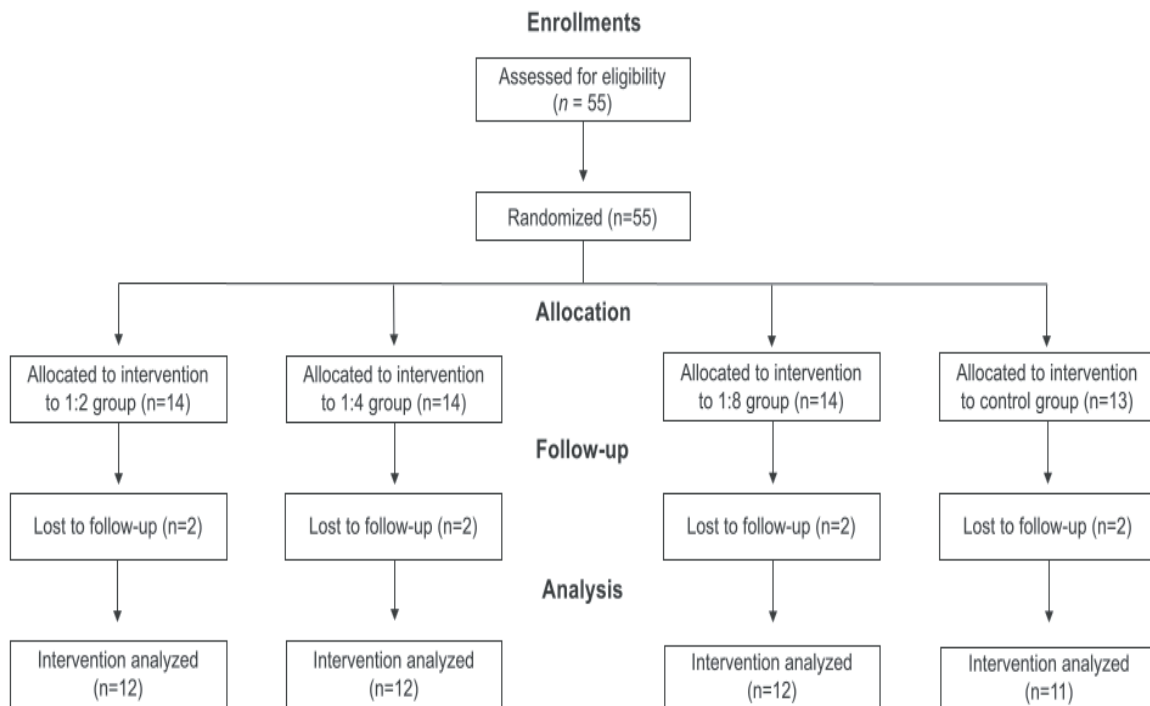


Figure 1. Flow chart of the study.

Table 1. HIIT protocol

Exercise type	Weeks	Intensity	Reps	Total work time	Total volume		
					1:2 group (30s:60s)	1:4 group (30s:120s)	1:8 group (30s:240s)
30-s running	1-2	All-out	6	3min	9min	15min	27min
sprint	3-4		8	4min	12min	20min	36min

Anthropometric measurements and body composition

Participants' standing body height and weight were measured to the nearest 0.1 cm using a stadiometer (T.K.K. Takei Scientific Ins Co., Tokyo, Japan) and balance beam scale (Seca 841, GmbH & Co. KG, Hamburg, Germany) to the nearest 0.1 kg, respectively. Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m²). Body composition variables (i.e., % body fat, fat tissue, and lean tissue) were measured by dual X-ray absorptiometry (DXA: QDR-4500W, Hologic, Marlborough, MA, USA). All participants were measured while wearing light clothing, barefoot, and after removing all metal from their person in a whole-body scan. The coefficient of variance of scanning was 1.5 % or less, which was in accordance with that indicated by the manufacturer. The reliability of the repeated measurements and intra-class correlation coefficient (ICC) was 0.99.

Muscle function

Muscle function was assessed by isokinetic muscle strength and endurance of the quadriceps (knee extensor) and hamstrings (knee flexor) with an isokinetic dynamometer (Cybex Humac Norm Model 770, Computer Sports Medicine Inc., New York, NY, USA). Prior to the measurement, the chair rotation scale, dynamometer rotation scale, and morale scale-secure chair were adjusted according to participants' preferences. Participants performed a full range of motion for peak torque with five maximal effort contractions at 60°·s⁻¹. Total work done was measured with twenty maximal effort contractions at 180°·s⁻¹. The data were normalized with each participant's body mass and calculated as peak torque and total work done ([Nm] ÷ [kg]).

