

The Effects of BCAA Supplementation on Muscle Damage Following a Lower-Body Resistance Exercise Bout in Soccer Players

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The purpose of this study was to determine the effect of two different dosages of BCAA supplementation on muscle damage in conjunction with a lower-body resistance exercise bout in soccer players. 30 male soccer players participated in a double blinded designed study conducted during exercise protocol. All subjects in a randomized and double-blind design were divided into three groups. All subjects were participated in lower- body resistance exercise bout (6 sets, 10 repetitions, 70% 1RM). The BCAA was given at doses of 200, 450 mg.kg⁻¹ BW for supplemental groups 1 and 2, respectively, 30 minutes before and after to exercise tests and carbohydrate (dextrin) was given at dose of 200 mg.kg⁻¹ BW for placebo group. To identify enzymes activity (IU/L), venous blood samples were obtained 30 minutes prior to exercise and at 24 and 48 hours post exercise.

Data were statistically analyzed using 2-way repeated measure ANOVA and Bonfferoni test. Baseline CK and LDH were determined 30 minutes before the exercise test. Baseline serum values for CK, LDH were not different between groups. However there were significant increases ($p < 0.05$) between the pre exercise and post exercise values for CK, LDH from 24 hours to 48 hours posttest, but there were no significant differences between two groups ($p < 0.05$).

These results suggested that two different dosages BCAA supplementation did not affect muscle damage during lower- body resistance exercise bout 48 hours later in soccer players.

Keywords: branch-chain amino acid, resistance exercise, soccer, lower body, muscle damage

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1. Introduction

Exercise-induced muscle damage has been reported to result in a number of local and systematic changes including disruptions to the sarcolemma, myofibres and excitation-contraction coupling processes, swollen mitochondria and an elevation of muscle proteins in the blood and as well as force production decrements and neuromuscular deficits (muscle inactivation) (Friden et al., 1983; Behm et al., 2001).

The area of exercise-induced muscle fibre injury has been discussed in detail in several recent reviews

(Allen et al., 2005; Close et al., 2005). It is clear from the literature that unaccustomed or intense exercise such as extended periods of running, strength training or sprinting, especially those incorporating eccentric exercises, can lead to disruption of the normal muscle ultrastructure and impairment of muscle function (Proske and Morgan, 2001; Huard et al, 2002). These changes are accompanied by an increase in muscle proteins in blood, which are useful markers of skeletal muscle injury. It is thought to result from damage to muscle and/or connective tissue. In attempting to quantify this damage, creatine kinase (CK) and lactate

dehydrogenase (LDH) have been used as blood measurements of intramuscular enzymes (Candow et al., 2006; Koba et al., 2007)

Resistance exercise induces various physiological events to occur within the body, with many of these changes occurring within skeletal muscle. Muscle protein synthesis is stimulated in the recovery period after resistance exercise. However, the rate of muscle protein breakdown is also increased, thereby blunting the change in the net balance between synthesis and breakdown. Although net muscle protein balance is generally improved after resistance exercise, it remains negative. In addition, nutrient intake is necessary to achieve positive net muscle protein balance, leading to muscle protein breakdown and damage (Borsheim et al., 2002; Cramer et al., 2004). Therefore, a nutritional supplement that can achieve a positive net muscle protein balance after eccentric exercise has the potential to improve muscle recovery after injury. Dietary proteins, in particular branched chain amino acids (BCAA), have an important role in the regulation of protein metabolism in skeletal muscle (Evans, 2001; Karlsson et al., 2004; Hakan et al., 2004). BCAA (leucine, isoleucine, and valine), and in particular leucine, have anabolic effects on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation in resting human skeletal muscle. During recovery from exercise, BCAA have an anabolic effect in human skeletal muscle (Blomstrand and Saltin, 2001; Hakan et al., 2004).

