



## **Effect of Ramadan Fasting on the Blood Coagulation System in a Session Soccer Match**

Fatemeh Kami<sup>1</sup>, Mohammad Reza Kordi<sup>2\*</sup>, AmirHossein Saffar Kohneh Quchan<sup>2</sup>, Seyed Houtan Shahidi<sup>3,4</sup>, Fatemeh Shabkhiz<sup>2</sup>

1. Department of Exercise Physiology, Alborz Campus, University of Tehran, Alborz, Iran.

2. Department of Exercise Physiology, Faculty of Physical Education and Sport Sciences, University of Tehran, Tehran, Iran.

3. Department of Community Medicine and Rehabilitation, Section of Sports Medicine, Umeå University, Umeå, Sweden.

4. Department of Education, Faculty of Social Science, Umeå University, Umeå, Sweden.

ARTICLE INFO	ABSTRACT
<i>Article type:</i> Research Paper	<b>Introduction:</b> Ramadan fasting is associated with modifications in athletes' metabolic, physiological, and psychological responses, which may affect their physical performance. The present study aimed to assess the changes in some of the risk factors for thrombosis in trained men after one soccer session in fasting and non-fasting states.
<i>Article History:</i> Received: 24 Feb 2021 Accepted: 08 Oct 2021 Published: 20 May 2022	<b>Methods:</b> This study was conducted on 11 amateur soccer players with the mean age of 42±16 years and mean body mass index of 26.49±2.86 kg/m <sup>2</sup> . The subjects played in a soccer match with the duration of ~100 minutes, including 10 minutes of warm-up and 90 minutes of a soccer match, in two sessions (one week before and on day four of Ramadan). Blood samples were collected before and after the soccer game. Fibrinogen and D-dimer were analyzed using the Clauss clotting method and the turbid metric assay, respectively. In addition, plasminogen and alpha 2-antiplasmin were analyzed via spectrophotometry, and homocysteine was examined using the ELISA assay. Data analysis was performed using the Shapiro-Wilk test and independent and dependent t-test at the significance level of P≤0.05.
<i>Keywords:</i> Exercise Acute Coagulation Blood Fibrinolysis Fasting	<b>Result:</b> One session of soccer match increased homocysteine in the fasting (P=0.006) and non-fasting subjects (P=0.042). Alpha 2-antiplasmin decreased in the fasting (P=0.031) and non-fasting subjects (P=0.001), while plasminogen decreased only in the non-fasting subjects (P=0.012). One session of soccer match had no significant impact on fibrinogen and D-dimer in both states, as well as plasminogen in the fasting state (P≥0.05). Furthermore, no significant differences were observed between the fasting and non-fasting subjects in terms of homocysteine, alpha 2-antiplasmin, fibrinogen, plasminogen, and D-dimer in response to one session of soccer match (P≥0.05).
	<b>Conclusion:</b> According to the results, one session of soccer match in the fasting state was parallel to the non-fasting state, and fasting led to no adverse consequences in the coagulation system of the subjects.

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### **Introduction**

Each year, millions of healthy adult Muslims observe obligatory fasting from sunrise to sunset during the month of Ramadan for 29-30 days. While fasting, people refrain from eating and drinking over periods of 13-18 hours a day depending on the season (1). The fasting state induces various physiological and psychological changes (2). The different physiological responses in Ramadan probably result from disturbances in the sleep-wake cycle and changes in the timing and type of meals (3).

Lifestyle changes for one month may influence and modify human health. For instance, dehydration is a significant challenge associated with Ramadan fasting. Signs of dehydration have been categorized by an increased hematocrit rate or hemoglobin concentration and plasma osmolality, which increase blood viscosity. High blood viscosity is a secondary effect of dehydration, which may increase the risk of thrombosis (4). Moreover, dehydration increases the level of coagulation factors (clot formation) and reduces fibrinolysis (clot degradation) (5). Blood coagulation and fibrinolysis contain two primary physiological systems that are

\* Corresponding author: Mohammad Reza Kordi, Department of Exercise Physiology, University of Tehran, Tehran, Iran. Tel: +989123300076, E-mail: mrkordi@ut.ac.ir.

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monitored by a balance between activators and inhibitors (6). These factors are strongly associated with the risk of thrombosis. Thrombosis is a hemostatic barrier, which blocks the arteries (7). The fibrinolytic system in the blood is responsible for eliminating and destroying the clot, and fibrinolysis is activated upon the activation of the coagulation system (8). Hemostasis is a physiological process associated with the functional balance between the blood coagulation and fibrinolytic systems (9). These systems play a pivotal role in the human inflammatory response, which also reflects blood viscosity and the risk of thrombosis (8).