Fatigue resistance

The fatigue resistance was assessed using an isokinetic dynamometer (Dipla, et al., 2009). The participant performed three sets of twenty maximal extensions and flexion contraction at 120°·s⁻¹ with 1-minute intervals, and the researcher provided verbal feedback of encouragement. Only the dominant leg of preference was assessed. Total work done was calculated as the sum of work done during the three sets, and each set was recorded. Rating of perceived exertion (Borg scale; baseline RPE: 17.0±1.16, post-test RPE: 16.7±6.77) and heart rate were recorded at baseline and post-tests of each set (baseline peak heart rate: 155.1±14.05 bpm, post-test peak heart rate: 155.1±13.06 bpm). Blood samples for lactate concentration (mmol/L) were collected from fingertip with strips (Accutrend® lactate, Roche Diagnostics, Mannheim, Germany) by Accutrend® Plus (Roche Diagnostics, Mannheim Germany), before, immediately after, and 5-min

and 10 min after the rest (baseline peak lactate: 8.9 mmol/L, post-test peak lactate: 8.6 mmol/L). The reliability of the repeated measurements assessed by the ICCs ranged from 0.92 to 0.97.

Stress-to-recovery biomarkers

Fasting venous blood samples were obtained at the beginning of baseline, after two weeks (i.e., mid-term test), and three days after the intervention period. We performed an additional analysis for a blood sample at mid-term period in order to identify the changes between exercise load changes (number of repetitions, weeks 1 to 2: six times; weeks 3 to 4: eight times) in HIIT programs. Participants arrived at the laboratory at a standardized time (between 08:00 and 08:30) after a 12 hour overnight fast and avoided moderate to vigorous physical activity or training 48 hours after training. Fasting venous blood samples (5 ml) were taken by a medical laboratory technician from the left arm's antecubital vein area and separated into individual serum separator tubes. Participants rested in the seated position for 10 to 15 min before blood collection. The clotted blood was separated using centrifugation at 3000 rpm for 15 min and was stored at -80°C in a mechanical freezer for later analysis. The blood-borne markers assays were performed at the national committee for experiments in the clinic laboratory (Green Cross Lab Cell, certified by the Korea laboratory accreditation scheme, South Korea). Stress-to-recovery biomarkers, including FT, C, CK, and urea U, were analyzed. FT was analyzed by an automatic radioimmunoassay (RIA) analyzer system (R counter, Packard, Meriden, USA) with free Testosterone RIA CT kit (Asbach Medical Products, Obrigheim, Germany). C was assessed using an automatic ECLIA (Electro-chemiluminescence Immunoassay) analyzer (Cobas 8000, Roche Diagnostics, Mannheim, Germany) with Cortisol II kit (Roche Diagnostics, Mannheim, Germany). CK, a marker of muscle damage, was analyzed with a creatine kinase kit (Roche Diagnostics, Mannheim, Germany) and estimated on an auto-analyzer (Roche Diagnostics, Roche Cobas 8000 Modular, Mannheim, Germany), which is a UV (ultraviolet) assay. U was analyzed with a UREAL kit (Roche Diagnostics, Mannheim Germany), a kinetic UV assay. The inter- and intra-class coefficient of variance was between 0.2 and 4.8%.

Statistical analyses

The data were analyzed using SPSS, version 26 for Windows (SPSS Inc, Chicago, IL, USA). All data were presented as means (M), standard deviations (SD), and 95% confidence interval (CI). Repeated measures ANOVAs were used to assess the interaction effect for the group by the time. In addition, one-way repeated-measures ANOVAs

with Bonferroni correction and dependent *t*-test were used to compare within-group values. If there were any significant differences in baseline variables, repeated measures ANCOVAs were conducted adjusted for baseline covariate. Effect sizes were calculated as dependent Cohen's *d* and partial eta-squared values. The statistical significance level was set at 0.05.

Results

Anthropometric measurements and body composition

Table 2 summarizes the baseline characteristics of the participants. There were no significant differences in height, weight, and BMI at baseline among the four groups. Table 3 displays changes for body composition of the participants during four weeks of HIIT training. There were no significant interaction effects for the group by time in body weight,

percent body fat, fat tissue, and lean tissue during the training period.

