



Combination of High-Intensity Interval Training and Creatine Intake Enhances Leg Strength and Anaerobic Power Without Changes in Body Composition in Physically Active Adult Men - A Randomized Controlled Study

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ABSTRACT

Various training methods and ergogenic aids have been employed to enhance athletic performance. This study aimed to understand the effect of combining six weeks of high-intensity interval training (HIIT) with creatine supplementation on body composition, leg strength, and anaerobic power in physically active male adults. In this six-week study, a total of fifteen physically active men, with a mean age of 21.13 ± 1.68 years, were divided into two groups: the Wingate-based HIIT training group (HIIT) and the Wingate-based HIIT training + creatine supplementation group (C-HIIT). Both groups performed HIIT training based on the Wingate protocol, three days a week for six weeks, with the intensity increasing during the second three-week period. In addition to the training, the C-HIIT group took a total of 10 g of creatine each training day, 5 grams 30 minutes before the exercise and 5 grams immediately after exercise. The body composition, leg strength, and anaerobic power measurements of the subjects were taken three days before the study started and three days after it ended. According to the findings, although there was no significant difference in body composition values between the two groups, it was observed that leg strength ($p < 0.05$) and anaerobic power parameters such as peak power, average power, and minimum power improved significantly in the C-HIIT group ($p < 0.01$). The addition of 10 grams of creatine monohydrate to HIIT training appears to be effective in improving leg strength and anaerobic power in physically active adult men.

INTRODUCTION

Exercise routines and nutritional intake have a significant impact on physical performance (Guest et al., 2019; Thomas et al., 2016). The improvement of performance and capacity among athletes of different levels (professional, amateur, recreational, etc.) has been enhanced through the use of nutritional supplements and ergogenic aids (Buckley et al., 1988; Maughan, 1999; Sobal & Marquart, 1994). Creatine monohydrate is one of the ergogenic aids used for this purpose. Creatine monohydrate consumption has been reported to provide multiple benefits including increasing muscle size, strength, and power (Becque et al., 2000), enhancing performance during short bursts of high-intensity exercises (Cooper et al., 2012), and promoting faster post-exercise recovery (Cooke & Barnes, 1997). In the literature, several studies have examined the effects of creatine intake on enhancing performance levels and body composition in various sports branches (Becque et al., 2000; Birch et al., 1994; Devries & Phillips, 2014). Some of these studies have shown an increase in lean body mass (Becque et al., 2000; Nissen & Sharp, 2003) and type II muscle fiber area (Burke et al., 2003; Hespel et al., 2001) in addition to performance improvements. However, other studies have focused solely on the effects of creatine supplementation on anaerobic power and performance levels in sports like swimming (Grindstaff & Kreider, 1997), rowing (Rossiter et al., 1996), and cycling (Balsom et al., 1993).

Apart from nutritional ergogenic aids, various training methods are also employed to enhance athletic performance. As a training model, high-intensity interval training (HIIT) has gained popularity in recent years due to its effects on improving both aerobic and anaerobic performance (Buchheit & Laursen, 2013). High-intensity interval training is defined as interval training performed at a high intensity above the anaerobic threshold, consisting of repetitions of short- to medium-duration exercises interspersed with intermittent passive and/or active rest periods (Graef et al., 2009). Inducing intermittent physiological stress, promoting chronic adaptation, and increasing energy efficiency are cited as key reasons why HIIT is efficacious in improving performance (Kendall et al., 2009). HIIT attracts more attention than traditional aerobic training programs because repetitive short-term high-intensity training, combined with low-intensity recovery, tends to further improve both aerobic and anaerobic energy systems, metabolic functions and physical performance (Bayati et al., 2011; Greeley et al., 2013). For example a study comparing the effects of HIIT and moderate continuous training in badminton players found that HIIT training increased anaerobic power and improved body

composition by reducing body fat percentage. Therefore, HIIT has been reported as an effective method for improving strength and physical performance (Ko et al., 2021). High intensity interval training can be applied using various methods and equipment, including the Wingate ergometer (Kendall et al., 2009), circuit training method (Schleppenbach et al., 2017), treadmill (Biwer et al., 2003), Tabata training method (Tabata, 2019) and other bicycle ergometers (Schaun & Del Vecchio, 2018).

