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Investigation of the Effects of an 8-Week Training Program on HIF-1 Levels in Football Players Across Different Energy Systems

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ABSTRACT

The aim of this study is to compare the effects of training in different energy systems on Wingate anaerobic power and Hypoxia-Inducible Factor-1 in soccer players. The study involved players from the U-19 football team competing in the 2020-2021. A total of 30 footballers participated and were randomly assigned to aerobic (n:10), anaerobic (n:10), and control (n:10) groups. On the first day, all players underwent body composition measurement and an aerobic test. A 20-minute incremental test protocol was applied, and 5 cc of blood was collected after the test. On the second day, a 30-second Wingate anaerobic power test protocol was conducted, and 5 cc of blood was collected post-test. Subsequently, using the "Research Randomizer" program, participants were randomly assigned to aerobic, anaerobic, and control groups. After grouping, in addition to team training, both aerobic and anaerobic groups underwent a total of 24 training sessions over 8 weeks, three days a week. The control group did not receive any additional training and only continued with team practices. Data were analyzed using SPSS v26. According to the data obtained from the footballers, the 8-week intervention positively contributed to PP, AP, MP, and PD values in both aerobic and anaerobic groups. Significant differences in HIF-1 values were observed in both aerobic and anaerobic groups compared to the control group. The applied training protocols were found to have effects on the study groups. Workouts conducted on both aerobic and anaerobic energy systems were determined to increase both HIF-1 and anaerobic power values.

1. INTRODUCTION

Soccer, with its captivating appeal and profound influence, stands as the most popular sport globally, surpassing other sporting disciplines. With approximately 1000 movements involved, soccer is a dynamic game characterized by rapid and sequential changes in actions. It encompasses irregular intervals where strength, speed, strength endurance, speed endurance, explosiveness, and coordination are exhibited through technical and tactical skills [1].

The study of energy systems has become a fundamental topic in sports sciences due to its direct correlation with the physiological impact mechanism of exercise on metabolism. Different types of exercises are known to have distinct physiological effects. While exercise duration is considered a crucial component in energy metabolism, exercise type, intensity, volume, and individual readiness are other factors with the potential to alter energy system contributions. Studies using muscle biopsy techniques indicate that approximately 52% of energy system contribution in a maximal 6-second exercise is met by the phosphagen system, 40% by anaerobic glycolysis, and 8% by the aerobic system. Despite the phosphagen system meeting roughly half of the energy demand in a 6-second exercise, about 60% of the existing stores can be utilized [2]. Hypoxia adaptation involves the synchronized transcriptional regulation of various genes and is largely coordinated by oxygen-sensitive transcription factors known as Hypoxia-Inducible Factors (HIFs), described as the "primary regulators of oxygen homeostasis." The protein responsible for cellular-level oxygen level adjustments, HIF-1, is a transcription factor. In

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situations of pathological and physiological hypoxia, new vessels are formed to ensure oxygenation. In response to hypoxia, HIF-1 activates cellular molecular programs to stimulate cell proliferation, survival, and angiogenesis. Semenza and Wang first observed an increase in the concentration of a transcriptional protein when cells entered a hypoxic state in 1992, naming this structure HIF [3].

HIF-1 is a structure composed of alpha and beta subunits. Physiologically, the beta subunit is not affected by intracellular oxygen levels, but the alpha subunit plays a role in the development of certain intracellular events depending on oxygen levels. Under oxygen-rich conditions, HIF-1 beta is expressed openly everywhere, while HIF-1a protein expression is typically very low or completely absent, rapidly degrading and moving away from the environment. In hypoxic conditions, it stabilizes, moves from the cytoplasm to the nucleus, and combines with the other subunit, HIF-1 beta, forming HIF-1. As oxygen levels decrease, HIF-1a protein levels and HIF-1 DNA binding activity increase. Both alpha and beta subunits have similar structures, including basic helix-loop-helix (bHLH) and PER-ARNT-SIM (PAS) regions [4].

This study aims to determine the effects of training in two different energy systems on HIF-1 and anaerobic power in athletes.

2. MATERIALS AND METHODS

In this study, a quasi-experimental design with pretest-posttest control group was used.