Muscle function and fatigue resistance

There were no significant group by time interaction effects for all groups in the lower limb muscle strength and endurance (Tables 4 and 5). However, repeated-measure ANOVA confirmed significant interaction effects in the fatigue resistance (Figure 2). There was a significant interaction effect for group by time on the first ($p < .05$, $\eta^2_p = .45$) and second set in fatigue resistance ($p < .05$, $\eta^2_p = .50$). The first set of fatigue resistance increased significantly in the 1:4 and 1:8 groups. However, the second set of fatigue resistance improved significantly in the 1:4 group only, while the 1:2, 1:8, and CON groups did not show any significant changes (Figure 3). No significant interaction effect for the group by time on the third set in fatigue resistance was found.

Table 2. Baseline characteristics of the participants

Variables	1:2 group (n=12)	1:4 group (n=12)	1:8 group (n=12)	Control group (n=11)	<i>p</i> -value
Age (years)	16.7±0.78	16.9±0.67	16.5±0.90	16.5±1.04	.64
Body height (cm)	174.7±7.10	176.1±6.98	175.4±5.36	174.7±4.64	.94
Body weight (kg)	66.3±11.80	66.4±12.51	65.4±6.04	66.7±11.71	.99
BMI (kg·m ⁻²)	21.7±2.99	21.3±2.89	21.3±1.27	21.8±2.88	.95

Note. Values are expressed as mean ± SD. BMI – body mass index.

Table 3. Change in body weight and body composition between baseline and post-test in adolescent athletes

Variables	Group	Baseline (95% CI)	Post (95% CI)	Cohen's <i>d</i>	Group (ES)	Time (ES)	Group × Time (ES)
Body weight (kg)	1:2	66.3±11.80 (58.8-73.8)	66.1±11.22 (59.0-73.3)	0.15			
	1:4	66.4±12.51 (58.5-74.4)	66.3±12.11 (58.6-74.0)	0.16	0.06 (0.06)	0.85 (0.14)	2.59 (0.43)
	1:8	65.4±6.04 (61.6-69.3)	65.4±6.13 (61.5-69.3)	0.06			
	CON	66.7±11.71 (58.8-74.6)	67.7±11.63 (59.9-75.5)	0.56			
Percent body fat (%)	1:2	12.6±4.74 (9.5-15.6)	12.3±5.10 (9.1-15.5)	0.29			
	1:4	11.6±2.46 (10.1-13.2)	11.1±2.36 (9.6-12.6)	0.94	1.06 (0.27)	5.71* (0.36)	1.45 (0.32)
	1:8	13.7±3.03 (11.8-15.6)	13.8±2.39 (12.3-15.3)	0.10			
	CON	13.6±3.71 (58.8-74.6)	13.1±3.59 (10.8-15.8)	0.72			
Fat tissue (kg)	1:2	8.7±5.61 (5.2-12.3)	8.6±5.58 (5.1-12.2)	0.23			
	1:4	7.9±2.86 (6.1-9.7)	7.6±2.71 (5.9-9.3)	0.59	0.30 (0.15)	2.39 (0.24)	0.27 (0.14)
	1:8	9.0±2.32 (7.5-10.5)	8.9±2.17 (7.6-10.3)	0.04			
	CON	9.2±4.29 (6.4-12.1)	9.1±4.17 (6.3-11.9)	0.27			
Lean tissue (kg)	1:2	54.8±6.57 (50.6-59.0)	55.5±6.30 (51.5-59.5)	0.48			
	1:4	56.0±9.77 (49.8-62.2)	56.8±9.21 (50.9-62.7)	0.51	0.17 (0.11)	14.50*** (0.58)	0.01 (0.03)
	1:8	53.8±5.07 (50.6-57.0)	54.5±5.75 (50.9-58.2)	0.61			
	CON	55.3±7.97 (49.9-60.6)	56.0±8.12 (50.5-61.5)	0.63			

Note. Values are expressed as mean ± SD. ES – partial eta squared. *Significant main effect, * $p < .05$, *** $p < .001$.