As a result, research into dairy protein supplementation as an ergogenic aid has received an increasing amount of attention in recent years (Blomstrand et al., 2006; Jackman et al., 2009; Shimomura et al., 2010). Recent studies have demonstrated that BCAA supplementation administration before and during endurance exercise may attenuate muscle damage. As an example, Greer et al. (2007) study has been shown that acute ingestion of 50g of BCAAs resulted in significantly lower CK levels at 4, 24, and 48 hours following a cycling protocol of 90 minutes at 55% of VO₂ peak. In addition, results from Koba et al. (2005, 2007) reported that consuming BCAAs during and in the days following running exercise had effect on CK and LDH levels. In a more recent study Shimomura et al. (2010), administered 100 mg/kg body weight of BCAAs or a placebo to women (21–24 years of age) prior to resistance exercise and found that muscle

soreness was lower after BCAA ingestion compared to a placebo. Conversely, Jackman et al. (2009) reported that acute BCAA ingestion did not attenuate serum levels of CK following a bout of unilateral eccentric exercise. Study of Zebblin et al. (2007) showed that BCAA supplementation administration before mild resistance exercise had no effect on serum CK activity. However, few studies have examined the effects of multiple doses BCAA supplementation on sermic indices of muscle damage after lower-body resistance exercise. Therefore, the purpose of this research was to determine the effects of two different doses of BCAA supplementation on sermic indicators of muscle damage following a lower-body resistance exercise in soccer players.

2. Methods

2.1. Subjects

Thirty young soccer players (age: 20.2±0.6 yr, weight: 74.2±2.4 kg, height: 177.4±1.1 cm, skin fold %16.2±%0.8, Mean ±SE) took part in this research. All subjects in a randomized and double-blind design were divided into three groups: supplemental groups 1 and 2 and placebo group. They had three training sessions per week. They also were nonsmokers, and used no medicinal drugs, dietary supplements, or anabolic steroids within the previous six month. All subjects signed the written informed consent to participate in the study. The study protocol was approved by the ethics committee of Tehran University of Medical Sciences. The study was conducted in accordance with Helsinki declaration and guideline of Iranian Ministry of Health and Medical education.

2.2. Nutritional design and supplements

Approximately one week after completion of the initial measurements the 9 days testing period began. The supplemental groups were required to take the BCAA supplementation, comprised of 50% leucine, 25% isoleucine, 25% valine (Pooyan Nutrition Company), for 9 days. The dosage were 68 mg.kg⁻¹ three times a day (with breakfast, lunch and dinner), with an additional 200 and 450 mg.kg⁻¹ given directly 30 minutes before and after the exercise test for supplement groups 1 and 2, respectively. The placebo group consumed 68 mg.kg⁻¹ (carbohydrate)

dextrin three times a day, instead of BCAA, with an additional 200 mg.kg⁻¹ given directly 30 minutes before and after the exercise test. Dosages were based on manufacturer's recommendations and previous human BCAA supplementation studies (Coombes et al., 2000). The exercise test was administered on day seven.

2.3. Dietary Analysis

The dietary food logs were evaluated using a dietary assessment food software program (Food Processor® SQL, ESHA Research, Salem, OR) to determine the average daily caloric (CAL) intake and macronutrient breakdown of carbohydrates (CHO), protein (PRO) and fat (FAT). The subject's dietary intake was not standardized for this investigation; however, subjects were instructed to maintain their normal dietary habits for the duration of the study. Subjects in all groups were instructed to keep a 9-day food logs, which began 6 days prior to the resistance exercise protocol and was continued until the final post-testing session. This was done to ensure that there was no significant difference in macronutrient intakes between experimental groups, particularly protein levels that could significantly alter total BCAA intake during the study.

2.4. Exercise protocol

Subjects were requested to avoid exercise the day of the test session. Subjects were free of systemic illness at the day of testing. The exercise session consisted of 3 different resistance exercises: the exercise order consisted of the leg press, knee extension and knee flexion. Subjects performed six sets of 10 repetitions at 70% 1RM for each resistance exercise. One-minute rest intervals separated sets for each sets and three-minute rest intervals were given after the completion of each exercise.

