Research Paper: Effects of Ramadan Fasting on Apolipoproteins A and B and Atherogenic Index in Fasting and Non-Fasting Students

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ABSTRACT

Purpose: Several studies have been done on the effects of fasting on human health indicating the beneficial effects of fasting on weight control, lipid metabolism, and lowering blood pressure in healthy people. The present study aimed at investigating the effects of Ramadan fasting on apolipoproteins A and B (Apo A and Apo B) and the atherogenic index of the fasting and non-fasting students.

Methods: This quasi-experimental study was conducted on 29 men aged 20-25 years. The samples were divided into the fasting (n=15) and non-fasting (n=14) groups. Serum levels of apolipoproteins A and B, biochemical-hematological factors, and atherogenic index were measured three days before the fasting month and after Ramadan. The inter-group and intra-group comparison was performed using student's t-test, and one-way Analysis of Variance (ANOVA) was used to assess the differences between the groups.

Results: In the fasting group, a significant reduction was observed in Apo B, triglyceride, total cholesterol, low-density lipoprotein, low-density lipoprotein to high-density, and triglycerides to high-density lipoprotein cholesterol ratio. However, the Apo A (P=0.001) and high-density lipoprotein (P=0.004) significantly increased after the intervention. The Atherogenic index, white blood cells, red blood cells, hemoglobin, hematocrit, and platelet count significantly decreased in the fasting group (P≤0.05).

Conclusion: According to the results, fasting during Ramadan could improve the biochemical and hematological factors. Therefore, it is recommended to use some biochemical and hematological indices to compare the effects of fasting to improve in of students these parameters.

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Highlights

- A reduction in apolipoproteins A and B can be optimally achieved with a multimodality approach by adopting a healthy lifestyle through diet modification, physical activity, and possible pharmacological therapy.

- Ramadan fasting has beneficial effects on HDL and LDL levels and could lead to a reduction in abdominal obesity, waist circumference, and waist to hip ratio Ramadan.

Plain Language Summary

Ramadan is a holy month in the Islamic calendar (lunar calendar varies between 29 and 30 days) once a year. About 1.5 billion Muslims worldwide are religiously abstained from eating foods, oral intakes, such as medicine (unless in necessary cases) or smoking during the daylight starting from dawn to sunset. Muslims break their fasting just after sunset by having the main meal and then they may have two or three meals during the night until the dawn time. Body Mass Index (BMI) and Waist Circumference (WC) are decreased gradually, especially in the last week of Ramadan compared with before Ramadan. The role of Ramadan fasting positively affect the metabolic syndrome markers, including central obesity, WC, fasting plasma glucose level, triglycerides level, High-Density Lipoprotein (HDL), and Blood Pressure (BP). In terms of metabolism and serum hormonal levels, Ramadan fasting may affect the metabolism of lipids, carbohydrates, and proteins, as well as related hormones levels. Also, Ramadan fasting has beneficial effects on HDL and LDL levels. Therefore, intermittent fasting, such as Ramadan fasting could be one of the treatment alternatives, especially in people with metastasis or cardiovascular or metabolic diseases considering their physician supervision. In general, Ramadan fasting is associated with positive improvements in different associated lipids, such as apolipoproteins, biochemical index, etc. that may directly or indirectly affect metabolic syndrome markers.

1. Introduction

Fasting during the holy month of Ramadan is the fourth pillar of Islam. More than 1.5 billion Muslims fast during this month [1]. Eating habits and sleep/wake cycles are adjusted according to the circadian rhythm. Ramadan fasting, due to changes in eating habits and sleep-wakefulness pattern, disrupts the hormones involved in this process. In addition to the intrinsic factors that influence the circadian rhythm, environmental factors, such as exercise, diet, and ambient light also affect this rhythm [2-4]. Changes in the sleep-wake cycle, eating habits, and subsequent changes in biochemical and hematological factors are observed during Ramadan [5]. In this regard, many studies have indicated that fasting without causing any problems for people's health is useful for people's health. In this regard, fasting causes significant changes in body weight, hematological parameters, glucose levels, and lipids [6, 7].

Hormonal changes, decreased metabolic rate, and dehydration are other changes that have been reported following lifestyle changes in the fasting month [8, 9]. Fasting during Ramadan mobilizes triglycerides from adipose tissue and transports them into the blood circulation to make this fuel available to metabolically active tissues [10]. During fasting, biochemical pathways involved in mobilization and fat burning are activated to increase the consumption of free fatty acids. Short periods of fasting, such as fasting during Ramadan, regularly expose active tissue to increased fat consumption [11].

