



The Supplementation of L-Arginine, When Combined with High-Intensity Interval Training, Leads to Superior Improvements in Inflammatory Status among Football Players

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ABSTRACT

Introduction: Optimizing post-exercise recovery and mitigating metabolic stress are central challenges in competitive sports. Limited data currently exist concerning the synergistic impact of high-intensity interval training (HIIT) and L-Arginine on immune-metabolic regulation in footballer players. This study aimed to compare the effects of six weeks of combined HIIT and L-Arginine supplementation on inflammatory/anti-inflammatory indices, hepatic enzymes, and key physical and skill performance factors.

Methods: Twenty male football players were randomly divided into two intervention groups: HIIT plus L-Arginine (HIIT+ARG) and HIIT plus placebo (HIIT+PL). The six-week intervention involved three training sessions per week, with progressive HIIT intensity reaching 110% of maximal aerobic speed (MAS) by weeks 5-6. The HIIT+ARG group consumed 6 grams of L-Arginine daily. Data were analyzed using covariance (ANCOVA). All statistical analyses for this study were conducted using SPSS software (version 26). The statistical significance level was set at $P < 0.05$.

Results: The results of the analysis of covariance, controlling for pre-test values, revealed a significant between-group difference at the post-test stage regarding interleukin-10 levels (IL-10) ($F = 11.31, P = 0.004$) and alanine aminotransferase ($F = 4.94, P = 0.04$); specifically, the 'exercise + supplement' group exhibited more favorable improvements compared to the placebo group. However, after adjusting for baseline values, no significant between-group differences were observed in interleukin-6 ($F = 3.20, P = 0.09$) or aspartate aminotransferase levels ($F = 2.88, P = 0.107$). Within the HIIT+ARG group, IL-6 ($P=0.002$), ALT ($P= 0.043$), and AST ($P= 0.005$) all significantly decreased, while IL-10 significantly increased ($P= 0.001$). Concurrently, this combined regimen resulted in significant functional gains: anaerobic power and VO_{2max} significantly improved, and agility and dribbling/shooting time significantly decreased (indicating performance enhancement). The HIIT+PL group showed no significant change in these markers.

Conclusion: The simultaneous use of HIIT and L-Arginine induced a significant dual-directional modulation of immune and metabolic markers, alongside notable functional gains in anaerobic capacity and agility performance. These findings underscore the specific benefit of L-Arginine as an ergogenic aid; therefore, coaches and athletes are recommended to incorporate this combined approach to enhance metabolic health and functional capacity.

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Introduction

The effective formulation of training programs tailored to specific sports relies on two essential elements: identifying the distinct physiological requirements of the sport and incorporating effective training methods to enhance athletic performance. Consequently, HIIT has garnered

considerable interest from both practitioners and researchers as a powerful approach that can simultaneously improve aerobic and anaerobic capabilities. It has firmly established itself as a fundamental conditioning method for elite football players [1]. The HIIT framework consists of repeated work intervals, typically lasting from

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short to moderate durations (10 seconds to 5 minutes), performed at an intensity that exceeds the anaerobic threshold. These work intervals are alternated with periods of low-intensity active or passive recovery, which aid in the necessary partial restoration of physiological balance [2]. A key feature of these training programs is their generally low overall training volume [3]. Research has shown that even low-volume HIIT can induce extensive systemic adaptations. These adaptations include improved substrate utilization efficiency, a significant increase in muscle glycogen reserves, and heightened maximal activity of both glycolytic and oxidative enzyme systems [4, 5]. However, published research remains inconsistent regarding changes in maximal oxygen consumption (VO_{2max}) following interventions, with some studies reporting increases while others find no significant changes. Despite the established effectiveness of HIIT, the existing literature presents mixed results concerning its overall influence on athletes' endurance and sprint performance metrics [4, 6]. Moreover, the inherently high-intensity nature of these workouts leads to considerable mechanical and metabolic stress, often resulting in significant muscle damage, biochemical disturbances, and pronounced inflammatory responses [7]. These undesirable responses can ultimately lead to decreased athletic performance, delayed recovery, and in chronic cases, overtraining syndrome [8]. One of the key ways to monitor systemic stress and fatigue resulting from training loads is by observing changes in biochemical markers. Among these markers, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are primarily recognized as hepatic enzymes but also serve a dual purpose. Their assessment is used as a marker for muscular injury and acute fatigue due to their release—albeit in smaller amounts—from damaged muscle cells, in addition to their essential role in hepatic amino acid metabolism [9]. Research indicates that athletic activities, particularly sports like football, can lead to a statistically significant increase in circulating ALT and AST levels [10, 11].