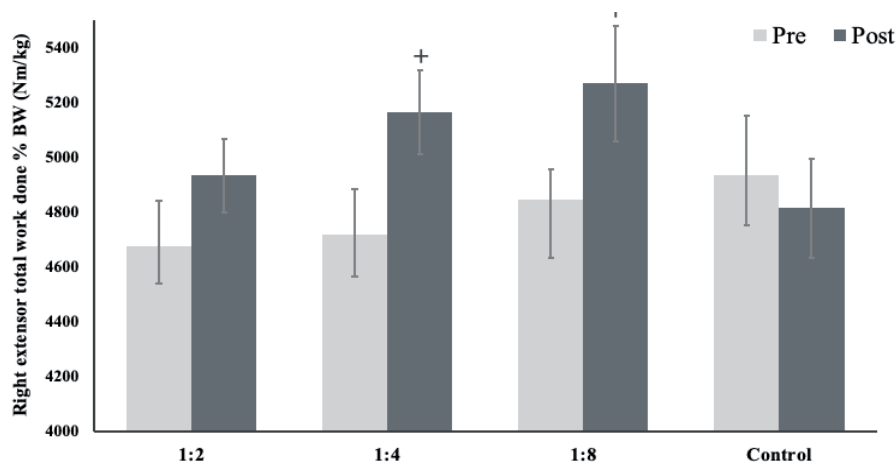


Figure 2. Comparisons of the first set of fatigue resistance following various protocols with HIIT. Values are expressed as mean and standard error of mean. Significant difference between pre- and post-test, ⁺*p*<.05

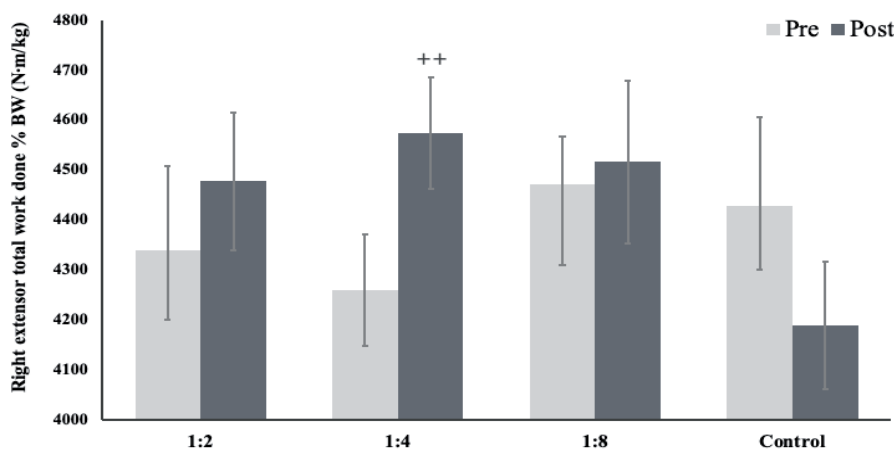


Figure 3. Comparisons of the second set of fatigue resistance following various protocols with HIIT. Values are expressed as mean and standard error of mean. Significant difference between pre- and post-test, ⁺⁺*p*<.01

Table 4. Change in bilateral isokinetic muscular strength between baseline and post-test in adolescent athletes

Variables	Group	Baseline (95% CI)	Post (95% CI)	Cohen's <i>d</i>	Group (ES)	Time (ES)	Group × Time (ES)
Right extensor peak torque %BW (N·m/kg)	1:2	333.3±43.3 (302.0-364.7)	336.1±47.5 (305.9-366.3)	0.16			
	1:4	345.1±51.0 (312.7-377.5)	354.0±48.12 (323.4-384.6)	0.27	2.32	0.03	0.93
	1:8	302.1±52.76 (268.6-335.6)	296.0±36.84 (272.6-319.4)	0.18	(0.40)	(0.03)	(0.25)
	CON	326.0±56.00 (288.4-363.6)	317.46±61.88 (275.9-359.0)	0.28			
Right flexor peak torque % BW (N·m/kg)	1:2	184.3±20.89 (171.0-197.5)	196.6±28.49 (178.5-214.7)	0.58			
	1:4	197.0±22.63 (182.6-211.4)	219.7±19.22 (207.5-231.9)	1.12	2.82	12.91 ⁺⁺⁺	1.15
	1:8	183.0±20.72 (169.8-196.2)	192.1±29.81 (173.1-211.0)	0.33	(0.44)	(0.59)	(0.28)
	CON	176.8±32.87 (154.7-198.9)	182.3±39.90 (155.5-209.1)	0.21			
Left extensor peak torque % BW (N·m/kg)	1:2	340.1±45.25 (311.3-368.8)	339.3±52.46 (305.9-372.6)	0.03			
	1:4	349.6±25.98 (333.1-366.1)	349.4±35.20 (327.1-371.8)	0.01	1.79	2.84	2.56
	1:8	312.2±39.99 (286.8-337.6)	312.4±37.77 (288.4-336.4)	0.01	(0.35)	(2.56)	(0.42)
	CON	343.1±44.82 (313.0-373.2)	311.5±66.81 (266.6-356.3)	0.64			
Left flexor peak torque% BW (N·m/kg)	1:2	178.5±19.81 (165.9-191.1)	186.4±21.79 (172.6-200.3)	0.40			
	1:4	195.9±33.85 (174.4-217.4)	209.8±24.88 (194.0-225.6)	0.40	2.37	4.16 ⁺	0.01
	1:8	181.5±22.91 (166.9-196.1)	186.5±17.38 (175.5-197.5)	0.20	(0.41)	(0.31)	(0.03)
	CON	183.4±25.01 (166.6-200.2)	186.5±30.37 (166.1-206.9)	0.17			