Lipoproteins are particles composed of lipids and proteins. Four lipids, including free cholesterol, esterified cholesterol, triglycerides, and phospholipids are present in the structure of lipoproteins. The proteins in the lipoprotein structure are called apoprotein and apolipoprotein [12]. It has been shown that the ratio of apolipoprotein B (Apo B) to apolipoprotein A (Apo A) is an important predictor for cardiac muscle injury [13]. Moreover, apolipoprotein plays a major role in reversing the cholesterol cycle to reduc atherosclerosis. Studies have shown an inverse correlation between Apo A and risk of cardiovascular disease [14].

Numerous studies have been conducted in this regard. The results of the studies have been different and sometimes contradictory. In this regard, Saada et al. showed that there is no significant change in Body Mass Index (BMI) between fasting and non-fasting individuals due to fasting during Ramadan. However, the levels of glucose and high-density lipoprotein cholesterol (HDL-C) were significantly increased, whereas the levels of hemoglobin,

Total Cholesterol (TC), triglycerides, Low-Density Lipoprotein Cholesterol (LDL-C), and Very-Low-Density Lipoprotein Cholesterol (VLDL-C) decreased considerably during the third week of Ramadan [15]. Salehi et al. observed a significant decrease in body weight, BMI, glucose, and TC after a fasting period during Ramadan by comparing the blood sample of 19 fasting men during the first days and 23 months of Ramadan [16]. Indral et al. found that serum urea, triglyceride, TC, and LDL-C levels significantly reduced following Ramadan fasting [17].

In summary, contradictory findings have been reported regarding the effect of fasting on the lipid profile. Some studies have reported elevated levels of TC and LDL-C due to weight loss during fasting [18], whereas others have reported a decrease in cholesterol in the early days during Ramadan [19], and some have also reported no changes [20, 21]. Given the importance of fasting in the holy month of Ramadan, comparing the effects of one month fasting on the biochemical and hematological factors of fasting and non-fasting inactive men can yield desirable results and is helpful for better understanding the physiological conditions during Ramadan. Therefore, the present study aimed at investigating the effects of Ramadan fasting on Apo A and B and the atherogenic index of the fasting and non-fasting students.

2. Materials and Methods

Subjects

This quasi-experimental study was conducted on 29 inactive male students divided into two experimental groups. The students had a BMI of 25-30 kg/m^2 and were selected voluntarily and objectively. At the first stage of the research, the students were introduced to the concept and approaches of cooperation. Important notes were also provided to the subjects regarding nutrition, diseases, drug consumption, supplements, drug abuse, no smoking habits, and lack of participation in other studies for a minimum of six months prior to the research schedule. Participation in the study was voluntary and the written informed consent was obtained from the cases. Afterward, the subjects were randomly assigned to the fasting (n=15) and non-fasting (n=14) groups. The following equation was used to determine the sample size:

$$n=\frac{2\sigma^2(Z_{1-\alpha/2}+Z_{1-\beta})^2}{d^2}=\frac{2(2.5)^2(2+1.28)^2}{3.5^2}=10.97\cong 1$$

Body composition

At the second stage of the research, the height of the students was measured in centimeters using the Seca height meter (Germany) with the sensitivity of five millimeters, and their weight was calculated using a digital scale (-PS06, Beurer, Germany). Next, the Waist-to-Hip Ratio (WHR) was determined and BMI was calculated by dividing the body weight by the squared height in meters (kg/m^2). In order to measure the waist of the participants, a tape measure was used (MABIS, Japan) at the midway between the lowest rib and top of hips (above the navel). The hips were measured at the widest point around the buttocks and the tape was held snugly without pulling and leveled around, and the value was divided for the WHR. All measurements were performed while the subjects had refrained from eating and drinking four hours prior to the test, and their bladder, stomach, and intestines had been emptied.