The acute inflammatory response to exercise is primarily regulated by messenger cytokines such as IL-6. This molecule is released by myotubes (muscle cells) and has historically been classified as a pro-inflammatory factor [12]. However, recent evidence redefines the post-exercise increase in IL-6—now recognized as a myokine—as a signal that effectively initiates

anti-inflammatory pathways [12, 13]. In this context, IL-10 emerges as the key anti-inflammatory cytokine, playing a crucial role in mitigating and regulating inflammation by suppressing its pro-inflammatory counterparts [14]. Consequently, tracking the dynamic balance between IL-6 and IL-10 is increasingly recognized as an essential measure for assessing an athlete's physiological recovery status and overall immunological preparedness [15]. In the quest to alleviate stress induced by training while enhancing recovery efficiency, nutritional supplementation has emerged as a key area of interest for researchers. L-Arginine, a semi-essential amino acid, plays a direct role in the synthesis pathways of nitric oxide (NO), thus improving its bioavailability [16]. Due to its strong vasodilatory properties, L-Arginine promotes enhanced oxygen delivery to active tissues and accelerates the removal of metabolic byproducts [17]. Additionally, recent studies have explored L-Arginine's ability to influence inflammatory mediators, particularly IL-6 [18]. Some research suggests that L-Arginine supplementation may reduce the significant increase in IL-6 that occurs after intense resistance training and may help restore the ideal IL-6/IL-10 ratio [17]. Despite the extensive literature outlining the performance-enhancing benefits of HIIT, the existing evidence regarding its long-term and adaptive effects on the balance of inflammatory cytokines, such as the IL-6 to IL-10 ratio, remains contentious and inconsistent, particularly in highly specialized groups like professional football players [18, 19]. Currently, biochemical and nutritional supplements are widely used in the athletic community, highlighting the need for a thorough investigation into their true effects on human performance. Among the most commonly used supplements are amino acids, particularly L-Arginine, which is recognized as a conditionally essential amino acid increasingly marketed to enhance athletes' exercise efficiency and reduce fatigue [20, 21]. L-Arginine is a vital metabolic precursor for the production of endogenous nitric oxide (NO), which promotes vasodilation by relaxing blood vessel walls and increasing blood flow to active muscles during exercise. This mechanism is expected to enhance nutrient delivery, improve oxygen distribution, and overall boost aerobic performance [22, 23]. However, despite these plausible physiological mechanisms, existing research on L-Arginine's impact on key performance metrics, such as blood lactate levels and VO_{2max} in athletes, has

consistently produced contradictory and inconsistent results [24-26].

Moreover, the specific regulatory function of L-Arginine in influencing the systemic stress response triggered by HIIT—a stress often indicated by increased hepatic enzymes (ALT and AST)—has yet to be thoroughly examined alongside inflammatory markers (IL-6 and IL-10). The main scientific gap lies in the lack of studies evaluating the effects of a combined intervention (HIIT + Arginine supplementation) on both inflammatory balance (IL-6 and IL-10) and the health of recovery-related organs, as indicated by hepatic enzymes. Such an integrated assessment is crucial for developing precise nutritional strategies aimed at enhancing training load management and expediting post-exercise recovery in elite athletes. To date, the simultaneous impact of HIIT and L-Arginine supplementation on both physical conditioning and skill-related aspects has not been investigated specifically in male football players. Consequently, this study aims to address this gap by evaluating the effects of a six-week HIIT regimen combined with L-Arginine supplementation on the levels of Interleukins 6 and 10, hepatic enzymes, physical conditioning, and skill-related factors in football players.

Materials and Methods

Study Design

The present study employed an applied research methodology, utilizing a quasi-experimental design characterized by a pre-test/post-test design. All research activities commenced following approval from Hakim Sabzevari University (Code: IR.HSU.REC.1404.012) and were registered under the clinical trial identification number IRCT20120129008863N12, in strict compliance with the Declaration of Helsinki guidelines. Before the study began, the research aims, procedural details, and potential risks and benefits were thoroughly explained to all participants, and written informed consent was obtained from each individual. The statistical population for this investigation comprised male football players aged between 18 and 20 years. After an initial screening phase, twenty semi-professional volunteer football players were selected. The inclusion criteria required participants to have at least six months of continuous football training experience, be free from any acute or chronic health conditions, and have abstained from tobacco use, as well as any non-essential sports or pharmacological supplements, for the six months preceding the study.

The final sample size was initially determined using G*Power software (version 3.1) for the primary outcome variable (changes in Interleukin 6). By configuring the analysis for a repeated measures ANOVA, with an effect size of 0.3, an alpha level of 0.05, and a statistical power of 0.80, the suggested sample size was calculated to be 24 individuals. However, due to practical constraints, the study was conducted with 20 participants. A subsequent recalculation confirmed that the statistical power of the study remains above 75% for detecting an effect size of 0.35.

The homogeneity of the two experimental groups regarding baseline descriptive characteristics (age, height, weight, and body mass index [BMI]) was evaluated through an Independent Samples T-Test during the pre-test phase. The findings indicated no statistically significant differences between the groups in these baseline variables ($P > 0.05$), thereby validating the efficacy of the randomization process. Consequently, subjects were randomized using simple random sampling, resulting in the formation of two groups, each comprising 10 participants: the HIIT+ARG group and the HIIT+PL (Placebo) group. To maintain allocation concealment, a technique involving sealed, numbered, opaque envelopes was employed. Random codes for Groups A and B were generated by an independent researcher and subsequently inserted into these envelopes. The envelopes were opened in sequence as participants entered the study, ensuring that the researchers conducting the study remained blinded to each subject's assignment until the intervention occurred. This study was designed as a double-blind trial, meaning that both the participants and the researchers were unaware of the contents of the capsules ingested (L-Arginine or Placebo) and the specific group assignment (A or B) of the subjects. Additionally, all blood samples intended for measuring markers such as IL-6, IL-10, ALT, and AST were labeled with random numerical codes before being sent to the laboratory. This crucial measure guaranteed that the laboratory analyst was also completely blinded to the participants' group allocation, effectively eliminating the possibility of measurement bias.