Despite the time-efficiency of HIIT in inducing chronic physiological adaptation, it depletes phosphocreatine stores in the muscles and decreases power output by inducing fatigue (Bogdanis et al., 1995). Additionally, it has been observed that high-intensity exercise performance improves with an increase in intramuscular phosphocreatine, suggesting that creatine supplementation may be beneficial in this process (Syrotaik & Bell, 2004). It is thought that creatine intake may also be adequate in this process. It is predicted that phosphocreatine synthesis and the rate of mitochondrial Adenosine triphosphate (ATP) transfer to the cytoplasm may increase with an adequate level of creatine supplementation (Harris et al., 1992; Hultman et al., 1996). This indicates that performing HIIT with creatine supplementation could further enhance performance. Therefore, this current study aimed to compare the effectiveness of HIIT alone with HIIT combined with 10 grams of creatine intake per session in physically active adult men over the six week training period. We hypothesized that creatine supplementation with Wingate-based HIIT would positively impact lean body mass, leg strength, and anaerobic power parameters.

METHODS

Study Design

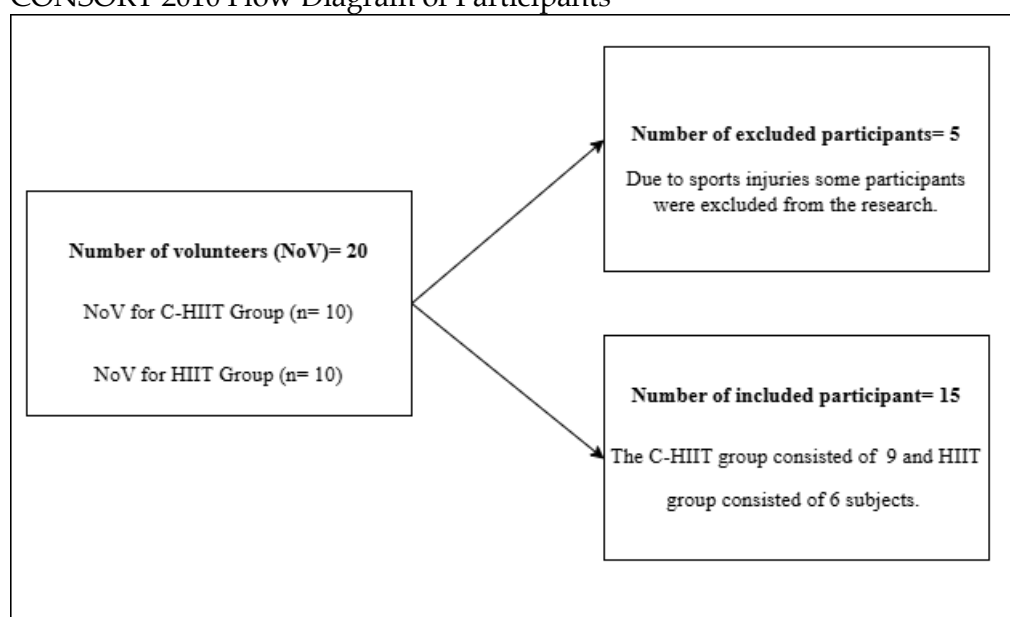
The research was conducted using the experimental method, a quantitative research approach. This study was conducted in accordance with the CONSORT 2010 (Figure 1) statement. In this experimental research design the effects of creatine supplementation on body composition, leg strength, and anaerobic power values in physically active adult men were examined. After obtaining the necessary permissions, the participants visited the measurement site for the first time for familiarization, where the study details were explained to them, and the tests were demonstrated. Three days later, the participants made their second visit for pre-tests. First, body composition and leg strength were evaluated, and after a 20 minute rest, the Wingate Anaerobic Power Test was administered. Throughout the six-week study, both groups participated in the same Wingate-based HIIT program, with the duration

and number of sets increasing during the second three weeks. During this period, one of the groups took 10 g of creatine per training session. Three days after the study concluded, participants visited the measurement site for post-tests, and the study was completed. Training and measurements were conducted in the sports science laboratory of Ankara University from March to June 2022.