Blood Sample Collection

Sampling was performed between 15:00 and 16:00 PM. After 10-12 hours of fasting in the laboratory, blood samples were collected from the left vein of each subject in the sitting position and at rest. Serum biochemical concentrations were determined using an autoanalyzer spectrophotometer and various kits at different wavelengths. Moreover, serum triglyceride concentration (mg/dl) was determined using the Man kits and GPO-PAP enzymatic method at the wavelength of 546 nm. HDL and LDL were also measured using the enzymatic method (Pishtaz Teb kit, Tehran, Iran). Apo A and B were measured by carbometer and ROCSH kits (Germany). The Atherogenic index was calculated by differences in the TC/HDL-C ratio [22].

Statistical analysis

The obtained results were analyzed using the Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL). Data were presented as Mean±SD. The distribution of data was found normal confirmed by the Shapiro-Wilk test and homogeneity of variance was checked by Leven's test. The inter-group and intra-group comparison was performed using student's t-test, and one-way Analysis of Variance (ANOVA) was used to assess the differences between the groups. In all the statistical analyses, the significance level was considered to be less than 0.05.

3. Results

Table 1 shows the characteristics of the subjects. The results of Shapiro-Wilk and Levene's tests are shown

in Table 2. According to Table 3, a significant reduction was observed in triglyceride, TC, low-density lipoprotein, low-density lipoprotein to high-density lipoprotein ratio, triglyceride to HDL ratio, and TC to HDL ratio in the fasting group. On the other hand, a significant increase was denoted in HDL after intervention in the fasting group. According to the findings, the atherogenic index and Apo B significantly reduced only in the fasting group, whereas a significant increase was denoted in the Apo A levels. According to the results of Tables 4, there was a significant decrease in White Blood Cells (WBCs), Red Blood Cells (RBCs), hemoglobin, hematocrit, and platelet count after the intervention.

According to the results of Tables 3 and 4, significant differences were observed in between the fasting and non-fasting groups in terms of the TG, TC, HDL-C, LDL-C/HDL-C, TG/HDL-C, TC/HDL-C, Apo A, Atherogenic index, WBCs and RBCs levels ($P<0.05$), whereas no significant changes were observed in LDL-C, Apo B, hematological indices (such as hemoglobin), hematocrit, and platelets levels.

4. Discussion

According to the results of the present study, triglyceride, TC, LDL, LDL-C/HDL-C, HDL-C, and atherogenic index significantly decreased in the fasting group. Furthermore, fasting led to an increase in HDL. The results of the present study are consistent with those of Al Hourani et al. and Mohammadzade et al. [23, 24], whereas they are inconsistent with the findings reported by Roy et al. and Khan et al. [25, 26].

The limited release of free fatty acid from adipose tissue triglyceride mass into the bloodstream is considered as a fuel, triggering the activation of the lipase-stimulating enzyme via cellular signaling. An enzyme stimulating phosphorylation moves from the cytoplasm of adipose cells to lipid particles within the cell [27]. Before the Hormone-Sensitive Lipase (HSL) can catalyze the hydrolysis of triglycerides within lipid particles, the protein-based lipid nanoparticles must be phosphorylated. The action of the HSL on triglycerides results in the formation of two molars of non-esterified fatty acids and one mole of monoglycerides. The hydrolysis of this monoglyceride residue to glycerol and fatty acid is accomplished by the activity of monoglyceride lipase [28].

Triglycerides in the bloodstream are hydrolyzed by lipoproteins and lipases in the capillary endothelium of the muscles, and they release fatty acids. These released fatty acids are not directly absorbed in the lipolysis [29]. It has suggested that the mechanism, by which these fatty acids can be transmitted from the skeletal muscle is facilitated by the increased capacity of the muscle to absorb and burn fat through increased capillary density in the muscle. An increase in the surface area to remove more free fatty acids from the blood allows for the increased activity of the enzymes that move the fats and metabolize them. Catabolism of fats can also be influenced by factors, such as increased lipid oxidation to carbohydrates, increased use of intramuscular triglycerides, and decreased muscle glycogen content [30].