Note. Values are expressed as mean ± SD. ES – partial eta squared. ⁺Significant main effect, ⁺*p*<.05, ⁺⁺⁺*p*<.001.

Table 5. Change in bilateral isokinetic muscular endurance at 180°s between baseline and post-test in adolescent athletes

Variables	Group	Baseline (95% CI)	Post (95% CI)	Cohen's d	Group (ES)	Time (ES)	Group × Time (ES)
Right extensor Total work %BW (N·m/kg)	1:2	3809.7±702.22 (3363.5-4255.8)	4230.8±487.12 (3921.3-4540.2)	0.81	0.44 (0.18)	23.22 ⁺⁺⁺ (0.74)	1.28 (0.30)
	1:4	3978.7±448.33 (3693.8-4263.5)	4433.2±388.87 (4159.1-4980.2)	1.07			
	1:8	3954.3±445.84 (3671.0-4237.5)	4182.6±363.35 (3951.7-4413.4)	0.80			
	CON	3945.9±574.5 (3560.0-4331.8)	4094.5±566.54 (3713.9-4475.2)	0.28			
Right flexor Total work %BW (N·m/kg)	1:2	2551.8±534.47 (2212.2-2891.4)	2758.1±419.33 (2491.7-3024-5)	0.57	0.44 (0.18)	6.43 ⁺ (0.39)	1.32 (0.30)
	1:4	2461.0±437.41 (2183.1-2738.9)	2724.4±438.73 (2445.7-3003.2)	1.09			
	1:8	2527.4±348.66 (2305.9-2748.9)	2588.3±537.01 (2227-1-2909-5)	0.08			
	CON	2450.0±410.45 (2174.3-2725.7)	2470.6±476.20 (2150.7-2790.6)	0.07			
Left extensor Total work %BW (N·m/kg)	1:2	3921.9±568.33 (3560.8-4283.0)	4209.8±492.47 (3895.9-4255.7)	1.10	0.11 (0.08)	14.63 ⁺⁺⁺ (0.58)	1.59 (0.33)
	1:4	3836.3±422.74 (3567.7-4104.9)	4164.6±394.62 (3913.9-4415.3)	0.80			
	1:8	4030.8±485.38 (3722.4-4339.2)	4155.1±340.14 (3939.0-4371.2)	0.38			
	CON	4014.5±419.12 (3733.0-4296.1)	4066.5±481.62 (3743.0-4390.1)	0.12			
Left flexor Total work %BW (N·m/kg)	1:2	2636.3±633.45 (2233.8-3038.7)	2660.8±356.57 (2434.3-2887.4)	0.06	1.37 (0.31)	0.41 (0.10)	0.45 (0.18)
	1:4	2573.3±413.74 (2310.5-2836.2)	2697.3±323.16 (2492.0-2902.7)	0.28			
	1:8	2571.6±251.10 (2412.0-2731.1)	2510.6±339.21 (2295.1-2726.1)	0.19			
	CON	2343.5±418.05 (2062.6-2624.3)	2404.3±472.30 (2087.0-2721.6)	0.13			

Note. Values are expressed as mean ± SD. ES – partial eta squared. *Significant main effect, *p<.05, ***p<.001.