2.5. Experimental design

The protocol was designed to determine whether an oral BCAA supplementation effect on muscle damage if given before and after a resistance exercise bout. Body composition was estimated using the sum of three skinfolds (chest, abdomen, and thigh) following the procedures outlined by Jackson and Pollock. At least 1 week before the initial infusion

study, each subject was familiarized with resistance exercise, and their one-repetition maximum (1RM, the maximum weight that can be lifted for one repetition) was determined for each exercise. A warm-up protocol was consisted of loads equal to 30% (8-10 repetitions), 50% (4-6 repetitions), 70% (2-4 repetitions) and 90% (1 repetition) of an estimated 1RM. Subjects were given up to four maximal attempts to achieve their 1RM. Rest periods of 3 to 5 minutes were given between trials. Subjects were instructed to perform a 10 minute warm-up on a cycle ergometer. Baseline muscle damage was evaluated using the measurements of serum CK, LDH levels and muscle soreness. Baseline muscle soreness was evaluated using the visual analogue scale (VAS) which has been utilized as a valid indicator of pain in several studies (Nosaka et al., 2006; James et al., 2008; Shimomura et al., 2010) has correlated with other indices of muscle damage including MVC, and CK (Nosaka et al., 2006), and has obtained reported reliability scores as high as $r = 0.97$ for assessing soreness (Bijur et al., 2001).

2.6. Serum analyses

Serum was obtained from centrifugation of whole blood samples after allowing for clotting (1 hour) at room temperature (25 °C). A spectrophotometer (Kodak Ektachem DT60) was used to determine serum CK and LDH from 10 µl of serum. Analyses were carried out according to the Kodak Ektachem protocol manual with the analyzer calibrated daily using Kodak standards. In our hands, the coefficients of variation for CK and LDH assays are 3 and 5% respectively.

2.7. Collection of blood samples

Venous blood samples were drawn by antecubital venipuncture 30 minutes before the bout and at the 24 and 48 hours after the bout. The blood was immediately centrifuged at 1500 RCF for 10 minutes at 4°C, and the plasma was separated and stored in Eppendorf tubes at -70°C for subsequent use. Plasma samples were used for measurements of LDH and CK activity.

2.8. Statistical Analyses

The Kolmogorov-Smirnov test of normality

revealed that none of the variables studied required logarithmic transformation. Values are expressed as the mean \pm SE. and were compared with 2-way repeated measure ANOVA. Bonferroni test was used in order to learn which measurement time the difference comes from. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Subject characteristics

There were no significant differences among groups for age, height, bodyweight, percent body fat, 1RM leg press, 1RM knee extension, and 1RM knee flexion (**Table 1**). 9-day dietary analysis (excluding supplementation) revealed no difference in energy, protein, fat and carbohydrate intake between groups throughout the study (**Table 2**).

3.2. Serum activity of Creatine kinase

Mean serum CK levels before and 24 and 48 hours after the lower-body resistance exercise is presented in **Figure 1**. A significant main effect was seen ($F = 10.629$, $p < 0.01$) with no significant interaction ($F = 1.246$, $p = 0.324$). Serum CK activity was elevated in three groups 24, 48 hours after lower-body resistance exercise. Approximately 24 hours post resistance exercise, CK activity reached peak levels of 659.8 ± 154.8 U/l for the placebo-supplemented group, 607.5

± 155.4 U/l for the high dosage BCAA supplemented group and 625.4 ± 153.4 U/l for the low dosage BCAA supplemented group. CK was significantly elevated in all groups at the 24 and 48 hours, but did not reach statistical significance. However, there was a highly significant group by time interaction for CK activity ($p < 0.01$), BCAA supplementation intake did not affect serum CK levels.

3.3. Serum activity of lactate dehydrogenase

Similar elevations were also observed in the serum LDH activity. Mean serum LDH levels before and 24 and 48 hours after the lower-body resistance exercise is presented in **Figure 2**. A significant main effect was seen ($F = 6.566$, $p < 0.01$) with no significant interaction ($F = 1.397$, $p = 0.265$). Serum LDH activity was elevated in three groups 24 and 48 hours after lower-body resistance exercise bout. Approximately 24 hours post resistance exercise, LDH activity reached peak levels of 470.8 ± 41.4 U/l for the placebo-supplemented group and 447.6 ± 155.4 U/l for the high dosage BCAA supplemented group and 457.3 ± 30.06 U/l for the low dosage BCAA supplemented group. LDH was significantly elevated in the all groups at the 24 and 48 hours, but did not reach statistical significance. However, there was a highly significant group by time interaction for LDH activity ($p < 0.01$), BCAA supplementation intake did not affect serum LDH levels.