According to the results of the present study, WBCs, RBCs, hemoglobin, hematocrit, and platelet count decreased significantly in the fasting group. The results of the present study are consistent with those of Sedaghat et al. [5], but inconsistent with the findings of Ahmad et al. [31]. It was shown that prolonged starvation could dramatically decrease WBC count, and following a refeeding process by a coordinated operation, the immune system is rebuilt through a significant increase in hematopoietic cells to balance the cell population. It occurs even after severe suppression of WBCs and a balance in the cell population [32]. Even after severe suppression of WBCs and following damages, such as chemotherapy, or aging, prolonged starvation cycles can restore normal WBC count and the balance in WBCs. Therefore, regardless of the cause of the deficiency, the organism

Table 1. The characteristic of subjects in this study

Groups	Parameters			
	Mean±SD			
	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m²)
Fasting	20.40±0.63	179.00±6.30	79.96±5.32	25.3±2.33
Non-fasting	22.07±2.05	178.28±4.33	79.45±5.05	25.03±1.94
Inter-group P-value	-	P=0.72	P=0.79	P=0.99

Table 2. The result of the Shapiro-Wilk and Levene's tests to check the normality of data and variance homogeneity, respectively

Variables	Groups	Shapiro-Wilk		Levene's Test	
		Pre-test	Post-test	Pre-test	Post-test
TG (mg/dL)	Fasting	0.40	0.56	0.71	0.77
	Non-fasting	0.95	0.37		
TC (mg/dL)	Fasting	0.77	0.67	0.63	0.97
	Non-fasting	0.16	0.86		
LDL (mg/dl)	Fasting	0.66	0.98	0.85	0.57
	Non-fasting	061	0.86		
HDL (mg/dL)	Fasting	0.12	0.38	0.07	0.22
	Non-fasting	0.11	0.51		
LDL/HDL (mg/dL)	Fasting	0.31	0.37	0.31	0.66
	Non-fasting	0.45	0.21		
TG/HDL (mg/dL)	Fasting	0.63	0.85	0.72	0.58
	Non-fasting	0.76	0.11		
TC/HDL (mg/dL)	Fasting	0.57	0.17	0.09	0.88
	Non-fasting	0.74	0.86		
Apo A (mg/dL)	Fasting	0.27	0.51	0.16	0.13
	Non-fasting	0.65	0.18		
Apo B (mg/dL)	Fasting	0.23	0.14	0.78	0.16
	Non-fasting	0.13	0.09		
Atherogenic index	Fasting	0.77	0.67	0.63	0.97
	Non-fasting	0.16	0.86		
White blood cell count (x106/mm^3)	Fasting	0.74	0.83	0.81	0.11
	Non-fasting	0.06	0.51		
Red blood cell count (x106/mm^3)	Fasting	0.51	0.99	0.21	0.19
	Non-fasting	0.30	0.12		
Hemoglobin (gm/dl)	Fasting	0.11	0.13	0.99	0.32
	Non-fasting	0.40	0.78		
Hematocrit (%)	Fasting	0.33	0.06	0.59	0.57
	Non-fasting	0.12	0.11		
Platelets (1000)	Fasting	0.14	0.06	0.27	0.64
	Non-fasting	0.73	0.42		

Table 3. The levels of some biochemical variables measured before and after Ramadan

Variables	Groups	Variations				
		Stages		** P	*** P	
		* Mean±SD		P	F	P
		Pre-test	Post-test			
TG (mg/dl)	Fasting	75.00±15.15	67.93±16.26	0.03‡	0.01	0.03‡
	Non-fasting	68.85±17.27	71.35±15.01	0.44		
TC (mg/dl)	Fasting	135.26±25.83	126.40±24.00	0.001‡	7.53	0.03‡
	Non-fasting	135.35±20.49	142.78±20.68	0.11		
LDL (mg/dL)	Fasting	69.40±15.55	63.33±15.08	0.02‡	0.23	0.53
	Non-fasting	73.64±13.66	69.78±12.35	0.16		
HDL (mg/dL)	Fasting	38.66±4.09	42.46±5.19	0.004‡	0.58	0.01‡
	Non-fasting	47.21±8.03	46.35±7.09	0.57		
LDL/HDL (mg/dL)	Fasting	1.81±0.46	1.51±0.41	0.001‡	0.33	0.04‡
	Non-fasting	1.60±0.40	1.55±0.41	0.56		
TG/HDL (mg/dL)	Fasting	1.95±0.38	1.62±0.43	0.005‡	2.63	0.003‡
	Non-fasting	1.49±0.41	1.57±0.45	0.28		
TC/HDL (mg/dL)	Fasting	3.52±0.77	3.02±0.77	0.001‡	0.33	0.001‡
	Non-fasting	2.91±0.51	3.14±0.66	0.04		
Apo A (mg/dl)	Fasting	128.80±4.10	132.33±3.26	0.001‡	1.45	0.001‡
	Non-fasting	131.71±1.97	131.21±2.08	0.27		
Apo B (mg/dl)	Fasting	132.53±1.45	131.53±1.59	0.03‡	1.21	0.194
	Non-fasting	132.35±1.64	132.28±2.39	0.90		
Atherogenic index	Fasting	134.26±25.83	125.40±24.00	0.001‡	7.53	0.02‡
	Non-fasting	134.35±20.49	141.78±20.68	0.11		