Participants

G-power analysis was performed to determine the sample size. A total of 20 physically active adult men, aged 18-30 years, with no medical history and not using drugs or ergogenic aids, who exercised at an intensity of 3-6 METs for 150 minutes per week, were included in the study. The ethical approval for the research was obtained from the Ethical Committee of Ankara University Rectorate (Number: E-56786525-050.04.04/378028 Decision Number: 2022/02) and was carried out in compliance with the principles of the Declaration of Helsinki (2013). Written informed consent forms that explain the study to the participants and any rights and risks were obtained prior to data collection. After obtaining these official permissions, the participants were randomly assigned into two groups as those who performed Wingate-based HIIT with creatine (C-HIIT; age: 21.78 ± 1.79 years; height: 179.56 ± 8.26 cm; weight: 76.86 ± 10.66 kg) and those who performed only Wingate-based HIIT (HIIT) (age: 20.167 ± 0.98 years; height: 172.33 ± 9.88 cm; weight: 66.53 ± 9.51 kg) randomly. Only 15 participants (HIIT, C-HIIT) were able to complete the study, as 5 of the participants were excluded due to sports injuries (Figure 1).

Figure 1
CONSORT 2010 Flow Diagram of Participants



Procedures

Wingate-based HIIT Program

In the study to observe the effects of creatine, a short-term HIIT exercise program was planned, and participants performed the exercise using a stationary bicycle. Participants in the C-HIIT and HIIT groups performed the same Wingate-based HIIT with a resistance equal to 7.5% of their body weight, three times a week for six weeks. The Wingate-based HIIT program is outlined in Table 1 and illustrated in Figure 2.

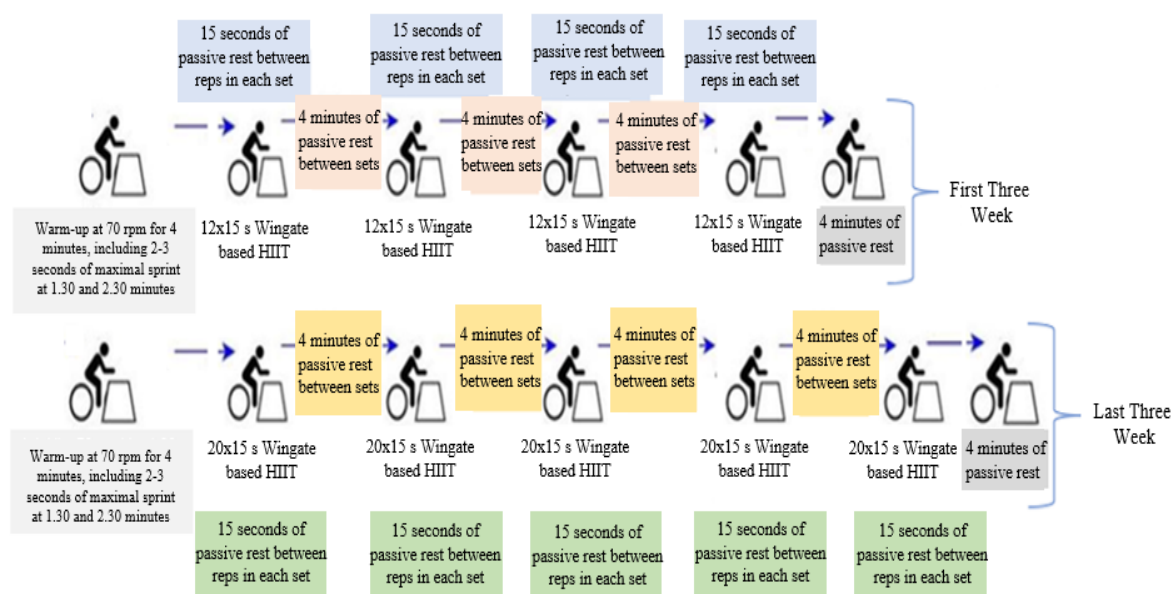
Table 1

Wingate-based HIIT Program.

First Three Week	Last Three Week
Warm-up: Warm-up at 70 rpm for 4 minutes, including 2-3 seconds of maximal sprint at 1.30 and 2.30 minutes	Warm-up: Warm-up at 70 rpm for 4 minutes, including 2-3 seconds of maximal sprint at 1.30 and 2.30 minutes
Wingate-based HIIT: HIIT 4 sets of 12x15 seconds of maximal repetitions with 15 seconds of passive rest between reps in each set	Wingate-based HIIT: HIIT 5 sets of 20x15 seconds of maximal repetitions with 15 seconds of passive rest between reps in each set
Cool-down: 4 minutes of passive rest between sets	Cool-down: 4 minutes of passive rest between sets

Figure 2

Wingate-based HIIT Program.



