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Evaluation of Changes in Blood Hematological and Biochemical Parameters in Response to Islamic Fasting and Regular Physical Activity in Male and Female Subjects

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ABSTRACT

Introduction: Fasting during Ramadan is a religious obligation for healthy adult Muslims. This study aimed to investigate the effects of Ramadan fasting and physical activity on blood hematological and biochemical parameters in male and female subjects.
Methods: In this study, 50 healthy subjects were randomly assigned to four groups: male control fasting (MCF) group (n=13), male training fasting (MTF) group (n=13), female control fasting (FCF) group (n=12), and female training fasting (FTF) group (n=12), and were compared in two stages (before and after the intervention). For inter- and intra-group comparisons, a repeated measure ANOVA was applied.
Results: The results showed a significant decline in body weight, body mass index, body fat percentage, and waist-to-hip ratio after Ramadan fasting, compared to the pre-Ramadan period. Also, significant changes were observed in red blood cell count and hematocrit level (P<0.05). Additionally, serum total cholesterol (TC), low-density lipoprotein (LDL), and TC/high-density lipoprotein (HDL) ratio significantly decreased after Ramadan, compared to the pre-Ramadan period. The two groups were significantly different in terms of weight, body mass index, body fat percentage, and waist-to-hip ratio after Ramadan (P<0.05). Moreover, the two groups were significantly different in terms of changes in mean triglyceride level, TC concentration, LDL-cholesterol, LDL/HDL ratio, TG/HDL ratio, and TC/HDL ratio.
Conclusion: Ramadan fasting, accompanied by regular physical activity, can induce positive changes in hematological and biochemical parameters. These variations may be due to dietary changes, biological responses of the body to starvation, or physical activity during Ramadan.

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Introduction

Fasting in Islam is an obligatory religious practice for all adults, who have reached the age of puberty. Almost 400 million out of 1 billion Muslims adhere to this religious practice during Ramadan (1). In this month, Muslims abstain from eating, drinking, and smoking from sunrise (Sahar) to sunset (Iftar) (2). Fasting in this month leads to some changes in one's eating habits, the amount of received energy, and physiological parameters in the blood (e.g., hematological and biochemical indices) (2, 3).

Physical exercise in Ramadan generally accelerates the metabolism in the body and prevents the accumulation of body fats. According to the literature, athletes, who fast during Ramadan, less encounter digestive problems and blood glucose changes, compared to non-athletes (4). On the contrary, based on a previous study, fasting in teenage soccer players reduced the amount of used oxygen and decreased their agility and performance (5).

Ramadan fasting has been shown to cause some changes in blood hematological and biochemical parameters (6). Contradictory findings have been reported regarding the effects of fasting on blood hematological and biochemical parameters. In this regard, Khaled et al. examined the effects of fasting on 60 Muslims (7). A significant decline was reported in fasting blood sugar (FBS) and high-density lipoprotein cholesterol (HDL-C), while

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triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) significantly increased.

On the other hand, Qujeq et al. reported a significant decline in LDL concentration and a significant increase in HDL level in the middle and end of Ramadan, compared to the previous months (8). Additionally, Farshidfar et al. noticed a significant decline in hemoglobin and FBS levels on the 3rd and 15th days of Ramadan. However, on the 28th day of this month, LDL and FBS levels significantly decreased, while HDL and TC concentrations considerably increased (9).

On the contrary, Saada et al. indicated a noticeable increase in FBS and HDL levels and a significant decline in TG, TC, hemoglobin, LDL, and very-low-density lipoprotein (VLDL) levels during Ramadan (10). Moreover, Indral et al. concluded that TG, TC, and LDL levels significantly decreased in 19 fasting male subjects, whose blood samples had been drawn on the 1st and 23rd days of Ramadan (6).

Furuncuoglu et al. suggested a decline in hemoglobin and plasma protein levels and a significant increase in the number of lymph nodes after Ramadan (11). Moreover, Huda et al. indicated a significant decrease in the platelet count, hemoglobin level, red blood cell count, and hematocrit level by the end of Ramadan (12). Therefore, on the whole, conflicting results have been reported on the effects of fasting on blood hematological parameters.