It is widely acknowledged that fasting, exercise training, and dehydration cause various consequences in the coagulation and fibrinolytic systems (10). In a study in this regard, Darzabi et al. (2020) reported that Ramadan fasting increases osmolarity and serum electrolytes (11). In addition, Javanmardi et al (2018) stated that cerebral venous sinus thrombosis increases during Ramadan due to dehydration (12). Some studies have also proposed that a small degree of dehydration induced by acute exercise and fluid restriction increases homocysteine and significantly impairs the endothelial function (13).

Many fasting individuals and athletes continue their exercise training during Ramadan. Furthermore, important world competitions may be scheduled during this month, such as the FIFA World Cup 2014 and 2018 and the Olympic Games in London in 2012. Therefore, researchers and coaches are concerned about the fact that prolonged fasting and exercising while fasting harms the health and performance of athletes (14, 15). To date, no exercise training studies have been focused on the impact of acute exercise training on the function of the coagulation system while fasting.

The present study aimed to investigate the impact of one soccer training session in the fasting and non-fasting states on some of the risk factors for thrombosis, including fibrinogen, homocysteine, D-dimer, alpha 2-antiplasmin, and plasminogen, in fasting men.

## Materials and Methods

This study was conducted on 11 amateur soccer players with the mean age of  $42 \pm 16$  years and mean body mass index of  $26.49 \pm 2.86$  kg/m<sup>2</sup>. The subjects played a soccer match in two sessions

one week before and on day four of Ramadan. All the participants trained at least two days per week for an average of 90 minutes per day. The match sessions were scheduled at 17:30-19:30 during the control period (before fast breaking in Ramadan). In both periods, the match sessions lasted for ~100 minutes, including 10 minutes of warm-up and 90 minutes of a soccer match (45+45 minutes).

The physical performance parameters of the match were recorded using a global positioning system (GPS). The study was conducted in Tehran, Iran in 2019. In this period, Ramadan started on May 6<sup>th</sup> and ended on June 4<sup>th</sup>. The length of each daytime fast was approximately 15 hours, starting at ~03:50 and ending at ~19:50. Written informed consent was obtained from the participants, and data were collected using the physical activity readiness questionnaire. The inclusion criteria of the study were no history of cardiovascular diseases, coagulation disorders, and chronic disorders and no smoking habits. The study protocol was approved by the Ethics Committee of the Department of Physical Education at Tehran University (IR.UT.SPORT.REC.1398.042).

The current research was performed in three stages. At the first stage, blood samples were collected before starting the soccer game. The second stage was playing the soccer game, and the third stage involved blood sample collection after the intervention. Due to the sensitivity of the tests, the blood samples were collected before and after the intervention on the football field and immediately centrifuged. The plasma was separated and transferred to a freezer.

For the analysis of homocysteine, the blood samples were poured in tubes containing lyophilized EDTA powder for the removal of water from the materials to prolong the life of moisture-sensitive materials. The samples were placed in ice immediately after blood sampling, and the plasma was rapidly separated via centrifugation (Eppendorf centrifuge 5702; made in Germany). In addition, coagulation test samples were collected in plastic or silicone tubes containing 3.2% sodium citrate at the ratio of 1:9 (1: volume of sodium citrate, 9: volume of blood samples) and separated via centrifugation at 2500-2000 grams for 15 minutes in order to isolate the plasma samples. On the day of the measurements, the samples were taken out of the freezer and allowed to thaw. Each parameter

was analyzed in accordance with the instructions of the kits' manufacturer.

The D-dimer assay was implemented based on the change in the turbidity of a microparticle suspension, which was measured via photometry using the STA®-Liatest® D-Di kit and the STA compact coagulation analyzer (made in France). Before performing the test with two different levels of control (N & P), the accuracy and reproducibility of the results were assessed (i.e., quality control). Moreover, the D-dimer assay of the plasma samples was automatically carried out using the analyzer at 540 nanometers immediately after the samples were loaded.

The alpha 2-antiplasmin and plasminogen assays were implemented based on a colorimetric (chromogenic) assay, which was measured via photometry using the Stachrom Antiplasmin kit (made in France) and the STA compact coagulation analyzer. Before running the test with two different levels of control (N & P), the accuracy and reproducibility of the results were evaluated (i.e., quality control). The alpha 2-antiplasmin assay of the plasma was automatically carried out using the analyzer at 405 nanometers immediately after the samples were loaded.