* Data presented as Mean±SD

** Paired sample t-test

*** Inter-group P-value

‡ The mean difference is significant at the 0.05 level

PHYSICAL TREATMENTS

can regain its ability to rebuild the bloodstream through a period of starvation [33].

One of the reasons for the decrease in hematocrit is the decrease in the number of RBCs, which is due to the lack of RBC precursors [34]. In the current study, the RBC count decreased. Older blood cells damaged by foot kicking mechanically, muscle contraction, vessel wall collision, and gastrointestinal bleeding reduce the number of RBCs. Another cause for a decrease in hematocrit is due to the increased plasma volume, which also can be owing to a decline in blood concentration [35, 36]. In addition to the limitations, such as varied diet, different adaptation responses to physical activity, small sample size (due to withdrawal from the study), individual differences, and fasting time during Ramadan, the subjects' eating habits, gender, fat status, and race may also influence the interpretation of these results; therefore, caution should be exercised.

Table 4. The levels of some hematological indices measured before and after Ramadan

Variables	Groups	Stages		Variations		
		* Mean±SD		** P	*** P	
		Pre-test	Post-test	P	F	P
White blood cell count (x106/mm^3)	Fasting	7.16±1.87	6.46±1.28	0.04‡	1.13	0.02‡
	Non-fasting	6.64±1.84	7.25±2.52	0.18		
Red blood cell count (x106/mm^3)	Fasting	4.89±0.32	4.77±0.35	0.002‡	3.09	0.002‡
	Non-fasting	4.81±0.39	4.89±0.47	0.137		
Hemoglobin (gm/dL)	Fasting	13.90±1.07	13.39±1.60	0.02‡	0.29	0.08
	Non-fasting	13.95±1.01	13.88±0.90	0.61		
Hematocrit (%)	Fasting	42.66±3.34	41.44±2.73	0.02‡	0.11	0.82
	Non-fasting	41.51±4.56	41.72±4.08	0.79		
Platelets (1000)	Fasting	235.13±63.91	217.73±45.01	0.04‡	0.48	0.25
	Non-fasting	213.78±48.48	210.18±32.01	0.62		

* Data presented as Mean±SD

PHYSICAL TREATMENTS

** Paired sample t-test

*** Inter-group P-value

‡ The mean difference is significant at the 0.05 level

According to the findings of the current research, the Apo A and B levels increased and decreased, respectively in the fasting group It has been reported that the elevated Apo A level can be due to increased HDL, activation of the Lipoprotein Lipase (LPL), lysistine, and cholesterol acyltransferase enzymes, and decreased hepatic lipase activity [37, 38]. Fasting increases catecholamines and sympathetic nerve activity and decreases insulin secretion. It also increases the level of stress hormones (growth hormone, cortisol, epinephrine, and glucagon) secreted. This provides a suitable basis for mobilizing fats and releasing free fatty acids from the adipose tissue [39].

LPL activity increases in response to fasting, which can justify the rise in triglycerides and other serum lipids of the subjects following fasting [40]. The findings of this study showed that regardless of the highly valuable spiritual effects of the holy month of Ramadan, Islamic fasting through diet modification by changing the number and timing of meals and observing the precondition for proper calorie intake causes favorable changes in biochemical and hematological parameters in fasting individuals.

Ethical Considerations

Compliance with ethical guidelines

This randomized clinical trial was approved by the Ethics Committee of Iran University of Medical Sciences (Code: IR.MUMS.REC.1398.42401) and its proposal was registered at the Iranian Registry of Clinical Trials (No.: IRCT20120129008863N9).

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Authors' contributions

Design: All authors; Conceptualization, implementation, data analysis, Writing-original draft: Keyvan Hejazi; Data interpretation, writing – review & editing: Teimour Darzabi.

Conflict of interest

The authors declared no conflicts of interest.

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