Considering the conflicting results on the effects of fasting on biochemical and hematologi-cal parameters, scarcity of data on the effects of sports activities in Ramadan and the prolonged duration of fasting on hot summer days, evaluation of fasting, accompanied with or without regular physical activity, is of great significance.

Concerning the importance of fasting (particularly accompanied by regular physical activity) and understanding athletes' physiology-ical conditions during Ramadan, researchers have been encouraged to compare the effects of fasting and regular physical activity on biochemical and hematological parameters. Therefore, the aim of this study was to assess the effects of fasting and regular physical activity on biochemical and hematological parameters in male and female subjects.

Material and methods

Study design

This quasi-experimental study was conducted in two phases, i.e., before and after Ramadan, on two experimental and two control groups. The inclusion criteria were as follows: 1) participation in the first stage of the study; 2) familiarity with the study concepts; and 3) willingness to participate in the study. Suggestions on sport activities, nutrition, diseases, and drug use/abuse were given to the participants so that they could take the required measures.

All subjects were asked to complete a medical examination form and a questionnaire to ensure that they were not taking any medications regularly and were free of cardiac, respiratory, renal, or metabolic diseases. If the subjects had any of the mentioned problems, they were excluded from the study.

The study protocol was approved by the Human Research Ethics Committee of the Academy of Physical Education at Ferdowsi University of Mashhad, Iran (grant No.: 23246). The subjects were randomly assigned to four groups: male control fasting (MCF) group (n=13), male training fasting (MTF) group (n=13), female control fasting (FCF) group (n=12), and female training fasting (FTF) group (n=12).

During the second stage of the study, the subjects' height was measured in centimeters, and their weight was calculated, using a digital scale (PS07-PS06, Beurer, Germany). Afterwards, the waist-to-hip (WHR) ratio was determined. Body fat percentage (BFP) was calculated, using a body compound determiner (InBody-720, Korea), based on bioelectrical impedance analysis method. All the measurements were carried out while the volunteers had stopped eating or drinking for four hours prior to the tests, and their bladder and stomach were empty.

Exercise programs

The training sessions started in the evening of the first day and continued until the 30th day of Ramadan (15-16:30 pm). The MTF and FTF groups were asked to perform aerobic training three sessions per week (90 min per session). In this study, regular exercise included 15 min of general warm-up (i.e., walking, stretching, and exercise), followed by 50-60 min of specific

Table 1. The within and between groups of body composition in the active and inactive subjects

Variables	Gender	Group(s)	Pre-test M±SD*	Post-test M±SD*	Variations: Within Groups *P*-value	Variations: Interaction (group × phase) *P*-value	Variations: Interaction (gender × phase) *P*-value	Variations: Between groups *P*-value
Weight (kg)	Male	Exercise	65.7±7.7	64.9±7.5	†0.000	0.124	0.931	†0.037
		Control	69.3±6.7	68.5±6.8				
	Female	Exercise	63.97±6.53	63.00±6.91				
		Control	69.26±6.23	69.05±5.99				
Body mass index (kg/m2)	Male	Exercise	20.9±2.1	20.5±2.0	†0.000	0.630	0.135	†0.018
		Control	24.9±4.0	24.3±3.9				
	Female	Exercise	21.55±2.81	21.55±2.85				
		Control	22.49±2.79	22.42±2.76				
Body fat Percentage (%)	Male	Exercise	11.4±4.1	10.8±3.7	†0.004	0.968	0.464	†0.002
		Control	20.0±6.9	19.0±7.9				
	Female	Exercise	23.66±2.91	21.87±2.47				
		Control	24.00±2.59	22.83±2.97				
Waist-to-hip ratio (cm)	Male	Exercise	0.7±0.6	0.6±0.5	†0.005	0.061	†0.002	†0.001
		Control	0.8±0.7	0.7±0.6				
	Female	Exercise	0.81±4.41	0.81±4.18				
		Control	0.82±2.91	0.83±2.15				

*: Data are presented as mean±standard deviation; ‡: The mean difference is significant at 0.05

training, consisting of rhythmic movements, rotational motions of the joints, forward and backward leaps (carried out aerobically), and strength movements. These movements were performed alternatively at an increasing, gradual rate, with an intensity of 60-75% of maximum heart rate reserve (MHRR).