Table 6. Change in stress-to-recovery indicate biomarkers between baseline, mid-, and post-tests in adolescent athletes

Variables	Group	Baseline (95% CI)	Mid (95% CI)	Post (95% CI)	Within groups post-hoc (ES)	Group (ES)	Time (ES)	Group × Time (ES)
Free testosterone (pmol/L)	1:2	40.8±7.23 (36.3-45.5)	43.3±7.67 (38.5-48.1)	41.1±9.69 (34.9-47.0)	N/A (0.02)	1.46 (0.32)	0.95 (0.15)	3.34 ^{**} (0.48)
	1:4	47.4±13.7 (38.5-56.2)	48.1±11.3 (41.1-55.4)	47.7±10.80 (41.1-54.7)	N/A (0.00)			
	1:8	41.5±6.94 (37.1-45.9)	49.2±10.9 (42.2-56.2)	40.4±9.65 (34.1-46.3)	Mid > Baseline, Post (0.16)			
	CON	44.1±15.16 (33.8-54.3)	36.3±9.1 (30.1-42.2)	40.4±13.36 (31.6-49.2)	N/A (0.12)			
	Between groups post-hoc (ES)	N/A (0.09)	1:4, 1:8 > CON (0.27)	N/A (0.13)				

Cortisol (µg/dL)	1:2	9.2±2.30 (7.7-10.6)	11.3±3.57 (9.0-13.6)	10.6±2.34 (9.2-12.1)	Mid, Post > Baseline (0.09)			
	1:4	8.6±1.99 (7.4-9.9)	12.2±2.92 (10.3-14.0)	11.2±2.56 (9.5-12.8)	N/A (0.27)			
	1:8	9.8±2.22 (8.4-11.2)	8.9±3.13 (6.9-10.8)	11.3±3.47 (9.1-13.5)	N/A (0.11)	1.11 (0.39)	6.60** (0.62)	3.46** (0.49)
	CON	9.0±2.14 (8.4-11.2)	8.1±3.94 (5.5-10.8)	10.0±3.10 (7.9-12.1)	N/A (0.16)			
	Between groups <i>post-hoc</i> (ES)	N/A (0.06)	1:2, 1:4 > CON, 1:4 > 1:8 (0.28)	N/A (0.04)				
Creatine kinase (U/L)	1:2	453.4±178.60 (325.6-581.2)	524.0±179.49 (410.0-638.0)	320.7±124.9 (241.3-400.0)	N/A (0.04)			
	1:4	506.4±176.1 (388.1-624.7)	554.1±359.0 (326.0-782.2)	535.6±507.85 (145.2-928.9)	N/A (0.00)			
	1:8	222.4±89.76 (165.4-279.4)	283.9±96.07 (222.9-345.0)	248.9±91.09 (191.0-306.8)	N/A (0.08)	6.41** (0.73)	3.55* (0.50)	2.98* (0.32)
	CON	213.3±65.26 (169.4-257.1)	315.9±97.27 (250.6-381.3)	336.8±180.08 (208.0-465.6)	N/A (0.05)			
	Between groups <i>post-hoc</i> (ES)	1:2, 1:4 > 1:8, CON (0.49)	1:2, 1:4 > 1:8, CON (0.27)	N/A (0.09)				
Urea (mg/dL)	1:2	32.1±7.59 (25.3-35.6)	29.9±7.92 (22.8-34.3)	27.9±5.31 (23.4-31.3)	N/A (0.06)			
	1:4	32.9±7.17 (28.6-40.5)	29.6±6.41 (27.1-36.1)	29.0±7.14 (23.9-33.5)	N/A (0.05)			
	1:8	28.3±5.94 (24.5-32.1)	26.9±3.89 (24.4-29.4)	29.0±6.80 (24.6-33.3)	N/A (0.02)	0.51 (0.19)	2.98 (0.26)	0.92 (0.25)
	CON	29.9±8.15 (23.9-36.2)	28.1±5.28 (25.5-32.4)	29.8±6.12 (25.0-34.1)	N/A (0.01)			
	Between groups <i>post-hoc</i> (ES)	N/A (0.09)	N/A (0.08)	N/A (0.01)				

Note. Values are expressed as mean ± SD. ES – partial eta squared, N/A – not applicable. *Significant main and interaction effect, *p<.05, **p<.01.