Training Program

Both groups of participants followed the same training regimen for six weeks, which included three sessions each week, totaling 18 sessions, with each session lasting 30 minutes. It is important to note that on days without intervention, participants engaged solely in their

regular football training. This standardized training protocol was structured around two sets throughout the entire six-week duration. The principle of overload was applied by modifying the number of repetitions over time: during Weeks 1 and 2, participants completed six one-minute repetitions per set, while from Weeks 3 to 6, this number increased to eight one-minute repetitions per set. The intensity of the work intervals within each one-minute repetition was systematically increased over the weeks: the

initial 30 seconds of each one-minute bout were executed at 100% of maximal aerobic speed (MAS) during Weeks 1 and 2, at 105% MAS during Weeks 3 and 4, and at 110% MAS during Weeks 5 and 6. The second 30 seconds of each one-minute bout, maintained throughout all six weeks, was consistently performed at 50% of MAS, serving as active recovery within the repetition. The rest period between each set was consistently set at 4 minutes (Table 1) [27].

Table 1. Training program characteristics

Group	Weeks 1-2	Weeks 3-4	Weeks 5-6
High-Intensity Interval Training	2 sets, 6 repetitions, 1 minute (30 seconds at 100% MAS and 30 seconds at 50% MAS) with 4 minutes rest between sets.	2 sets, 8 repetitions, 1 minute (30 seconds at 105% MAS and 30 seconds at 50% MAS) with 4 minutes rest between sets.	2 sets, 8 repetitions, 1 minute (30 seconds at 110% MAS and 30 seconds at 50% MAS) with 4 minutes rest between sets.

MAS Test (Maximum Aerobic Speed Test): The MAS Test evaluates the intensity of exercise. To set up the test, place 11 blue cones 20 meters apart along a 200-meter track, with red cones positioned 2 meters behind each blue cone. An examiner uses a stopwatch and a whistle to conduct the test, while a pre-recorded tape plays an auditory signal. The signals begin at a speed of 8.5 km/h and increase by 0.5 km/h every minute until the participant reaches exhaustion. Participants must cross the red cone before hearing the whistle at each 20-meter interval.

Supplementation Protocol and Compliance Monitoring

In the HIIT+ARG group, participants ingested a daily dosage of 6 grams of L-arginine capsules along with 200 ml of water. In contrast, the HIIT+PL group consumed an equivalent quantity of a placebo (cellulose) [18]. The supplement used in this research was a powder provided by the British firm Balk. The exact quantities of both the supplement and the placebo were measured using a BL2 experimental scale with a precision of 0.01 grams, produced by Kia, Iran. All supplement and placebo capsules were indistinguishable in terms of weight, size, shape, and color. The schedule for supplement consumption was as follows: on training days, capsules were taken immediately after the exercise session, while on rest days, the capsules were ingested with the main meals (breakfast and lunch). To ensure the integrity of the study, participants' compliance with the protocols was rigorously monitored. Attendance at all training sessions was carefully documented, and a minimum adherence rate of 90% (at least 17 out of 18 sessions) was required for a participant to remain in the study. Additionally, the remaining capsules were counted at the end of each week. Any participant who consumed less than 90% of the assigned capsules was subsequently excluded from the final data analysis.

Physical and Physiological Performance Assessments

The 20-meter Shuttle Run Test was used to estimate maximal oxygen consumption (VO₂ max). This test requires participants to run back and forth over a 20-meter course, following pre-recorded auditory signals. The initial running speed starts at 8.5 km/h and increases by 0.5 km/h at the end of each minute. Participants must touch the 20-meter lines with the tips of their feet.

The test ends when a participant is unable to maintain the required pace for two consecutive signals. The last completed stage is recorded, and VO₂max (expressed in ml/kg/min) is predicted using a regression equation that incorporates speed and age [28].

To estimate maximum oxygen uptake (Y ml/kg/min) from the 20-meter Shuttle Run Test, the speed (X, in km/h) corresponding to the stage (Speed = 8 + 0.5 × Stage Number) and the subject's age (A) are used in the following regression equation (Eq. 1):

$$\text{Eq. 1. } \text{VO}_{2\text{max}} = Y = 31.02 + (3.23 \times X) - (3.24 \times A) + (0.1536 \times X \times A)$$

The Illinois agility test was used to assess participants' movement capacity and their ability to quickly change direction (agility). Participants started in a prone (face-down) position and navigated a designated course that included a 10-meter sprint followed by a return and a zigzag route around four cones. Timing began on the

'Go' command and ended when the final 10-meter finish line was crossed. The total duration was recorded in seconds [29].

The 100-meter Sprint Test was conducted to measure maximal running speed and evaluate pure speed performance. Participants started from a standard position (one foot behind the line) and covered the 100-meter distance at maximal exertion. Timing began with either an electronic or manual stopwatch and stopped upon crossing the finish line. The recorded time was noted in seconds [30].

The running anaerobic sprint test (RAST) was employed to evaluate anaerobic power and fatigue capacity. This protocol consisted of six maximal 35-meter sprints (out and back runs) with a 10-second recovery interval between each repetition ($6 \times 35\text{m}$). Data from this test were used to compute key indices such as peak power, mean power, and the fatigue index [31].