At the end of each training session, jogging, walking, and stretching exercises were performed for 15 minutes to return the body to its normal status (13). Accordingly, MHRR variables were respectively calculated, based on Karvonen Formula for every single athlete [Equation 1]. The variables were controlled during exercises by a heart rate monitor (Polar, Finland) (14):

Target heart rate= [%60 or %70+ [(220-age)-Resting heart rate]]+Resting heart rate (Equation 1)

Biochemical tests

Blood samples were collected via venipuncture from the forearm vein after at least 15 minutes of sitting at rest or in the supine position. Blood samples were transferred into a tube containing K2EDTA and mixed for 15 min before analysis. Afterwards, the samples were centrifuged in plastic capillary tubes, using the Haemato Spin Centrifuge device (Hct, Hawksley, Sussex, UK).

Serum biochemical concentrations were determined, using an automated spectrophotometric analyzer and different kits at various wavelengths. Serum cholesterol concentration was determined as mg/dL by Pars Azmoon kits, using cholesterol oxidase/para-amino-phenazone (CHOD-PAP) method at 546 nm wavelength.

Serum glucose concentration was determined as mg/dL by Pars Azmoon kits via CHOD-PAP method at 500-546 nm wavelengths. Moreover, serum TG concentration was determined as mg/dL by Pars Azmoon kits, using enzymatic GPO-PAP method at 546 nm wavelength. HDL and LDL levels were measured by Man kit (Tehran, Iran), using the enzymatic method. Hemoglobin and hematocrit concentrations were analyzed by K-4500 Automated Hematology Analyzer.

Statistical analysis

SPSS version 15 was used at the end of the experiment to analyze the data. After ensuring the normal distribution of the data via

Table 2. The within and between groups of hematological indices in active and inactive subjects

Variables	Gender	Group(s)	Pre-test M±SD*	Post-test M±SD*	Variations: Within groups	Variations: Interaction (group × phase)	Variations: Interaction (gender × phase)	Variations: Between groups
					P-value	*P*-value	*P*-value	*P*-value
White blood cell count (×106/mm³)	Male	Exercise	5.6±1.7	5.2±1.0	0.062	†0.010	0.293	0.160
		Control	5.9±0.9	6.0±1.0				
	Female	Exercise	7.05±2.00	5.55±1.11				
		Control	6.91±1.20	7.07±2.46				
Red blood cell count (×106/mm³)	Male	Exercise	5.0±0.2	4.9±0.2	†0.013	0.288	0.238	†0.036
		Control	4.9±0.2	4.8±0.2				
	Female	Exercise	5.30±0.22	5.19±0.30				
		Control	5.05±0.35	5.01±0.27				
Hemoglobin (gm/dL)	Male	Exercise	14.5±1.1	14.2±1.0	0.406	†0.000	0.881	0.515
		Control	14.8±1.1	14.9±1.0				
	Female	Exercise	14.95±0.89	14.30±0.80				
		Control	14.21±0.82	14.8±1.1				
Hematocrit (%)	Male	Exercise	42.1±2.8	41.4±2.4	†0.003	0.374	0.915	0.943
		Control	42.8±2.5	41.9±2.2				
	Female	Exercise	43.91±1.63	42.75±1.52				
		Control	42.83±1.73	42.60±2.54				
Platelet count (1000)	Male	Exercise	202.9±39.6	203.4±44.7	0.343	0.676	0.154	0.752
		Control	206.2±51.3	209.2±53.2				
	Female	Exercise	207.91±40.07	207.60±24.64				
		Control	225.16±38.56	206.36±34.47				

*Data are presented as mean±standard deviation; ‡: The mean difference is significant at 0.05

Kolmogorov-Smirnov test and evaluation of variances by Levene's test, repeated measures ANOVA was applied to make intra- and inter-group comparisons (group×phase). P-value less than 0.05 was considered statistically significant.