Stress-to-recovery biomarkers

A significant interaction effect for group by time on FT ($p<.01$, $\eta^2_p=.48$), C ($p<.01$, $\eta^2_p=.049$), and CK ($p<.05$, $\eta^2_p=.32$) (Table 6) was observed. FT increased significantly in the 1:8 group at the mid-term test (mid > baseline and post), while the 1:2, 1:4, and CON groups did not show any significant changes. The *post-hoc* results revealed the 1:4 and 1:8 groups had a significantly higher value of FT than the CON group in the mid-term test ($\eta^2_p=.27$). The C levels of the 1:2 group increased significantly at mid-term and post-test when compared to baseline (mid, post > baseline, $\eta^2_p=.16$), while the 1:4, 1:8, and CON groups did not show any significant improvements. There was a significant difference in C at the mid-test among the groups (1:2, 1:4 > CON, 1:4 > 1:8, $\eta^2_p=.28$). CK increased significantly in the 1:2 group (baseline, mid > post, $\eta^2_p=.20$) during the intervention. Moreover, there were no significant changes from baseline, mid-term and post-test in CK in the 1:4, 1:8, and CON groups, and the 1:2 and 1:4 groups had a significantly higher level of CK than the 1:8 and CON groups at baseline

($\eta^2_p=.49$) and mid-test ($\eta^2_p=.27$). However, repeated ANCOVA with baseline as a covariate showed no significant interaction effects for the group by time on creatine kinase.

Discussion and conclusions

The optimal HIIT protocol that maximally increases muscle function and fatigue resistance still remains unknown. The present study compared the effects of various work-to-rest ratios of HIIT on muscle function and fatigue resistance in adolescent athletes. The major findings of the study are as follows: (1) the HIIT 1:4 group improved fatigue resistance at the first and second sets over a 4-week period, and (2) the 1:4 group better maintained a stress-to-recovery balance compared to the other groups.

This study found that various work-to-rest ratios of HIIT displayed no changes in lower limb muscle strength and endurance measured by an isokinetic dynamometer in adolescent athletes. This finding was in line with previous literature where six sessions of HIIT over 2-3 weeks were insuffi-

cient to improve the isokinetic muscle strength and endurance of healthy young adults (Astorino, Allen, Roberson, & Jurancich, 2012). Based on these results, it is assumed that an insufficient training period was provided for well-trained athletes to elicit positive effects (DeWeese, Hornsby, Stone, & Stone, 2015; Handsfield, et al., 2017). Previous studies have also found that resistance training is a potent stimulator of neuromuscular adaptations that increase muscle strength. Thus, further study is needed to establish optimal long-term (> 24 weeks) or mid-term (> 12 weeks) HIIT intervention with resistance training (Ross, et al., 2009; Sabag, et al., 2018; Seo, Jung, Song, & Kim, 2015).

Muscle fatigue is defined as the decline in muscle performance that occurs in response to repetitive muscle contractions and associated muscle activities such as accumulation of H⁺ ions, decrease in Ca²⁺ sensitivity, and reduction in shortening velocity (Allen, Lamb, & Westerblad, 2008). High-intensity repeated movements result in rapid fatigue, which directly causes a decrease in athletic performance (Goodall, Charlton, Howatson, & Thomas, 2015; Perrey, Racinais, Saimouaa, & Girard, 2010). In the present study, the 1:4 and 1:8 groups improved in the first sets compared with the 1:2 and CON groups. The 1:4 group increased significantly in the second set, while the 1:2, 1:8, and CON groups did not manifest any change from pre- to post-tests. These results indicated that improvements in fatigue resistance in the 1:4 group, indicated by increases in total work done, were only observed in both the first and second sets. It is possible that sufficient rest time during HIIT has allowed the enhancement of fatigue resistance and neuromuscular adaptations (MacInnis & Gibala, 2017; Mendez-Villanueva, Hamer, & Bishop, 2008; Racinais, et al., 2007; Torma, et al., 2019). A previous study suggested that muscle contraction is related to the peripheral nervous system (PNS) and that, although voluntary exercise activates the central nervous system (CNS) to a greater extent than PNS, it may have different effects depending on the stimulation type in the muscle (Billaut & Basset, 2007; Fernandez-del-Olmo, et al., 2013). The extent of voluntary muscle contractions brought about using the modified fatigue induction protocol could be assessed by the CNS and fatigue resistance (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). As a result, optimal HIIT protocols induced greater activation in the CNS compared with the PNS and might improve tolerance to afferents inhibitory (i.e., group III/IV afferents) (O'Leary, et al., 2017). In contrast to the hypothesis that fatigue resistance would be improved in the 1:2 group because of similar work-to-rest ratios, it did not show any change. These results might be caused by recovery type (i.e., dynamic vs. static), and differences arise in HIIT effectiveness on muscle endurance according to the

angular velocity. A great increase could be obtained if we set the protocol for the high angular velocity protocol by an isokinetic dynamometer.