The vertical jump test was conducted to assess the explosive strength of the leg muscles. Participants stood next to a wall and marked their maximum reach height (standing reach). They then jumped as high as possible without a run-up or step, marking the highest point they could reach. The difference between the maximum jump height and the standing reach height was recorded as the vertical jump height (in centimeters). The Lewis formula/nomogram was then applied to evaluate power output [32].

Football Technical Skills Assessments

The wall volley test was used to assess players' proficiency and precision in key aspects of ball control, rebound, and quick passing [33]. The implementation protocol was as follows: participants were instructed to strike or throw the ball against a wall, then control, retrieve (rebound), and send the ball back against the wall for the maximum number of repetitions possible within a designated 30-second timeframe. Players were allowed to receive and deliver the ball both from the air and the ground; however, the use of their arms or hands was strictly forbidden (except for the initiation of the trial). Each participant completed three distinct trials, and the best performance (the highest number of accurate strikes) was recorded for further data analysis.

The football dribbling test was conducted to measure the ability to perform rapid and precise ball manipulation while in motion. The subject, upon the "Go" signal, began dribbling the ball through a series of obstacles according to a predetermined course layout. After navigating around the fourth cone, the participant reversed direction and followed the identical course back

to the endpoint. Timing was stopped when the entire body of the subject had completely crossed the finish line. This assessment was repeated three times, and the fastest time recorded (measured in seconds and tenths of a second) was considered the individual's final score [34]. This comprehensive evaluation assessed the ability to dribble precisely and transition rapidly and efficiently into a final shooting execution. The testing procedure closely followed the dribbling test protocol (Section 3.4.2), with the exception that upon reaching the last obstacle of the dribbling course, the participant was required to immediately attempt a shot at a designated target area. This target area was located 14.9 meters from the end of the dribbling course, assuming that 14.9 meters was the intended measurement, as distances of 9.4 or 4.9 meters may be too close for a definitive football shooting evaluation. Performance metrics included the total time taken to navigate the dribbling course and the accuracy and speed of the shot directed at the goal, which were assessed based on a specific sub-protocol.

The 300-meter run with ball test was conducted to evaluate specific endurance, which combines running speed with the ability to maintain ball control while fatigued. At the "Go" command, the subject dribbled a ball along a 50-meter course marked by two cones. The participant completed this 50-meter segment six times (three laps out and three laps back) to cover the full 300 meters. The total time was measured in seconds and tenths of a second, with a lower time indicating better speed-endurance while controlling the ball.

Functional Movement Screening (FMS) Assessment

For the functional performance screening, the assessments developed by Cook et al. [35], known as the functional movement screen (FMS), were employed. This comprehensive screening consists of seven fundamental movement patterns: the deep squat, hurdle step, in-line lunge, shoulder mobility, active straight leg raise, trunk stability push-up, and rotary stability, along with three related clearing tests.

Prior to testing, each participant received essential explanations and verbal instructions for each movement pattern, followed by one practice trial. To ensure accurate evaluation, assessors positioned themselves equidistant from the participant in three directions: anterior, posterior, and lateral.

Participants executed each movement pattern three times. For unilateral patterns (performed on one side), the best score from the three

repetitions for each side was recorded, and the lower of the two best scores was taken as the overall score for that pattern. In contrast, for bilateral patterns, the highest score from the three attempts was noted.

Each movement pattern was assessed using a standardized four-level scoring rubric, resulting in scores ranging from zero to three. A score of 3 was assigned for perfect execution without compensatory actions. A score of 2 was given for successful completion that involved compensatory movements, indicating a compromise in functional efficiency. A score of 1 was assigned when the participant could not fully complete the movement or return to the starting position without compensation. The lowest score of 0 was given for movement execution accompanied by self-reported pain or a positive result from the associated clearing test.

For the deep squat, hurdle step, in-line lunge, and rotary stability tests, participants received 3 points for correct execution without compensatory actions and 2 points if compensatory movements were observed. A score of 1 was given for failure to perform the movement without compensations, while any report of pain during the movement or a failed clearing test resulted in a score of 0.

Blood Sampling and Biochemical Measurements

Blood sampling occurred at two key time points: the pre-test (72 hours before the intervention began) and the post-test (48 hours after the final training session). Both sampling phases were conducted at a specific time (8:00 a.m.) following a mandatory 12-hour overnight fast. The concentrations of IL-6 and IL-10 were measured using the enzyme-linked immunosorbent assay (ELISA) method. This procedure utilized a commercial zellBio kit (Germany, Serial No. ZB-15126C-H9648), which exhibited an intra-assay

coefficient of variation of less than 10% and a sensitivity below 0.5 pg/mL. The hepatic enzymes ALT and AST were assessed through standard photometric methods using an auto-analyzer and kits from Pars Azmoon.