2.1. Research Group

The study and control groups consisted of players from the B.B. Erzurumspor U-19 football team competing in the 2020-2021 T.F.F. Elite League. A total of 30 footballers participated. Initial assessments included body composition measurements and aerobic testing for all players. Blood samples (5 cc) were collected from the players after both aerobic and anaerobic tests. Subsequently, all participants were randomly allocated to aerobic (n:10), anaerobic (n:10), and control groups (n:10) using the "Research Randomizer" program.

This study was carried out after the approval of the Ethics Committee of Atatürk University. Participant provided informed consent, with the volunteer form covering research details, risks, benefits, confidentiality, and participant rights. The research strictly adhered to the ethical principles of the Declaration of Helsinki, prioritizing participant's rights and well-being in design, procedures, and confidentiality measures.

2.2. Performance Measurements

Performance measurements were conducted in the Atatürk University Sports Sciences Application and Research Center laboratories, while biochemical measurements were carried out by personnel from the Atatürk University Faculty of Medicine Blood Center.

2.2.1. Training intervention

After grouping, in addition to regular team training sessions, a total of 24 training sessions were implemented over 8 weeks, three days a week, for both the aerobic and anaerobic groups. The control group did not undergo any additional training and continued with regular team practices.

2.2.2. Anaerobic power test

To assess the anaerobic power of the footballers, the Wingate 894E test system (6 sensors) was utilized. The protocol included preparation, recovery, acceleration, Wingate test, and cool down phases. Pedal length and saddle weight adjustments were made for each player before the test. The test began with a 2-minute precautionary stretching exercise after completing the warm-up protocol. The saddle weight was automatically calculated by the device to be equivalent to 7.5% of the players' body weight. During the test, players were instructed to pedal maximally against the specified resistance for 30 seconds, with verbal encouragement provided.

2.2.3. Aerobic power test

For the assessment of aerobic power, the Wingate LC4 test system (6 sensors) was employed. Footballers warmed up for 10 minutes at 25 Watts before the test, followed by specific exercises for the lower extremities. An incremental (stepwise increase) test program was applied, starting at 50 Watts and increasing by 15 Watts every 90 seconds, concluding the test at 20 minutes. After completing the test, players were given 5 minutes of cool down at 10 Watts.

2.2.4. Blood sample collection procedure

Blood samples were collected by expert personnel from the Atatürk University Blood

Center. A tourniquet was applied 10-15 cm above the site where blood would be drawn. After the blood collection process, the collected blood was carefully transferred to tubes to prevent hemolysis, and the caps were securely closed. Blood samples were collected from the footballers in a rested state after both aerobic and anaerobic tests. The collected blood samples were centrifuged at 4000 rpm for 15 minutes, and the resulting sera were aliquoted into 5 cc Eppendorf tubes, then stored at -80 degrees Celsius.

2.2.5. HIF-1 measurement and analysis

On the day of analysis, sera were thawed under appropriate conditions, vortexed for homogenization, and HIF-1 ALPHA levels were analyzed using commercial ELISA kits according to the manufacturer's recommended standard protocol. ELISA analysis was performed in the Atatürk University Faculty of Medicine Medical Biochemistry Department Laboratory using the ELISA reader device (BIO-TEK Power Wave XS). The coefficient of variation (CV) for intra-assay and inter-assay variations of the kits was found to be below 8% and 10%, respectively.

2.3. Statistical Analysis

Data collected in the study were analyzed using SPSS 26 (Statistical Package for Social Sciences). Normality tests were conducted using skewness, kurtosis, histogram, and the Shapiro-Wilk test. As skewness and kurtosis values were between +2 and -2, indicating normal distribution, no correction was needed. Paired sample t test was used to compare the pre-test and post-test values within groups. One-way ANOVA test was used to determine the differences between the groups. Differences between groups were analyzed with post hoc (Bonferroni) test. A significance level of p<0.05 was considered in all hypothesis tests.

3. RESULTS

Table 1. Paired Sample t-Test Table for the PP, AP, MP, and Fatigue Index Values of the Groups.