Table 1 Subject Characteristics (N = 30)

Characteristics	Supplementation1	Supplementation2	Placebo	p
Age (yr)	20.2 \pm 0.9	20.1 \pm 0.7	20.3 \pm 0.6	0.63
Height (cm)	177.3 \pm 1.1	178.5 \pm 1.2	176.2 \pm 1.0	0.81
Weight (kg)	74.1 \pm 2.3	75.3 \pm 2.9	74.9 \pm 2.6	0.3
% Body Fat	16.1 \pm 0.9	16.3 \pm 0.8	16.2 \pm 0.7	0.7
1RM leg press (kg)	227.1 \pm 12	231.4 \pm 11.1	235.6 \pm 11.7	0.69
1RM knee extension (kg)	52.7 \pm 4.1	52.1 \pm 3.1	53.9 \pm 3.9	0.74
1RM knee flexion (kg)	86.1 \pm 5.3	84.3 \pm 3.7	83.4 \pm 5.1	0.51

Values are mean \pm SE

Table 2 Dietary Analyses

Characteristics	Supplementation1 (n=10)	Supplementation2 (n=10)	Placebo (n=10)	p
Energy Intake (kcal)	2460.1 + 303.8	2505.3 + 271	2464.2 + 292.1	0.51
Protein Intake (g)	67.4 + 9.6	68.5 + 11.3	68.9 + 9.7	0.91
Fat Intake (g)	70.1 + 11.3	74.5 + 10.5	71.9 + 9.7	0.54
Carbohydrate Intake (g)	365.3 + 72	366.4 + 73.6	362.7 + 74.1	0.92

Values are mean \pm SE

3.4. Muscle soreness

Pre exercise values were not different among groups for muscle soreness muscle soreness (VAS Score). There was no group by time interaction ($F=1.139$, $p=0.335$), but there was a main effect of time ($F=9.6$, $p=0.00$). Muscle soreness significantly increased above baseline levels for all groups at all time points ($p < 0.05$; **Figure 3**). Peak soreness occurred at 48 hours post exercise for all groups. Once again, the two different dosages of BCAA supplementation had no effect on soreness scores.

4. Discussion

The primary finding of this investigation is that two different dosages of BCAA supplementation have no effect on indices of muscle damage and soreness following lower-body resistance exercise. An increase in biochemical markers of muscle damage and muscle soreness following lower-body resistance exercise was observed in all groups at various time points. The increases in serum enzyme levels suggested that the exercise protocol used in this study resulted in skeletal muscle injury.

The pattern of change in CK in the current study was similar to Zebblin et al. (2007), who reported that BCAA supplementation administered before mild resistance exercise had no effect on serum CK activity. These data are in contrast to Coombes et al. (2000) and Koba et al. (2005, 2007), who showed that BCAA intake before and during endurance exercise reduces indirect markers of muscle damage. Using a similar muscle damage and supplementation protocol to the current investigation, Jackman et al. (2009) reported that consuming BCAAs during and in the days following unilateral eccentric exercise had no effect on CK levels. Ethnic, age, subjects, exercise protocol, intensity of exercise, timing and dosage of the supplementation may account for the discrepancy between the previous investigations and the current investigation. Total creatine kinase (CK) levels depend on age, gender, race, muscle mass, physical activity and climatic condition (Brancaccio et al., 2006; Brancaccio et al., 2007). High levels of serum CK in apparently healthy subjects may be correlated with physical training status, as they depend on sarcomeric damage; strenuous exercise that damages skeletal muscle cells results in increased total serum CK. Total serum CK activity is markedly elevated for

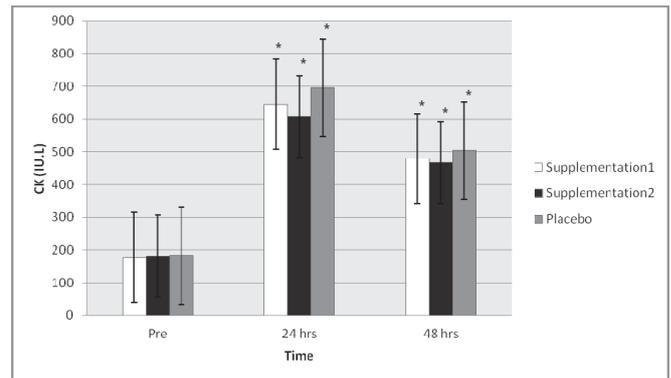


Figure 1 Serum creatine kinase (CK) concentration across the 48 hours

Values are means \pm standard errors. * $p < 0.05$, significantly different from pre exercise, there were no differences among groups. Error bars represent 95% confidence limits.