Results

The findings of the present study are summarized in Tables 1-3. The mean age of male subjects in the experimental and control groups was 19.38±0.5 and 21.07±1.55 years, respectively. Moreover, the mean age of female subjects was 22.33±2.30 and 20.00±1.34 years in the experimental and control groups, respectively.

Findings on the effects of Ramadan fasting on body composition are presented in Table 1. According to Table 1, a significant decline was reported in body weight, BMI, BFP, and WHR after Ramadan fasting, compared to the pre-Ramadan period. The difference between the groups was significant in terms of weight, BMI, BFP, and WHR after Ramadan fasting ($P<0.05$).

Findings on the effects of Ramadan fasting on hematological indices are shown in Table 2. A trivial but significant decline was reported in red blood cell count and hematocrit level in active men and female groups ($P<0.05$) (Table 2). However, white blood cell count, hemoglobin level, hematocrit level, and platelet count did not significantly change during Ramadan ($P<0.05$). Differences between the groups were not significant in terms of white blood cell count, hemoglobin level, hematocrit level, or platelet count.

According to Table 3, a significant decline was reported in serum TC level, LDL concentration, and TC/HDL ratio after Ramadan fasting, compared to the pre-Ramadan period. Moreover, the inter-group comparison showed significant changes in TG level, TC level, LDL-C,

Table 3. The within and between groups of some biochemical variables in the active and inactive subjects

Variables	Gender	Group(s)	Pre-test M±SD*	Post-test M±SD*	Variations: Within groups *P*-value	Variations: Interaction (group × phase) *P*-value	Variations: Interaction (gender × phase) *P*-value	Variations: Between groups *P*-value
Fast Blood Sugar (mg/dL)	Male	Exercise	91.1±5.6	85.1±5.0	0.211	0.024†	0.000†	0.192
		Control	88.3±6.3	84.4±7.5				
	Female	Exercise	85.75±11.03	89.30±7.08				
		Control	77.16±6.88	91.90±10.03				
Triglycerides (mg/dL)	Male	Exercise	51.9±14.2	51.9±13.0	0.746	0.027†	0.604	0.001†
		Control	97.1±57.4	107.3±52.5				
	Female	Exercise	80.63±36.86	59.33±21.13				
		Control	88.54±52.58	112.60±56.39				
Cholesterol total (mg/dL)	Male	Exercise	145.2±9.8	140.2±9.2	0.021†	0.766	0.503	0.030†
		Control	185.4±37.1	175.8±33.9				
	Female	Exercise	159.66±27.15	150.3±21.08				
		Control	160.16±27.35	145.56±46.47				
Low-density lipoprotein (mg/dL)	Male	Exercise	93.7±8.7	89.9±5.9	0.003†	0.322	0.510	†0.004†
		Control	128.0±30.5	117.0±27.3				
	Female	Exercise	95.63±10.98	92.00±14.04				
		Control	101.66±22.77	95.36±20.57				
High-density lipoprotein (mg/dL)	Male	Exercise	41.2±6.3	43.3±5.6	0.358	0.006†	0.162	0.204
		Control	37.9±3.9	38.9±3.7				
	Female	Exercise	37.41±4.44	40.40±6.25				
		Control	41.58±6.08	37.45±4.39				
LDL/HDL ratio (mg/dL)	Male	Exercise	2.3±0.3	2.1±0.2	0.203	0.026†	0.590	0.002†
		Control	3.4±0.9	3.0±0.7				
	Female	Exercise	2.57±0.42	2.39±0.61				
		Control	2.45±0.52	2.58±0.63				
TG/HDL ratio (mg/dL)	Male	Exercise	1.2±0.4	1.2±0.4	0.837	0.008†	0.800	0.001†
		Control	2.6±1.6	2.8±1.5				
	Female	Exercise	2.18±1.02	1.44±0.54				
		Control	2.18±1.35	3.05±1.62				
TC/HDL ratio (mg/dL)	Male	Exercise	3.5±0.4	3.2±0.3	0.025†	0.009†	0.028†	0.004†
		Control	4.9±1.2	4.5±0.9				
	Female	Exercise	4.19±0.61	3.80±0.83				
		Control	3.88±0.69	4.25±0.73				