Statistical Analysis

Data analysis was performed using SPSS statistical software (version 26). Initially, the Shapiro-Wilk test was conducted to evaluate the normality of data distribution, and Levene's test was employed to ensure the homogeneity of variances. Upon confirming the assumptions of parametric statistics, an independent samples t-test was utilized to compare demographic characteristics and baseline indices between the two groups during the pre-test phase. Furthermore, a paired samples t-test was applied to assess intra-group changes by comparing pre-test and post-test values. Finally, to compare the post-test means between the groups while controlling for the influence of pre-test scores as a covariate, analysis of covariance (ANCOVA) was performed. To evaluate the magnitude of the treatment effect, partial eta squared (η^2) was calculated and reported as the effect size index. The interpretation of these values was based on Cohen's benchmarks, where $\eta^2 = 0.01$ indicates a small effect, $\eta^2 = 0.06$ represents a medium effect, and $\eta^2 = 0.14$ denotes a large effect. This approach provides a robust assessment of the practical importance of the observed differences between the experimental and control groups. For all analyses, the level of statistical significance was set at $p < 0.05$.

Results

The descriptive characteristics of the study participants, including the mean and standard deviation for variables such as age, height, weight, and body mass index, are presented in Table 2.

Table 2. Descriptive characteristics of the research groups

Groups	Variables (Mean \pm SD)			
	Age (years)	Height (meters)	Weight (kg)	Body Mass Index (kg/m ²)
Exercise + Supplement	18.60 \pm 0.84	1.72 \pm 0.04	60.30 \pm 4.57	20.30 \pm 1.26
Exercise + Placebo	18.90 \pm 0.87	1.72 \pm 0.05	63.08 \pm 8.13	21.14 \pm 20.06
Significance Level*	0.445	0.896	0.359	0.287

*Acceptable significance level; The two groups were homogeneous in terms of descriptive characteristics at the pre-test.

To ensure the homogeneity of the groups prior to the intervention, an Independent Samples T-test was conducted for the comparison of all baseline variables. The results of this test clearly demonstrated that there were no statistically

significant differences ($P > 0.05$) between the HIIT+ARG (training + supplement) group and the HIIT+PL (training + placebo) group across any of the descriptive baseline variables. This confirmed initial homogeneity is crucial, as it

strengthens the internal validity of the quasi-experimental design for a more reliable assessment of the intervention effects. The results of the ANCOVA, after adjusting for pre-test values, revealed a statistically significant difference between the two groups in the post-test levels of IL-10 ($F = 11.31$, $P = 0.004$) and ALT ($F = 4.94$, $P = 0.04$). Specifically, the exercise + supplement group experienced significantly greater improvements compared to the placebo group. However, after controlling for baseline values, no significant between-group differences were observed in the levels of IL-6 ($F = 3.20$, $P =$

0.09) or AST ($F = 2.88$, $P = 0.107$). Furthermore, significant reductions were also exclusively observed in this group for the key hepatic enzymes, AST ($P=0.005$) and ALT ($P=0.043$). These findings strongly suggest that the L-Arginine supplementation plays a critical synergistic role alongside HIIT in modulating the inflammatory response and improving liver function indices. Full details of these findings, including effect sizes and statistical values, are provided in Table 3.

Table 3. Comparison of mean intra- and inter-group changes in inflammatory and pro-inflammatory indices in football players

Variables	Groups	Stages		Within group mean changes		η^2
		Pre-test M \pm SD*	6 th Week M \pm SD*	P-Value α	Percentage of changes	
Interleukin 6 (pg/ml)	Exercise + supplement	7.41 \pm 1.58	5.31 \pm 0.64	0.002 α	-39.54	0.159
	Exercise + placebo	6.06 \pm 1.33	6.55 \pm 1.97	0.531	7.48	
P-Value β (Between groups)		0.07	0.09			
Interleukin 10 (pg/ml)	Exercise + supplement	15.00 \pm 2.00	18.70 \pm 2.11	0.001 α	19.78	4.00
	Exercise + placebo	16.30 \pm 4.83	17.30 \pm 4.34	0.096	5.78	
P-Value β (Between groups)		0.37	0.004 β			
AST (UI/L)	Exercise + supplement	25.60 \pm 6.53	22.30 \pm 5.29	0.005 α	-14.79	0.145
	Exercise + placebo	23.20 \pm 3.32	23.10 \pm 4.67	0.938	-0.43	
P-Value β (Between groups)		0.72	0.10			
ALT (UI/L)	Exercise + supplement	17.50 \pm 5.56	14.40 \pm 2.83	0.043 α	-21.52	0.225
	Exercise + placebo	17.10 \pm 4.22	16.40 \pm 3.83	0.226	-4.26	
P-Value β (Between groups)		0.20	0.04 β			

† A significant level $P < 0.05$

*Data presented as mean \pm standard deviation

α : denotes the p-value derived from the paired samples t-test for assessing intra-group changes, while β : represents the p-value obtained from the independent samples t-test and ANCOVA for evaluating between-group comparisons.

The statistical analysis indicated that the times \times group interaction effect was not significant for the majority of the athletic performance indices, including 100-meter sprint speed, anaerobic power, VO₂max, and agility. Nevertheless, the examination of within-group changes revealed several notable improvements. Specifically, maximum anaerobic power significantly increased ($P=0.022$) exclusively within the HIIT+ARG (training + supplement) group. Maximal oxygen consumption VO₂max showed a significant increase in both groups ($P=0.001$), demonstrating the overall effectiveness of the HIIT protocol. Furthermore, a significant improvement in agility time was observed specifically in the HIIT+ARG group ($P=0.039$). These findings suggest that while the supplement did not universally enhance all performance measures across groups, it did confer specific benefits in maximum anaerobic power and

agility. The comprehensive details of these findings are summarized in Table 4.