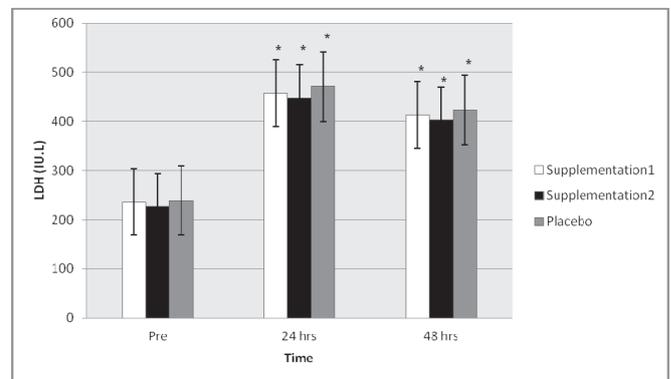


Figure 2 Serum lactate dehydrogenase (LDH) concentrations across the 48 hours

Values are means \pm standard errors. * $p < 0.05$, significantly different from pre exercise, there were no differences among groups. Error bars represent 95% confidence limits.

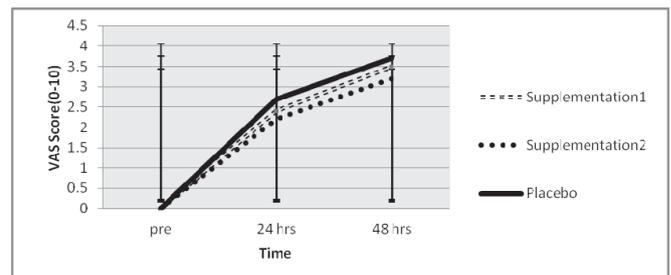


Figure 3 Visual analogue scales (VAS) across the 48 hours

Values are means \pm standard errors. * $p < 0.05$, significantly different from pre exercise, there were no differences among groups. Error bars represent 95% confidence limits.

24 hours after the exercise bout (Brancaccio et al., 2006; Brancaccio et al., 2007).

Research has shown that repetitive, intense exercise which involves a large eccentric component is typically associated with disruption and damage to skeletal muscle connective and/or contractile

tissue. Because injury to skeletal muscle is often characterized by impaired muscle function, attempts have been made to find mechanisms to reduce exercise-induced muscle protein breakdown and stimulate protein synthesis (Knitter et al., 2000).

Although significant differences were not found in regards to CK, the effect size appears much lower than in the study of Koba et al (2005) (η^2 , The CHO+BCAA group in the Koba et al. study received BCAA drink containing 2 g of total BCAA (Val; 0.5 g, Leu; 1 g, Ile; 0.5 g), 0.5 g of arginine and 20 g of carbohydrates in 500 mL more during the trial than the CHO alone group that may account for the larger effect size. However, it is also possible that amino acids and CHO ingestion have a synergistic effect in preventing damage. Because CHO intake during exercise produces a small but significant insulin response that would aid BCAA entry into the muscle cell, amino acid ingestion may not attenuate muscle damage as effectively without the co-ingestion of CHO. An approximately 10-fold increase in insulin sensitivity is reported when CHO is co-infused with BCAA as compared to CHO alone (Koba et al., 2007; Greer et al., 2007).

Functional assessment of athletes' fitness includes a variety of variables. Serum creatine kinase (CK) and lactate dehydrogenase (LDH) are indications of the degree of metabolic adaptation to physical training of skeletal muscles (Brancaccio et al., 2006; Brancaccio et al., 2007). Both enzymes are involved in muscle metabolism, and their serum concentration is normally very low, a result of physiological wears and tears of the cell. They increase considerably either after intensive exercise or in muscle pathology. Changes in serum activity of muscle enzymes have been reported in both normal subjects and athletes after strenuous exercise. The amount of enzyme efflux from muscle tissue to serum can be influenced by physical exercise. Also, there are ethnic differences, and the differences between the sexes have been attributed to the protective effects of estrogen on muscle cell membrane (Clarson, 2002; Brancaccio et al., 2006; Brancaccio et al., 2007).