In the present study, the fibrinogen assay was performed based on the Clauss clotting method. Clot detection by the STA compact coagulation analyzer involves an electromagnetic-

mechanical system and monitors the oscillation of a steel ball within the cuvette with the thrombin and diluted plasma. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time in seconds. In our study, the time was translated into fibrinogen concentration from a standard fibrinogen curve and stored on the STA compact. Before using the samples with two different levels of control (N and P), the accuracy and reproducibility of the results were assessed (i.e., quality control).

The concentration of homocysteine was determined using the ELISA assay (Axis® Homocysteine EIA kit; made in Germany). Following that, the samples were analyzed using the awareness technology INC reader (made in USA). Before performing the test with three levels of controls (low, normal, and high), the accuracy and reproducibility of the results were evaluated (i.e., quality control).

Data analysis was performed in SPSS version 21 using the Shapiro-Wilk test to assess the normality of data distribution and Levene test to evaluate the homogeneity of the obtained data. Since the ANCOVA defaults were not met, dependent t-test was employed for the intragroup comparison, and independent t-test was used for the intergroup comparison. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

**Table 1.** Anthropometric Characteristics of subjects

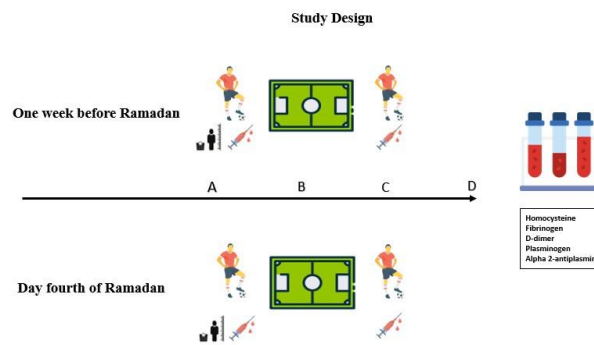
Conditions	n	Sex	Mean age ± SD	Body mass index (BMI)
Fasting	11	Male	16 ± 0.42	25.92 ± 1.64
Non-fasting	11	Male	16 ± 0.42	26.49 ± 2.86

**Table 2.** Physical performance values recorded by GPS

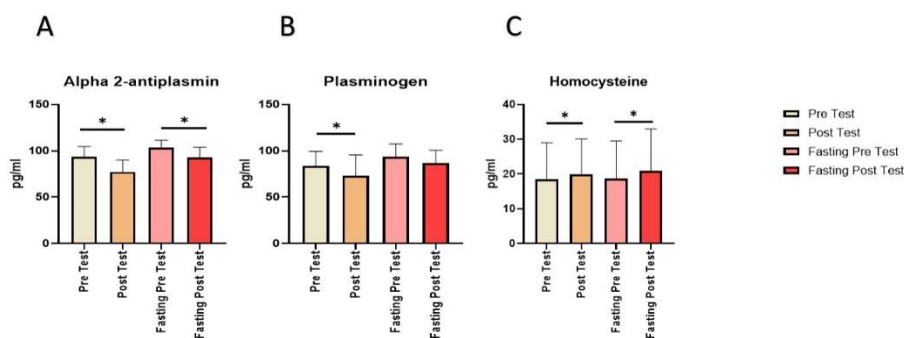
Variables	Fasting	Non-Fasting
Duration of the match (min)	100.18	101
The amount of calories consumed (Kcal)	586	688
The average distance traveled (m)	6500	7700
Maximum speed (Km/h)	25.7	25.9
Maximum heart rate (beats/min)	199	190