The analysis of factors related to technical skills and functional capacity indicated that the Time \times Group interaction effect did not reach statistical significance for any of the assessed variables. This included skill tests, dribbling and shooting time, 300-meter sprint with the ball, general dribbling performance, and the overall Functional Movement Screen score. However, despite the lack of a significant interaction, the examination of within-group changes revealed that the recorded time for dribbling and shooting as well as the general dribbling test significantly decreased in both groups (HIIT+ARG and HIIT+PL). These results suggest that the HIIT training protocol itself was effective in enhancing fundamental technical and motor skills, independent of the L-Arginine supplementation.

Table 4. Comparison of within-group and between-group changes in the means of physical fitness tests in football players

Variables	Groups	Stages		Within group mean changes		
		Pre-test M±SD*	6 th Week M±SD*	P-Value ^α	Percentage of changes	η ²
100-meter Speed (seconds)	Exercise + supplement	15.11±1.07	14.75±0.81	0.211	-2.44	0.04
	Exercise + placebo	15.35±1.07	15.20±1.29	0.328	-0.98	
P-Value ^β (Between groups)		0.627	0.412			
Anaerobic Power (Watts)	Exercise + supplement	520.25±112.91	559.14±115.20	0.022 ^α	6.95	0.02
	Exercise + placebo	500.48±100.88	526.39±118.93	0.153	4.92	
P-Value ^β (Between groups)		0.685	0.568			
VO2max (mL/kg/min)	Exercise + supplement	52.90±5.36	54.61±4.73	0.005 ^α	3.13	0.03
	Exercise + placebo	51.23±3.90	52.79±3.38	0.001 ^α	2.95	
P-Value ^β (Between groups)		0.437	0.419			
Vertical jump (cm)	Exercise + supplement	50.10±6.04	50.40±6.05	0.279	0.59	0.005
	Exercise + placebo	45.70±8.75	46.30±8.48	0.217	1.29	
P-Value ^β (Between groups)		0.207	0.775			
Agility (seconds)	Exercise + supplement	15.66±0.36	15.24±0.54	0.039 ^α	-2.75	0.01
	Exercise + placebo	15.37±0.54	15.10±0.71	0.087	-1.78	
P-Value ^β (Between groups)		0.181	0.679			

† A significant level P<0.05 *Data presented as mean ± standard deviation α: denotes the p-value derived from the paired samples t-test for assessing intra-group changes, while β: represents the p-value obtained from the independent samples t-test and ANCOVA for evaluating between-group comparisons.

Table 5. Comparison of within-group and between-group changes in the means of skill tests and functional movement screening scores in football players

Variables	Groups	Stages		Within group mean changes		
		Pre-test M±SD*	6 th Week M±SD*	P-Value ^α	Percentage of changes	η ²
Wall Volley (Repetitions in 30s)	Exercise + supplement	23.60±5.16	24.50±4.42	0.350	3.67	0.00
	Exercise + placebo	23.10±3.92	24.20±4.54	0.253	4.54	
P-Value ^β (Between groups)		0.810	0.942			
Dribbling and Shooting (Seconds)	Exercise + supplement	11.02±0.98	10.33±1.10	0.039 ^α	-6.67	0.038
	Exercise + placebo	11.71±1.38	11.19±1.35	0.018 ^α	-4.64	
P-Value ^β (Between groups)		0.215	0.421			
300-meter Run with Ball (Seconds)	Exercise + supplement	1.08±0.04	1.08±0.04	0.545	0.00	0.012
	Exercise + placebo	1.08±0.03	1.08±0.03	0.904	0.00	
P-Value ^β (Between groups)		0.870	0.656			
Dribbling (Seconds)	Exercise + supplement	9.16±0.80	8.67±0.89	0.016 ^α	-5.65	0.014
	Exercise + placebo	9.78±1.24	9.37±1.26	0.02 ^α	-5.50	
P-Value ^β (Between groups)		0.202	0.629			
FMS	Exercise + supplement	19.10±1.79	19.20±1.54	0.343	0.52	0.024
	Exercise + placebo	18.40±1.17	18.90±1.28	0.213	2.64	
P-Value ^β (Between groups)		0.315	0.523			

† A significant level P<0.05 *Data presented as mean ± standard deviation α: denotes the p-value derived from the paired samples t-test for assessing intra-group changes, while β: represents the p-value obtained from the independent samples t-test and ANCOVA for evaluating between-group comparisons.

The complete details of these findings, including all variables and their respective significance levels, are concisely presented in Table 5.