Creatine Supplement

In this study, to observe the effects of creatine, participants were given 10 g of creatine monohydrate dissolved in 200 ml of water only on training days throughout the study period.

The creatine supplementation was taken in two doses of five grams, 30 minutes before, and right after the training session.

Wingate Anaerobic Power Test

The Wingate anaerobic power test, a measure of lactic and alactic anaerobic power, was performed using a bicycle ergometer (Bar-Or, 1987). The specialist trainers made the measurements. Before the test started, detailed information was given to the participants. After the briefing, a four-minute warm-up protocol with a bicycle ergometer at 60-80 rpm pedal speed and 50 W workload was applied, followed by 4 minutes of passive resting. Seat and handlebar adjustments were made for each participant, considering their leg lengths (Inbar et al., 1996). Weights equal to 7.5% of each participant's body weight were placed on the pan of the bicycle ergometer, and anaerobic power testing was initiated (Bar-Or, 1987). When the participants reached a pedal speed of 150 rpm, the pan automatically lowered, and the Wingate Anaerobic Power Test began (Inbar et al., 1996). Participants' maximal effort pedaled against the weights for 30 seconds was collected. The Wingate software was used in which the program was set up with the ergometers. Information about the relevant power parameters during the test was transmitted to the software program on the computer. All absolute and relative power parameters (values such as peak power, minimum power, average power, power drop etc.) and total energy expenditure were calculated by the software program.

Body Composition

The measurements were made by the specialist dietitian. The body composition was determined using the PlusAvis 333 device (Jawon Medical, South Korea). Measurements were taken according to standard techniques while the participants wore only shorts and no shoes. Each participant was instructed to discontinue use of diuretic products at least 7 days prior to the measurement, to refrain from drinking alcohol for 48 hours, to cease exercising 12 hours prior, and to abstain from eating and drinking for at least 4 hours before the measurement. Additionally, participants were instructed to empty their bladders 30 minutes prior to the measurement. Finally, they were asked to remove the metal items they were wearing (Magyari et al., 2018). As body composition parameters, body weight (BW), percent body fat (PBF), mass of body fat (MBF), lean body weight (LBW), body mass index (BMI), total body water (TW), and basal metabolic rate (BMR) were used in the study.

Leg Strength

The measurements were made by the specialist trainers. A back and leg dynamometer (Takei-Back & Lift, Japan) was used to measure leg strength. Participants placed their feet on

the dynamometer stand. The knees were flexed at an angle of 130-140 and the back was straight. The participants pulled the dynamometer bar, which they held in pronation with their hands, until their knees were extended, using only their legs, without changing their current position. Participants repeated this pull twice, with three minutes between each attempt, and the best value for each participant was recorded (Heyward & Gibson, 2014).

Data Analysis

IBM SPSS 24.0 program (SPSS Inc., Chicago, IL, USA) was used to analyze the data obtained in the study statistically. Firstly, the distribution of the data was evaluated to determine whether the test used to compare the data was parametric or non-parametric. This normality distribution assessment was tested using the Shapiro-Wilk test, as both groups had fewer than 50 participants. The determination of the mean difference in within-group statistics was performed by using the Paired Sample t-Test for data with a normal distribution and the Wilcoxon signed-rank test for data that were not normally distributed. The independent sample t-test or Mann-Whitney U Test was utilized for comparisons between groups, depending on the data distribution. Groups are expressed as mean $\pm$ standard error. The alpha value was set at 0.05 for all statistical analyses.

RESULTS

The pre- and post-test findings for body composition and leg strength parameters are presented in Table 2. According to the findings a significant difference was observed only in the leg strength value in the C-HIIT group ($p < 0.05$). None of the body composition parameters were statistically different between the HIIT and C-HIIT groups ($p < 0.05$). The determination of the mean difference in within-group statistics was performed using a Paired Sample t-Test in data with a normal distribution.

As seen in Table 3, similar to the leg strength value significant differences in the C-HIIT group were found in almost all Wingate anaerobic test results when compared with the HIIT group. Both absolute and relative peak power, average power, and minimum power, and total energy expended showed increases in C-HIIT group ($p < 0.01$). The determination of the mean difference in within-group statistics was performed by Paired Sample T-Test in data with normal distribution.