**Table 3.** Independent t-test to compare the changes in plasma level factors between non-fasting and fasting groups

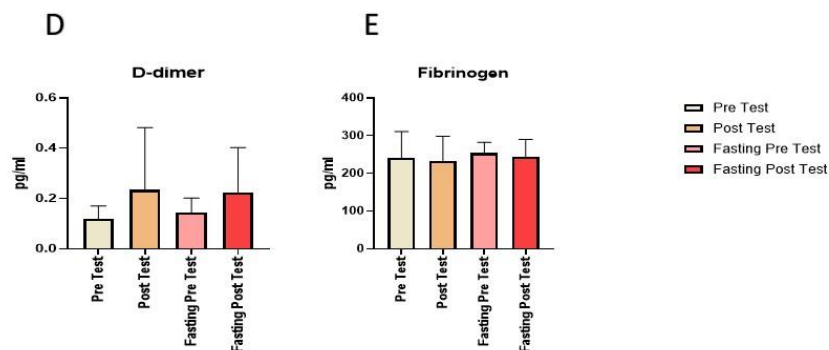
Variables	conditions	Timing	Mean ± SD (95% CI)	P-Value	
Homocysteine	Non-Fasting	Pre-Test	Post-Post	1.45 ± 0.62 (0.06 – 2.84)	0.042*
Fibrinogen	Non-Fasting	Pre-Test	Post-Post	-8 ± 12.30 (-35.42 – 19.42)	0.53
D-dimer	Non-Fasting	Pre-Test	Post-Post	0.11 ± 0.06 (-0.03 – 0.26)	0.111
Plasminogen	Non-Fasting	Pre-Test	Post-Post	-10.8 ± 3.53 (-18.68 – -2.95)	0.012*
Alpha 2-antiplasmin	Non-Fasting	Pre-Test	Post-Post	-16.27 ± 3.67 (-24.45 – -8.09)	0.001*
Homocysteine	Fasting	Pre-Test	Post-Post	2.18 ± 0.64 (0.74 – 3.61)	0.006*
Fibrinogen	Fasting	Pre-Test	Post-Post	-9 ± 13.47 (-39.01 – 21.01)	0.519
D-dimer	Fasting	Pre-Test	Post-Post	0.08 ± 0.04 (-0.02 – 0.19)	0.131
Plasminogen	Fasting	Pre-Test	Post-Post	-7.27 ± 4.38 (-17.04 – 2.49)	0.128
Alpha 2-antiplasmin	Fasting	Pre-Test	Post-Post	-10.54 ± 4.20 (-19.91 – -1.17)	0.031*



**Figure 1.** Note. A = Before starting the soccer match all participant measured blood samples and body mass index; B = Starting soccer match and it took about 100 minutes; C = Immediately after soccer match all participant blood sample got and froze; D = The data insert to the laboratory to analyze the blood sample biomarkers



**Figure 2.** Impact of one session soccer match during non-fasting and fasting on blood biomarkers. \* *P* at least < 0.05 from the analysis by indented t-test. Note. Pre-test and post-test refer to the non-fasting state.



**Figure 3.** Impact of one session soccer match throughout non-fasting and fasting on blood biomarkers. Note. Pre-test and post-test refers to the non-fasting state

**Results**

Tables 1 and 2 show the demographic characteristics of the participants and the football match recorded by GPS, respectively. Compared to the pretest and posttest in the fasting and non-fasting states, the mean level of plasma homocysteine increased significantly

(non-fasting:  $P=0.042$ ; fasting:  $P=0.006$ ) (Figure 2A), the mean level of alpha 2-antiplasmin significantly decreased (non-fasting:  $P=0.001$ ; fasting:  $P=0.031$ ) (Figure 2B), and the mean concentration of plasminogen decreased as well (non-fasting:  $P=0.012$ ) (Figure 2C) (Table 3).

Overall, data analysis indicated that the levels of the risk factors had no significant differences after one session of the soccer match in the fasting and non-fasting groups ( $P>0.05$ ) (Figure 3). By examining the percentage of the plasma volume, changes in the non-fasting and fasting groups were evaluated using the Dill and Costill equation (16), it was observed that these changes were not significant, and the effect of fasting on the number of thrombosis factors was not significant either (non-fasting: -6.52%; fasting: -5.6%;  $P>0.05$ ).

## Discussion

The most important findings of the current research indicated that partaking in a soccer match while fasting is similar to a non-fasting state. Interestingly, the number of the calories expended, the mean distance moved, maximum speed, and heart rate were nearly equal in both states. Fasting does not change the metabolic, coagulation, or fibrinolytic process, thereby leading to thrombosis in response to the elimination of a meal as long as there is proper and healthy nutrition throughout the year, and fasting is accompanied with exercise training. Furthermore, fasting modulates the inflammatory factors that influence thrombosis, and it is possible that the duration of fasting and the intensity and duration of exercise training affect these factors (10).

Ramadan fasting is a unique metabolic pattern in which an individual must avoid eating and drinking from dawn to dusk. It seems that changes in the number, type, and timing of the meals and reduced fat intake, adipose tissue loss, sleep patterns, and wakefulness during Ramadan have varied metabolic effects (10). Homocysteine is a common amino acid, which is produced during the metabolism of methionine (17). Elevated total homocysteine could increase the risk of neurodegenerative and cardiovascular diseases. Some of the main factors that affect homocysteine concentrations include age, gender, nutritional status, and physical activity. Our findings indicated that one soccer session match could increase homocysteine in the fasting and non-fasting states.