Discussion

The main objective of this study was to conduct a comparative analysis of the effects of a six-week HIIT program, supplemented with additional

nutrients, on inflammatory and anti-inflammatory markers, liver enzyme levels, physical fitness indicators, skill-related factors, and FMS scores among male football players. The findings of the present study demonstrated that the changes in IL-10 levels were statistically significant. Furthermore, at the conclusion of the intervention, IL-6 levels in the exercise plus supplement group exhibited a significant decrease ($P = 0.002$), representing a reduction of approximately 39.54%. Conversely, IL-10 levels significantly increased by the end of the intervention period in the same group ($P = 0.001$), reflecting an enhancement of approximately 19.78%. These findings align with the results reported by Irandoust et al. [18] and Nascimento et al. [17]. For instance, Irandoust et al. [18] investigated the impact of six weeks of HIIT combined with supplementation on IL-6 and body composition in young males, finding a significant reduction in inflammation in both the Exercise + and Exercise + Placebo groups compared to others. Similarly, Nascimento et al. [17] found that supplementation reduced the exercise-induced rise in cytokines after acute resistance training, significantly lowering peak IL-6 levels and lessening the exercise-induced drop in IL-10 levels. Conversely, the current results challenge the findings of Cullen et al. [19] and Martina et al. [36], the latter of whom noted no changes in inflammatory markers following treatment. The conflicting findings observed may stem from a combination of factors, particularly the inherent individual differences among participants, such as sex, baseline fitness level, and health status. Variations in the HIIT protocols used—such as exercise intensity, session duration, cycle repetition count, and rest intervals—also contribute, along with the overall duration of the training period. Researchers generally agree that exercise plays a crucial role in regulating and managing inflammation through three primary mechanisms: reducing visceral fat accumulation, increasing the synthesis and production of anti-inflammatory cytokines, and decreasing pro-inflammatory cytokines [37]. Additionally, weight loss achieved through physical activity serves as another mechanism for reducing inflammation [38], as this weight loss can lead to a decrease in cytokines derived from adipose tissue [39]. Therefore, it can be suggested that involving participants in a training program that effectively promotes weight loss will similarly lead to a reduction in systemic levels of inflammatory cytokines [19].

The concurrent modulation of IL-6 and IL-10 by L-Arginine suggests that the supplement

effectively activates a cellular signaling cascade. This effect primarily stems from L-Arginine's crucial role in producing Nitric Oxide (NO) and its related metabolic pathways. One way IL-6 levels are reduced is through the suppression of the NF- κ B pathway, which is typically activated in response to muscle damage and oxidative stress from HIIT. The increase in NO, generated by the Nitric Oxide Synthase (NOS) enzyme that utilizes L-Arginine, can inhibit the activation of the NF- κ B nuclear factor. Since NF- κ B acts as a master transcription factor for many pro-inflammatory genes, including IL-6, its suppression directly accounts for the observed anti-inflammatory effects [40]. As a result, the increase in Nitric Oxide directly inhibits the production signal within myocytes and immune cells, effectively reducing systemic inflammation. Additionally, a second, equally important pathway contributing to the observed rise is linked to the polarization of macrophages. This metabolic pathway not only diminishes the initial inflammatory response but also actively enhances anti-inflammatory and repair mechanisms. The subsequent increase in a powerful inhibitory cytokine is likely a result of the macrophage phenotype transition occurring at the site of tissue damage. This pathway is recognized for promoting the differentiation of macrophages from a pro-inflammatory to an anti-inflammatory phenotype [41]. Macrophages serve as the primary sources of the anti-inflammatory phenotype, which is essential for suppressing the inflammatory cascade and initiating tissue repair processes. This dual effect suggests that successfully regulates the two main phases of the immune response—shortening the acute inflammatory phase and hastening the transition into the recovery and repair phase—an outcome that is highly beneficial for athletic functional recovery.

The results of the present study indicated that the changes in AST levels were not statistically significant; however, ALT levels exhibited a significant change by the end of the intervention period. Nevertheless, the analysis of within-group changes revealed significant findings: by the conclusion of the six-week training intervention, the Exercise + Supplement cohort recorded a significant reduction in both AST ($p=0.005$) and ALT ($p=0.043$) levels. These decreases corresponded to approximate reductions of 14.79% and 21.52%, respectively, at the end of the intervention period. These specific results find partial corroboration in the systematic review conducted by Fateh et al. [42], which encompassed seventeen studies. Fateh and colleagues reported that supplementation

significantly ameliorated the lipid profile by reducing total cholesterol, LDL, HDL, and triglycerides. Crucially, their findings also showed a non-significant trend towards reduced levels of AST and ALT. Abassi et al. [43] examined the effects of a nine-week HIIT protocol, consisting of three sessions per week, on hepatic enzymes without caloric restriction. The notable within-group decline observed in the exercise + supplement group suggests stronger metabolic adaptations and enhanced cellular protection in this cohort. One of the documented protective mechanisms is its ability to safeguard muscle cell membranes. After intense exercise, such as HIIT, the release of AST and ALT into the bloodstream typically increases due to cellular membrane damage and the leakage of cytosolic contents. This effect is mitigated by enhancing Nitric Oxide production, which improves localized blood flow to the muscles [44].

This enhancement in vasodilation improves oxygen delivery and the supply of essential nutrients, such as glucose and amino acids, needed for tissue repair, ultimately reducing secondary injuries caused by ischemia and subsequent reperfusion. By limiting these primary insults, the leakage of intracellular enzymes is also minimized. Additionally, the metabolic byproducts associated with these processes have inherent antioxidant properties [45]. HIIT produces a significant amount of free radicals that can damage and destabilize cellular membranes. By reducing oxidative stress in both muscle and liver cells, the supplement helps maintain membrane integrity and decreases enzyme efflux. At the same time, HIIT acts as a strong stimulus for biochemical adaptations in muscles, leading to increased mitochondrial biogenesis and improved buffering system efficiency. This boost in mitochondrial density and function enhances the muscles' ability to utilize oxygen, thereby reducing reliance on anaerobic metabolism during high-intensity intervals [45, 46]. As a result, this metabolic conditioning lessens the systemic stress from each training session, decreasing the need for extensive muscle repair, which is crucial for minimizing the leakage of cellular damage markers.