Table 2

Comparison of Parameters and Average Differences in Body Composition and Leg Strength

Parameter	Group	Pre-test	Post-test	P ₋	Effect size
Body Weight (kg)	C-HIIT	76.86 ± 10.66	76.42 ± 10.96	0.128	0.04
	HIIT	66.53 ± 9.51	66.07 ± 9.26	0.270	0.05
	P ₋	0.964	0.902		
Percent Body Fat (%)	C-HIIT	19.66 ± 5.64	18.89 ± 5.27	0.225	0.14
	HIIT	17.47 ± 6.46	16.63 ± 7.03	0.058	0.12
	P ₋	0.587	0.217		
Mass of Body Fat (kg)	C-HIIT	15.64 ± 6.61	14.79 ± 6.14	0.086	0.13
	HIIT	11.95 ± 5.23	11.37 ± 5.53	0.063	0.11
	P ₋	0.743	0.909		
Lean Body Mass (kg)	C-HIIT	61.21 ± 5.87	61.63 ± 6.68	0.442	0.07
	HIIT	54.58 ± 6.01	54.70 ± 5.56	0.599	0.02
	P ₋	0.981	0.446		
Total Body Water (kg)	C-HIIT	44.07 ± 4.21	44.53 ± 4.64	0.246	0.10
	HIIT	39.37 ± 4.32	39.40 ± 3.99	0.588	0.01
	P ₋	0.978	0.507		
BMI (kg/m ²)	C-HIIT	23.97 ± 3.45	23.67 ± 3.47	0.336	0.09
	HIIT	22.53 ± 3.75	21.80 ± 3.06	0.346	0.21
	P ₋	0.676	0.778		
BMR (kcal)	C-HIIT	1672.22 ± 117.50	1679.22 ± 130.98	0.502	0.06
	HIIT	1554.67 ± 117.51	1562.33 ± 115.42	0.345	0.07
	P ₋	0.910	0.569		
Leg Strength (N)	C-HIIT	145.83 ± 35.32	166.20 ± 23.81	0.038*	0.68
	HIIT	128.00 ± 29.69	132.33 ± 30.28	0.650	0.14
	P ₋	0.951	0.553		

Note. BMI: Body mass index; BMR: Basal metabolic rate; C-HIIT: Wingate-based high intensity interval training with creatine intake; HIIT: Wingate-based high intensity interval training

Table 3

Comparison of Parameters and Average Differences in the Wingate Anaerobic Power Test

Parameter	Group	Pre-test	Post-test	P ₋	Effect size
Peak Power (W)	C-HIIT	892.78 ± 151.39	999.06 ± 182.22	0.007**	0.63
	HIIT	802.39 ± 137.66	803.37 ± 156.47	0.985	0.01
	P ₋	0.640	0.814		
Relative Peak Power (W/kg)	C-HIIT	11.58 ± 1.04	12.99 ± 1.42	0.008**	1.13
	HIIT	11.72 ± 0.86	12.36 ± 1.58	0.370	0.50
	P ₋	0.519	0.683		
Average Power (W)	C-HIIT	603.23 ± 119.68	682.65 ± 120.98	0.003**	0.66
	HIIT	577.70 ± 99.05	544.62 ± 119.08	0.512	0.30
	P ₋	0.627	0.824		
Relative Average Power (W/kg)	C-HIIT	7.84 ± 1.16	8.81 ± 0.85	0.004**	0.95
	HIIT	8.38 ± 0.35	8.38 ± 1.34	0.345	0
	P ₋	0.089	0.572		
Minimum Power (W)	C-HIIT	310.67 ± 136.25	396.38 ± 107.70	0.009**	0.71
	HIIT	309.25 ± 104.30	290.98 ± 105.73	0.750	0.17
	P ₋	0.211	0.666		
Relative Minimum Power (W/kg)	C-HIIT	3.99 ± 1.64	5.11 ± 1.14	0.009**	0.79
	HIIT	4.44 ± 1.28	4.48 ± 1.42	0.753	0.03
	P ₋	0.272	0.826		
Power Drop (%)	C-HIIT	65.84 ± 13.33	60.26 ± 8.91	0.154	0.49
	HIIT	62.32 ± 10.31	56.96 ± 23.61	0.706	0.29
	P ₋	0.298	0.197		
Total Energy (joule)	C-HIIT	17172.89 ± 3759.23	19716.33 ± 3634.66	0.008**	0.69
	HIIT	16736.33 ± 2847.97	15584.83 ± 3679.56	0.499	0.35
	P ₋	0.489	0.792		