Group	Variables	n	X̄	Sd	t	p
Aerobic	PP (W/kg)a	10	13.46	1.05	0.553	0.097
	PP (W/kg)b	10	14.31	1.55		
	AP (W/kg)a	10	8.72	0.88	0.375	0.286
	AP (W/kg)b	10	9.78	0.79		
	MP (W/kg)a	10	4.66	1.53	0.078	0.830
	MP (W/kg)b	10	5.69	2.10		
	PD (%)a	10	64.99	1.42	0.162	0.655
	PD (%)b	10	59.35	1.49		
Anaerobic	PP (W/kg)a	10	13.15	1.01	0.351	0.320
	PP (W/kg)b	10	13.17	0.78		
	AP (W/kg)a	10	8.35	0.37	0.914	0.000**
	AP (W/kg)b	10	8.44	0.40		
	MP (W/kg)a	10	3.70	1.49	0.876	0.001**
	MP (W/kg)b	10	4.19	1.53		
	PD (%)a	10	71.9	11.49	0.812	0.004**
	PD (%)b	10	68.45	11.14		
Control	PP (W/kg)a	10	12.51	1.48	0.708	0.022*
	PP (W/kg)b	10	12.42	2.00		
	AP (W/kg)a	10	8.35	0.41	0.945	0.000**
	AP (W/kg)b	10	8.29	0.46		
	MP (W/kg)a	10	5.21	0.43	0.738	0.015*
	MP (W/kg)b	10	5.04	0.57		
	PD (%)a	10	57.89	5.85	0.738	0.015*
	PD (%)b	10	60.58	6.91		

*p<0,05; **p<0,01; PP=Peak Power (W/kg); AP=Avarage Power (W/kg); MP=Minimum Power (W/kg); PD%=Fatigue Index; a=Pre Match; b=Post Match

Table 1 The values of Peak Power (PP W/kg), Average Power (AP W/kg), Minimum Power (MP W/kg), and Fatigue Index (PD%) of subjects in different groups are presented before and after exercise. According to the statistical analysis, in the Aerobic group, the PP value increased from

13.46±1.05 before the 8-week training program to 14.31±1.55 after, the AP value increased from 8.72±0.88 to 9.78±0.79, the MP value increased from 4.66±1.53 to 5.69±2.10, and the PD value decreased from 64.99±1.42 to 59.35±1.49. In the Anaerobic group, the PP value increased from 13.15±1.01 to 13.17±0.78, the AP value increased from 8.35±0.37 to 8.44±0.40, the MP value increased from 3.70±1.49 to 4.19±1.53, and the PD value decreased from 71.90±11.49 to 68.45±11.14 after the 8-week training program. In the Control group, the PP value increased from 12.51±1.48 to 12.42±2.00, the AP value decreased from 8.35±0.41 to 8.29±0.46, the MP value decreased from 5.21±0.43 to 5.04±0.57, and the PD value increased from 57.89±5.85 to 60.58±6.91 after the 8-week training program.

Table 2. ANOVA Table Showing the Intergroup Differences in HIF-1 Values After the Initial Test Applied as Aerobic and Anaerobic Exercises

Group	Variables	n	X̄	Sd	F	p	Dif.
20 min HIF	A -Aerobic	10	36.83	7.76	8.280	0.002*	A, B > C
	B - Anaerobic	10	36.20	6.35			
	C - Control	10	27.12	2.51			
30 sec HIF	A - Aerobic	10	116.23	25.19	11.198	0.000**	A, B > C
	B - Anaerobic	10	112.46	17.63			
	C - Control	10	77.71	14.24			

*p<0,05; **p<0,01; sec=Seconds; Dif.=Diffirence

Table 2 The table illustrates the intergroup differences in HIF-1 values of subjects in different groups after the pre-test following aerobic and anaerobic exercise applications. In the pre-test applied for 20 minutes, the post-aerobic exercise HIF-1 value in both the aerobic and anaerobic groups was found to be higher than the Control group value. Similarly, in the pre-test applied for 30 seconds, the post-anaerobic exercise HIF-1 value in both the aerobic and anaerobic groups was observed to be greater than the control group value.