*: Data are presented as mean±standard deviation; ‡: The mean difference is significant at 0.05

LDL/HDL ratio, TG/HDL ratio, and TC/HDL ratio, while no significant changes were observed in HDL or FBS level.

Discussion

The present study indicated significant changes in red blood cell count and hematocrit level. However, differences between the groups were not significant in terms of white blood cell count, hemoglobin level, platelet count or hematocrit level. The literature review indicated controversial findings on changes in hematological parameters in active and inactive subjects during Ramadan.

In a group of studies, subjects, who fasted during Ramadan, experienced a decline in hemoglobin and hematocrit levels (11, 12). Based on several studies, serum hemoglobin level either remains unchanged (15, 16) or increases (17) during Ramadan, whereas other studies have suggested a decline in hemoglobin level during this month (18-20).

Huda et al. showed a significant decline in hemoglobin level, platelet count, and hematocrit level by the end of Ramadan (12). Also, based on a study by Maughan et al., hemoglobin and hematocrit levels decreased during Ramadan fasting (20). Moreover, Schumacher et al. reported a significant decline in hemoglobin and hematocrit levels in a German team after the competition season (18).

Other investigations, including those by Al-Hourani et al. and Farshidfar et al., have reported contradictory findings (1, 9, 17, 21). For instance, Al Hourani and colleagues reported no significant changes in hemoglobin or hematocrit level after Ramadan fasting (1). Also, Farshidfar and colleagues examined the effects of Ramadan fasting on 62 students and reported a significant decline in hemoglobin level (9).

Athletes, especially those with physical stamina, normally have lower levels of hemoglobin and hematocrit, compared to non-athletes. Normally, after long, vigorous exercises, which require physical stamina, the amount of plasma increases. The increased plasma level is usually followed by a decrease in hematocrit level and the number of red blood cells. The increase in plasma makes the blood thinner, and therefore, it causes an increase in hematocrit and hemoglobin levels (22).

The displacement of fluids and proteins in the blood during exercise may balance the changes in plasma during recovery to the initial status. These changes might cause the thinning or thickening of the blood, which depends on the type of exercise, intensity of exercise, and its restrictions. In most cases, when exercise is performed in extremely warm climates, the thickening of the blood systematically occurs, which is a reaction to this type of exercise in and of itself.

Under these circumstances, the blood thickening mechanism is not only affected by the decrease in body fluids (which is caused by increased sweating), but is mainly caused by blood redistribution and the force for blood flow in the veins. Changes in hydrostatic pressure and increased swelling pressure in active tissues could be two examples (22). Myoglobins, electron-transferring proteins, and reduced ferritin density, which decreases the amount of iron in athletes and non-athletes, may be responsible for reduced hemoglobin synthesis. These changes probably occur due to lack of iron in the diet and reduced calories in the body while fasting (23, 24).

The present study showed a significant decline in serum LDL and TC/HDL ratio after Ramadan fasting, compared to the pre-Ramadan period. Moreover, differences between the groups were significant in terms of TG level, TC level, LDL-C concentration, LDL/HDL ratio, TG/HDL ratio, and TC/HDL ratio after Ramadan fasting.