Note. **p<0.01, C-HIIT: Wingate-based high intensity interval training with creatine intake; HIIT: Wingate-based high intensity interval training

DISCUSSIONS

The present study aimed to determine whether there was a difference in the effects of taking 10 grams of creatine supplementation per session with Wingate-based HIIT on body composition, leg strength, and anaerobic power parameters when compared to Wingate-based HIIT alone. For this purpose, fifteen physically active, healthy adult male participants were divided into two groups: C-HIIT and HIIT. They performed the same training for six weeks.

In the current research, it was found that six weeks of HIIT did not cause any changes in body composition independent of creatine intake. There was no significant change in any of the body weight, percent body weight, body fat mass, lean body mass, total body water, BMI, or BMR parameters in either group. Different results have been reported in other studies on this subject. In Forbes et al.'s study (2017), changes in body composition were observed alongside the change in performance values. Researchers examined the effects of HIIT training performed three days a week for a total of four weeks on $\text{VO}_{2\text{max}}$, ventilation threshold, lean body mass, body fat mass, anaerobic power and insulin sensitivity in 17 recreationally active adult women. As a result, they reported an increase in anaerobic power without any change in the body composition parameters. Unlike the current research, the increase in anaerobic power within four weeks without creatine supplementation may be due to the research group. A study group with a lower level of physical fitness than the group in the current study may be the reason for the significant increase in a short time. In another study, Alonso-Fernández et al. (2019) investigated the effect of HIIT-based functional body weight exercises that were performed with a 20/10 work/rest ratio for 7 weeks in 26 children. According to their results, while cardiorespiratory fitness increased, the percentage of body fat decreased. These findings differ from those obtained in the present research. The reason for this can be attributed to the fact that the research group consisted of children, rather than physically active adults, as in the current research, and the body composition values of children can change rapidly.

The primary objective of this research was to investigate the effects of creatine supplementation combined with Wingate-based HIIT on leg strength and anaerobic power values. According to the results, it was found that Wingate-based HIIT with creatine intake increases both absolute and relative anaerobic power values, such as peak power, average power, minimum power, and also total energy expended significantly ($p < 0.01$). An improvement in the same direction was also seen in leg strength ($p < 0.05$). Several studies in

the literature have examined the effects of activity types similar to or different from the current study, in combination with creatine intake, on various performance parameters. In one of these studies, Graef et al. (2009) compared the effects of 10 g of creatine intake per session with HIIT performed five days a week for four weeks, with HIIT alone, and concluded that creatine supplementation had no additional positive effects on maximal oxygen consumption despite an increase in ventilatory threshold. However, it is observed in the literature that the positive effects of creatine supplementation on performance tend to increase as the duration of activity shortens. Examining the effect of 20 g creatine supplementation for 5 days on kayak ergometer performance over different durations, McNaughton et al. (1998) divided the participants into two groups: creatine and placebo. They had them perform three maximum kayak ergometer tests of 90, 150, and 300 seconds. At the end of the research, they reported that creatine intake improves the amount of work completed during the kayak ergometer performance at durations ranging from 90 to 300 seconds.

In another study, Kendall et al. (2009) investigated the effects of a four-week HIIT program with five sets of two-minute exercise bouts with one minute rest interval on the cycle ergometer and creatine supplementation on critical power and anaerobic working capacity in college-aged men. The subjects were assigned to the creatine, placebo, and control groups. According to their results, it was observed that the maximum power output increased in the creatine group, while the anaerobic working capacity remained unchanged. Similarly, Romer et al. (2001) found positive effects of 5-day creatine monohydrate loading of 0.075 g per kilogram of body weight, four times a day and high-intensity, intermittent exercises consisting of 10 sets of two repetitions of a simulated game in squash players. As a result of the study, a significant reduction in sprint time was observed. Balsom et al. (1995) reported that six days of creatine loading (20 g per day) with HIIT sessions on a cycle ergometer did affect the power output in 10-second exercise periods and increase the total muscle creatine and phosphocreatine concentration. However, they did not alter jump performance in physically active males. Unlike the current research, the observed performance changes were accompanied by changes in body composition. This may be due to the amount and frequency of creatine loading. However, some studies also show that creatine intake with HIIT does not have any positive effect on anaerobic performance. For instance, Biwer et al. (2003) did not find any positive alterations in submaximal running interspersed with high-intensity intervals after creatine monohydrate loading of 0.3 g per kilogram of body weight over six days. The only significant change was the increase in body mass found among male participants.