Table 3. ANOVA Table Showing the Intergroup Differences in HIF-1 Values After the Final Test Applied as Aerobic and Anaerobic Exercises

Group	Variables	n	X̄	Sd	F	p	Dif.
20 min HIF	A -Aerobic	10	60.77	12.81	2.548	0.970	A, B > C
	B - Anaerobic	10	60.08	7.31			
	C - Control	10	51.27	10.62			
30 sec HIF	A - Aerobic	10	166.82	20.58	22.154	0.000**	A, B > C
	B - Anaerobic	10	153.60	21.39			
	C - Control	10	114.98	10.23			

*p<0,05; **p<0,01; sec=Seconds; Dif.=Diffirence

Table 3 The table illustrates the intergroup differences in HIF-1 values of subjects in different groups after the post-test following aerobic and anaerobic exercise applications. In the post-test applied for 20 minutes, there was no significant difference in the post-aerobic exercise HIF-1 value. However, in the post-test applied for 30 seconds, the post-anaerobic exercise HIF-1 value in both the aerobic and anaerobic groups was found to be higher than the control group value.

4. DISCUSSION

The aim of this study was to compare the effects of training in different energy systems on anaerobic power and Hypoxia Inducible Factor 1 (HIF-1) in football players. In this section, the obtained results will be discussed by comparing them with domestic and foreign literature.

According to the findings from the anaerobic power test at the end of the study, it was observed that the 8-week intervention had positive contributions to PP, AP, MP, and PD values in both aerobic and anaerobic groups. The 8-week aerobic and anaerobic interventions have significantly improved the athletes' performance and their ability to sustain performance without reducing it against fatigue. This result indicates the effectiveness of both training interventions.

A study conducted by Al'Hazza et al. (2001) [5] on elite football players in different leagues found significant differences among players in different leagues. This can be attributed to the increasing physical and physiological requirements of players as the league level increases.

In a study by Embiyaoğlu (2020) [6] examining the effects of creatine supplementation on anaerobic power in amateur football players, it was found that creatine supplementation had no effect on anaerobic power.

When examining the vertical jump test applied to determine the effects of an 8-week Tabata exercise on anaerobic power and other parameters, Ünver (2022) [7] found a significant difference in the experimental group after 8 weeks. This study seems to support the results of our study, possibly due to the applied training methods and continued team practices.

Sarı (2019) [8] conducted a study examining HIF-1 values of football players, kickboxers, and sedentary individuals after aerobic and anaerobic exercises, finding a significant difference in athletes performing anaerobic exercises. This is likely because hypoxic conditions occur in anaerobic environments, leading to an increase in HIF values. Our study supports this idea as there was a chronic improvement in both aerobic and anaerobic groups.

In a study on rugby players, Pramkratok et al. (2022) [9] found positive effects of repeated sprint training on aerobic performance over 6 weeks, and this result was closely related to increased HIF-1 and Vascular Endothelial Growth Factor. These results seem to support our study.

A study by Abe et al. (2015) [10] examining the effects of High-Intensity Interval Training (HIIT) on HIF-1 and glycolytic proteins found that HIF-1 plays a significant regulatory role in the metabolic adaptation of HIIT. The increase in HIF-1 value during high-intensity interval training may be attributed to the athlete participating in the next exercise without fully recovering.

Amalia et al. (2020) [11] found in their study that HIF-1 levels in stroke patients were significantly related to the severity of ischemic lesions in both large and small vessel diseases. The higher the HIF-1 value in acute stroke patients, the longer the recovery process.

In a study by Larruskain et al. (2018) [4] examining hamstring injuries in football players over 6 seasons, it was determined that there was a relationship between HIF-1 and CC and CT genes in athletes over the age of 24. HIF-1 had a strong association with the risk of injury between CC genes and a low risk with CT genes.

A study by Kammerer et al. (2020) [12] found that HIF-1 plays a significant role in the decrease of immune response, especially in colon cancer and T-cell immunity, in hypoxic conditions.

5. CONCLUSION

In conclusion, the applied training protocols had effects on the study groups. It was found that both aerobic and anaerobic exercise interventions increased both HIF-1 and anaerobic power values.

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Conflict of Interest

No conflict of interest is declared by tehe authors. In addition, no financial support was received.

Ethics Committee

The study protocol was approved by the Ethics Committee of the Atatürk University (Ethics Committee Approval: 2023/5).

Author Contributions

Study Design, GA, FK; Data Collection, GA; Statistical Analysis, FK; Data Interpretation, GA, FK; Manuscript Preparation, GA, FK; Literature Search, GA, FK. All authors have read and agreed to the published version of the manuscript.

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