The pattern of change in LDH in the current study was similar to that following high-force eccentric exercise reported by Nosaka and Clarkson (2006). Cooke et al. (2009) utilized a similar resistance protocol and found a significant increase in LDH activity 24, 48, 72 and 96 hours post-exercise. However, we found no differences between groups

for this enzyme activity of this protein in the blood. These data are in contrast to Koba et al. (2005, 2007), who reported reduced LDH concentrations in a BCAA-supplemented group as compared to those of a placebo group. The authors suggested that BCAA administration positively affected cellular injury. These differences recognized in the pattern for CK and LDH may be the result of the exercise protocol used by Coombes and McNaughton (2000) and Koba et al. (2005, 2007).

It is assumed that LDH concentrations are elevated when there is muscle damage because an increase in the permeability of muscle cell membranes, or the complete disruption of them, allows muscle enzymes to leak into the blood or lymphatic system (Brancaccio et al., 2006; Brancaccio et al., 2007).

Subjects' ratings of perceived soreness were elevated in all groups. While the exact mechanisms of muscular soreness have not fully been elucidated, it is generally accepted that soreness is associated with the cascade of events which follow initial mechanically induced perturbations (Proske et al., 2005). Following initial trauma an increase in intracellular Ca^{++} levels stimulates degradative pathways responsible for further Z-line disruption (Belcastro et al., 1998). The compromised state of the myofiber triggers an inflammatory response, partly characterized by edema and subsequent swelling of the muscle tissue. Indices of soreness typically rise in concert with the swelling response (Proske et al., 2005). The role of immunological changes and cytokine production on muscle soreness is still an area of debate, but it appears to some extent, that muscle soreness is moderately related to increases in certain inflammatory cytokines (Buford et al., 2009). In the present study muscle soreness increased above baseline levels for all groups at all time points. However, the two different dosages of BCAA supplementation had no effect on soreness scores. These results disagreed with the study of Shimomura et al. (2010) who found that 100 mg/kg body weight of BCAAs administered before the squat exercise lowered muscular soreness in participants relative to that of a placebo condition. The hypothesis that BCAA supplementation ingested before and after exercise (regardless of macronutrient composition) helps to prevent muscle damage is not supported by these results as there were no differences between all groups. However, since immunological changes were not measured in this investigation, the effect

of BCAA on cytokine production and subsequent DOMS remains purely speculative. Inflammation is known to be a critical component during the muscle repair and regeneration periods (Smith et al., 2008), but the effect of BCAA supplementation on these processes requires further investigation.

5. Conclusion

In conclusion, lower-body resistance exercise bout caused increases in indices of muscle damage and increases in VAS scores of muscle soreness. Based on these results, BCAA supplementation does not appear to be a viable option at attenuating muscle damage two days following lower-body resistance exercise bout. Additional investigations need to be conducted to determine if BCAA supplementation should be used when attempting to recover from a resistance exercise bout. Utilization of a resistance exercise model that induces damage levels comparable to those in BCAA supplementation studies may be able to determine whether BCAA supplementation can increase recovery following lower levels of damage.

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Visited lecturer, Technical & Vocational College Sama, Mahabad-Iran (February 2008 onward)
Part-time Trainer of sport teams, Islamic Azad University, Mahabad branch, Saghez branch (2006-2008)
Lecturer of I.R.Iran Federation of Sport Aerobic & Fitness

Main Works:

- Aminiaghdam, S. and Daneshmandi, H. (2011). The comparison of Navicular Drop and Genu Recurvatum values in Athletes with and without History of Anterior Cruciate Ligament Injury, *Research on Sport Sciences*; 8(2): 49-60.
- Aminiaghdam, S. Epidemiological Analysis of Lower Extremity Injuries during the Iranian Universities Futsal Championship. *Medicina dello Sport*, reference No. Med Sport-2372. [accepted to be published soon]

Membership in Learned Societies:

- European College of Sports Science (ECSS)
 - Iranian Association of Football Coaches
 - I.R.Iran Federation of Sport Aerobic & Fitness
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