The findings demonstrated that the interaction effect of time by group did not achieve statistical significance for most of the athletic performance variables assessed, including 100-meter sprint velocity, anaerobic power output, maximal oxygen uptake, lower-body explosive power, and overall agility performance. However, analysis of within-group changes yielded noteworthy

results: the Exercise + Supplement cohort recorded a significant increase in anaerobic power, reflecting a rise of approximately 6.95%. Additionally, significant gains in maximal oxygen uptake were observed in both cohorts by the end of the intervention period, with increases of approximately 3.13% and 2.95% for the Exercise + Supplement and Exercise + Placebo groups, respectively. Finally, a statistically significant decrease in agility time, indicating improved performance, was specifically noted within the Exercise + Supplement group. The enhanced aerobic capacity observed after implementing HIIT likely results from a combination of peripheral and central adaptations. These significant physiological changes include improved oxygen transport in the vascular system, increased capillary density, and a notable rise in mitochondrial content within active muscles. Additionally, low-volume HIIT protocols promote extensive metabolic adaptations, such as improved fuel efficiency, increased activity of both oxidative and glycolytic enzymes, and higher glycogen content in skeletal muscle. Together, these cellular changes enhance the muscles' ability to utilize oxygen effectively and generate energy efficiently through both aerobic and anaerobic pathways. At the same time, improvements in anaerobic power output are likely due to neuromuscular adaptations, including increased motor unit recruitment, as well as enhanced muscle buffering capacity and elevated glycolytic enzyme activity associated with anaerobic metabolism [5, 47, 48]. The current findings suggest that combining the supplement with HIIT has an additive effect on enhancing performance indicators. This effect can be explained by the supplement's presumed influence on the nitric oxide pathway. As a precursor to nitric oxide, it supports the production of this powerful vasodilator, which significantly increases blood flow to the active muscles. This enhanced blood flow improves the delivery of oxygen and essential nutrients. The increased oxygen supply, particularly during high-intensity exercise, likely helps delay the onset of fatigue, accelerates the clearance of harmful metabolites like lactate, and ultimately enhances athletes' ability to sustain power output. However, caution is warranted in interpreting these findings due to several limitations in the study design. These limitations include challenges in controlling for various dietary habits, differences in individual responses to physical activity, a small sample size due to participant attrition, and the lack of molecular investigations at the muscle tissue

level. One of the primary structural challenges in the present study is the limited diversity of control groups, specifically the absence of supplement-only or sedentary control groups. Although the current two-group design—comprising exercise plus supplementation versus exercise plus placebo—was centrally aimed at assessing the synergistic interaction between L-arginine and the HIIT protocol, the lack of additional reference groups precludes a precise isolation of the supplement's independent physiological effects in the absence of a training stimulus. Nevertheless, considering the competitive nature of semi-professional football and the imperative to maintain players' physical fitness during the season, establishing a non-training group would have presented significant ethical dilemmas and practical operational hurdles. Furthermore, the final sample size was reduced to 20 participants due to unforeseen subject attrition and the logistical complexities of coordinating with the team, which necessitates caution when generalizing the findings. Consequently, future research should utilize larger cohorts and employ factorial designs to more comprehensively explore the reciprocal effects of exercise and supplementation across varying levels of physical fitness.

Conclusion

In summary, this investigation's findings confirm the immunomodulatory potential of combining supplementation with HIIT. The Exercise + Supplement cohort demonstrated a statistically significant and crucial dual-directional shift in key biomarkers: levels of IL-6, ALT, and AST all significantly decreased, while the anti-inflammatory cytokine IL-10 significantly increased. Additionally, this group showed notable improvements in various performance metrics, including anaerobic power and agility. In contrast, the Placebo + Exercise group did not exhibit similar changes in these inflammatory and hepatic indices, underscoring the specific benefits of the supplement. Given the robust positive effects on enhancing anti-inflammatory status, improving liver enzyme profiles, and boosting athletic performance in football players, it is strongly recommended that sports scientists, coaches, and athletes incorporate this combined regimen into their training and nutritional strategies for optimal recovery and performance gains.

Declarations

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The execution of this research was entirely self-funded by the student.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the present research.

Author Contributions

MS: Project Management & Design, Project Execution, Data Collection, Results Interpretation, Manuscript Drafting, Final Manuscript Approval; KH: Project Management & Design, Data Analysis, Results Interpretation, Manuscript Drafting, Final Manuscript Approval; HM: Results Interpretation, Final Manuscript Approval

Ethical Considerations

All research activities commenced following approval from Hakim Sabzevari University (Code: IR.HSU.REC.1404.012) and were registered under the clinical trial identification number IRCT20120129008863N12, in strict compliance with the Declaration of Helsinki guidelines.

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Data Availability

The data used in this work are protected by confidentiality clauses from ethics approval and cannot be shared publicly.

Declaration of Artificial Intelligence (AI)

During the preparation of this work, the authors used ChatGPT 3.5 to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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