Findings reported in the literature on the influence of Ramadan fasting on changes in biochemical indices have been conflicting and inconsistent. Our results were consistent with the findings reported by Saleh et al. and Furuncuoglu et al. (11, 25). As Saleh et al. indicated, Ramadan fasting led to a significant decline in weight and LDL-C level, which could cause a significant increase in HDL-C level during Ramadan (25). Additionally, Furuncuoglu et al. reported a significant decline in TG, FBS, and TC levels in the intervention group (11).

The result of this study were inconsistent with the results reported by Ziaee et al. and Haghdoost et al. (26, 27). Ziaee et al. noted that Ramadan fasting resulted in a decline in HDL level, BMI, glucose level, and weight in both genders. Although LDL level significantly increased during Ramadan, there was no significant changes in VLDL, TG or TC level (26).

In a study by Haghdoost et al., HDL-C level decreased during and after Ramadan in both active and inactive groups. However, the decline was slightly greater in the inactive group, and the combination of physical activity and fasting had no significant effects on HDL level. Serum LDL level decreased in the active group during and after Ramadan. Moreover, HDL/LDL ratio decreased during Ramadan, while it significantly increased after this month (27).

Endurance exercise increases the lipase lipoprotein level. This enzyme has been shown to play an important role in transforming VLDL to HDL. Aerobic exercise has been also shown to increase lecithin-cholesterol acyltransferase (LCAT), which esterifies cholesterol to HDL in the muscles and may be a cause of increased HDL concentration (24, 28).

After some physical exercises, HDL-C, similar to TG, increases in terms of density (almost after a day of activity) and disappearance (almost after three days of activity). The interplay between these contradictory changes can possibly increase lipoprotein lipase activity, expedite the decomposition of glyceride in VLDL, and remove lipoprotein particles, making an additional layer of fat (free cholesterol and phospholipid) to be transferred to HDL-C. In addition, physical exercise leads to the formation of LCAT enzymes, which feed HDL-C particles (24).

LCAT is synthesized in the liver, is secreted in the plasma, and most of it connects to HDL. This enzyme helps form cholesteryl ester transfer protein (CETP) and facilitates the transfer of CETP to VLDL (sometimes to LDL-C). Also, LCAT, along with apolipoprotein A (co-factor), changes free cholesterols. Lack of this enzyme may be triggered by genetic malfunction or lack of apolipoprotein A; moreover, LCAT enzyme reduces CETP and HDL-C.

Chylomicrons including cholesterol, cholesteryl ester, phospholipids, and apoproteins are all first absorbed by the liver via endocitose and are then separated. Therefore, fatty acids, derived from foodstuffs or made in the liver via synthesis, transform into TG, are compacted into VLDL particles (along with cholesterol and cholesteryl ester), and finally empty into the blood stream (24).

In general, low muscle tissue density, high fat density, dissimilar fat distribution, baseline serum lipid level, and other factors such as gender, financial status, health status, diet, body weight, BMI, exercise type, intensity and time, physical strength, and amount of burned calories are some of the physiological and metabolic factors, which affect the body metabolism and significantly influence the lipid response to exercise (29). Furthermore, all these lipid forms are interwoven and it is impossible to examine them individually; therefore, there is a greater need for a conservative interpretation of the obtained findings.

However, in the present study, it was assumed that fasting, along with aerobic exercise during Ramadan, has a more significant impact on lipids and lipoproteins due to the use of fats as fuel, which is accompanied by an increase in maximal oxygen uptake and higher energy use, resulting in better changes in body weight, fat percentage, and fat distribution.

Since we faced many limitations such as diversity in diets, different responses to fasting, individual differences, diverse activity levels and physical strength, and disproportionate body composition in this study, we were forced to be more conservative in making the final conclusion.

This study demonstrated that Islamic fasting, regardless of its spiritual benefits, can cause positive changes in blood hematological and biochemical indices through modifying one's diet by causing changes in the frequency and time of meals especially in active individuals. This also helps restore the serum lipid level to the normal status. Nonetheless, further research is required to obtain more accurate results. The discrepancy in the findings may be due to differences in exercise protocols, exercise duration and intensity, type of exercise, and of course one's fitness level.

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