The best athletic performance can be achieved by meeting or exceeding the results of a predefined training plan in a multifaceted manner (Kellmann, 2010). Excessive training, however, causes overtraining syndrome (OTS) due to increased fatigue and nonfunctional overreaching (NFO), which in turn adversely affects athletic performance (Berryman, et al., 2018; Grivas, 2018). The results of stress-to-recovery indicators in this study showed that the 1:8 group observed a significantly increased FT following the 2-week intervention period; however, after four weeks, it decreased to the baseline values at pre-test ($p < .01$). C increased significantly in the 1:2 group only, between pre-, mid-, and post-tests. In addition, a significant increase was found in CK in the 1:2 group only, among baseline, mid- and post-tests. Julian et al. (2017) reported that after a 4-week low-intensity, high-volume training (LIHV) even a 5-week HIIT did not produce any change in the levels of FT, C, CK, and U. In the case of C levels (Julian, et al., 2017), it was considered that the 1:2 group showed an increase in C levels after only two and four weeks as compared with the results of baseline and that this group had a higher stress response than the other groups. However, there may be a wide range of stress-to-recovery indicators as per individual training levels and adaptability. Hence, the generalization of muscle fatigue across different populations could be limited because it varies with individuals. Consequently, the results of the stress-to-recovery indicators are likely to cause statistical errors due to the large difference range in the group-based statistical approach; thus, future studies need to be conducted to create algorithms or individualized reference range for variables that can provide a clear classification. Nevertheless, 30 seconds all-out running coupled with a 120s rest seems to maintain balance in stress-to-recovery indicators during the 4-week HIIT intervention.

Body composition is an important parameter responsible for attaining success in various sports (Reale, Burke, Cox, & Slater, 2020). However, no significant changes in body composition were founded in all groups. Monks, Seo, Kim, Jung, and Song (2017) observed that a 4-week HIIT intervention showed no significant interaction effects on body composition in collegiate athletes compared with high-intensity continuous training. Naimo et al. (2015) reported an increase in muscle thickness; however, it did not alter lean tissue, fat tissue, or percentage body fat in ice hockey players. A short-term HIIT (2-6 weeks) is focused on neuromuscular and skeletal adaptations. Thus, it is speculated that body composition during a short-term training period may be more influenced by nutrition

intake, age, and gender than the training effects. The status of lean tissue and fat tissue influences athletic performance and is an essential factor that warrant evaluation during the training. HIIT increases the release of cortisol, catecholamines, and growth hormone, which in turn stimulates fat metabolism (Boutcher, 2011). HIIT has a positive effect on the remodeling of the skeletal muscle. PGC-1 α can activate mitochondrial biogenesis and oxidative metabolism during HIIT and consequently can influence muscle protein synthesis (Gallo-Villegas, et al., 2018). However, a short-term HIIT may be less effective for improving body composition, such as muscle structure (Whyte, Gill, & Cathcart, 2010). If a systematic nutrition strategy program is followed along with a short-term HIIT, it may effectively improve the body composition of well-trained adolescent athletes; future studies should be conducted to investigate related issues.

The strength of this study was that it was well-designed with randomized control, and all training sessions were supervised by a CSCS from our research group. Nevertheless, limitations of the present study include a comparatively particular

sport, one gender only, and a small sample size, which may limit the generalizability of our findings. Although the present study assessed some stress-to-recovery indicators (FT, C, CK, and U), other variables should be measured to determine balance between training load and recovery of athletes such as growth hormone, insulin-like growth factor-1 (IGF-1), IGF-1 binding protein 3, c-reactive protein, tumor necrosis factor, and interleukin 6, etc. In addition, running-based HIIT is not a taekwondo-specific movement technique. Future research should be conducted to apply the HIIT program with specific taekwondo kicks and movements for adolescent athletes.

Our findings suggest that a 30-second all-out sprint running with a 120-second rest time may be more effective in improving muscle function in adolescent athletes. The present study confirmed that the HIIT of 1:4 work-to-rest ratio, lasting over a brief 4-week period, induced a significant improvement in fatigue resistance compared with the other work-to-rest ratios studied. The present data may provide useful, practical implications to develop a training program for HIIT in adolescent athletes.

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