In line with our findings, the results obtained by Herrmann et al. (2003b), König et al. (2003), Real et al. (2005), Gelecek et al. (2007), Venta et al. (2009), Bizheh and Jaafari (2011), Deminice et al. (2011, 2013), and Iglesias-Gutierrez et al. (2012)

demonstrated elevated total homocysteine after acute exercise, along with a continued increase reaching the maximum values within six hours post-exercise (18-26). During acute exercise, skeletal muscles increase protein and amino acid catabolism (27). This cortisol-dependent regulation results in the simultaneous uptake of amino acids into the liver to induce glucose synthesis (27, 28). Therefore, exercise increases plasma and muscle-free amino acids, which contribute to homocysteine formation from methionine (29). In addition, exercise while fasting leads to dehydration and haemoconcentration, which also increase homocysteine concentration. In this regard, Sánchez et al. (2019) reported that increased homocysteine following acute exercise was due to haemoconcentration, while rehydration during exercise prevents the increase in homocysteine concentrations (28).

Our findings demonstrated that the levels of plasminogen (insignificant decrease in the fasting state) and alpha 2-antiplasmin decreased following the soccer match. To date, no studies have examined plasminogen and alpha 2-antiplasmin levels during exercise training in the fasting state. Alpha 2-antiplasmin is the major plasma inhibitor of plasmin with a crucial function in diminishing plasmin activity and inhibiting fibrinolysis (30). The duration of fasting and exercise training are reported to alter its rate possibly due to the moderating effect of fasting on the immune system and the stimulation of the synthesis of this factor by the inflammatory cells in the liver, which modulate the production of alpha 2-antiplasmin. However, physical activity has been shown to be directly associated with TPA level of the tissue plasma activators. A study conducted by Ibrahim et al. (2011) showed that Ramadan fasting significantly reduced the inhibitory concentrations of type I plasminogen activator (PAI-1) (31). Based on these findings regarding simultaneous fasting with sports activity, it could be suggested that by increasing the level of TPA activator following exercise and reducing the inhibitory effect of PAI-1 on TPA due to fasting in response to plasminogen conversion, plasminogen conversion into plasmin is expected to increase, which ultimately reduces the plasminogen level.

Our findings indicated that one session of soccer match had no effect on the fibrinogen

concentration in the fasting and non-fasting states. Fibrinogen is a plasma glycoprotein synthesized by the liver and plays a key role in fibrin thrombus formation, while also increasing plasma viscosity (32). Research has yielded contradictory results regarding the acute effects of acute exercise on the levels of fibrinogen. For instance, Bizheh et al. (2011) observed that a single bout of circuit resistance exercise (intensity: 35% 1RM) caused no significant changes in the fibrinogen levels of sedentary middle-aged men (23). In another study, Mahmoodinezhad et al. (2016) reported the significant elevation of fibrinogen after an acute exhausting aerobic training session in female athletes (33). The findings of Kahraman et al. (2011) showed that an acute submaximal aerobic exercise caused no significant changes in the fibrinogen levels of sedentary young men (34). The differences in fibrinogen in response to acute exercise may be due to the differences in the training type, intensity, and duration (33). It is quite established that moderate exercise enhances blood fibrinolytic activity without the concomitant activation of blood coagulation mechanisms. In contrast, intense exercise induces the simultaneous activation of blood fibrinolysis and coagulation. Therefore, it seems that the intensity of the soccer match in the present study (not very intense) helped increase fibrinogen levels (23, 33, 34).

D-dimer is the end-product of the fibrinolysis pathway and the result of fibrin degradation. According to the literature, changes in the fibrinogen factor and d-dimer have an inverse correlation. In the activities that decrease the fibrinogen factor, the d-dimer factor will increase (35). Since the levels of fibrinogen (constituent of fibrin) did not change in the current research, D-dimer was not altered significantly.

One of the limitations of the present study was implementing a single soccer match without variations in terms of the mode of the exercise and physical fitness assessment. Therefore, further investigations are required to assess the long-term effects of fasting and the variations in the intensity of exercise training on physical fitness measurements, coagulation, and the fibrinolytic system.

## Conclusion

According to the results, one session of a soccer match exerted similar effects on the blood

coagulation factors in the fasting and non-fasting states. Therefore, proper nutrition and hydration strategies could be adopted to prevent the detrimental effects of fasting on thrombotic factors.

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## Conflicts of Interest

None declared.

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