There are also studies in the literature showing the positive effects of HIIT training without creatine intake on leg strength and anaerobic power. For example, in a study evaluating the effects of combined upper and lower body HIIT on muscular and cardiorespiratory fitness, leg extensor muscle strength, handgrip strength, and health-related quality of life in adults over 50 years of age, Hurst et al. (2019) concluded that the exercise program performed using a hydraulic resistance ergometer, twice a week for 12 weeks, resulted in significant differences in body weight, BMI, body fat thickness, maximum aerobic power and maximum anaerobic power. In Fatemeh et al.'s study (2016) subjects performed HIIT on a treadmill three times a week, for three weeks. According to the data obtained, there was a significant difference in body weight, BMI, body fat thickness, maximum aerobic power and maximum anaerobic power in the group performing HIIT ($p = 0.001$), while there was no change in waist-hip ratio (WHR; $p > 0.05$). Unlike the present research, the reason for reaching these results may be the differences in the training protocol and the initial performance levels of the participants.

Limitations

The current research has some limitations. Firstly, due to sports injuries experienced, the number of participants was reduced by one person compared to the initial plan. Secondly, this study was conducted only on female subjects. Adding both genders in future studies may provide more detailed results. Another suggestion is to expand the research group with professional athletes from sports disciplines where different energy metabolisms are active. In this way, different results arising from the research group in the literature can be addressed. Finally, the addition of non-invasive measurement methods such as heart rate variability, which also provides information about the autonomic nervous system, would offer a broader understanding of the chronic effects of creatine and HIIT training.

CONCLUSION

When the findings obtained as a result of the research were examined, it was seen that 10 grams of creatine supplement per session together with Wingate-based HIIT performed three days a week for six weeks, had an effect and increased the both absolute and relative anaerobic power parameters such as peak power, average power and minimum power values, as well as total energy expenditure. It also improved leg strength without affecting any body composition parameters. Although creatine supplementation has positive effects on anaerobic

performance parameters in many studies, different results can be observed in some studies when the literature is examined. The type of creatine used, the total amount, the amount of loading per unit time, the content of the training with creatine intake and the physical activity level of the participants may affect the results. Therefore, future studies may conduct more detailed research with a larger number of participants and experimental groups.

PRACTICAL IMPLICATIONS

The findings suggest that combining 10 grams of creatine supplementation per session with Wingate-based HIIT training (three days per week for six weeks) can enhance both absolute and relative anaerobic power parameters, including peak power, average power, minimum power, and total energy expenditure. This combined approach may be effective for improving leg strength without causing significant changes in body composition. Creatine supplementation can therefore be considered a beneficial nutritional strategy to support high-intensity training performance and anaerobic power development in athletes or active individuals. However, the variability in results observed across studies indicates that factors such as creatine type, dosage protocol, training design, and participants' fitness levels can influence outcomes. Future applications should focus on individualized supplementation protocols and training adjustments based on the athlete's needs and response. Further studies with larger sample sizes and controlled experimental designs are recommended to strengthen the generalizability of these findings.

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Authors' Contribution

The first author and the second author jointly generated the idea and hypothesis for the research and planned the methods to achieve the results. The overall supervision and responsibility for the organization and progress of the project and the article were undertaken by the second author. Both authors were responsible for conducting the experiments, monitoring participants, organizing, and reporting the data. The first author took responsibility for the logical explanation and presentation of the findings as well as for performing the necessary related functions. The creation of the entire article and its main

sections was a joint effort by both authors. The second author reworked the article before submission, improving not only the language and grammar but also the intellectual content.

Declaration of Conflict Interest

The authors declare no conflict interests.

Ethics Statement

The ethical permission for the research was obtained from the Ethics Committee of Ankara University Rectorate (Number: E-56786525-050.04.04/378028 decision number: 2022/02) and it was carried out in compliance with the Declaration of Helsinki's principles. Informed consent was obtained from all subjects involved in the study.

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