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Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects

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ABSTRACT

Intermittent fasting and caloric restriction have been shown to extend life expectancy and reduce inflammation and cancer promotion in animal models. It was hypothesized that intermittent prolonged fasting practiced during the month of Ramadan (RIF) could positively affect the inflammatory state. To investigate this hypothesis, a cross-sectional study was designed to investigate the impact of RIF on selected inflammatory cytokines and immune biomarkers in healthy subjects. Fifty (21 men and 29 women) healthy volunteers who practiced Ramadan fasting were recruited for the investigation of circulating proinflammatory cytokines (interleukin [IL]-1β, IL-6, and tumor necrosis factor α), immune cells (total leukocytes, monocytes, granulocytes, and lymphocytes), and anthropometric and dietary assessments. The investigations were conducted 1 week before Ramadan fasting, at the end of the third week of Ramadan, and 1 month after the cessation of Ramadan month. The proinflammatory cytokines IL-1β, IL-6, and tumor necrosis factor α; systolic and diastolic blood pressures; body weight; and body fat percentage were significantly lower ($P < .05$) during Ramadan as compared with before Ramadan or after the cessation of Ramadan fasting. Immune cells significantly decreased during Ramadan but still remained within the reference ranges. These results indicate that RIF attenuates inflammatory status of the body by suppressing proinflammatory cytokine expression and decreasing body fat and circulating levels of leukocytes.

Abbreviations: BMI, body mass index; CR, caloric restriction; DBP, diastolic blood pressure; HC, hip circumference; HDL, high-density lipoprotein; IF, intermittent fasting; IL-1β, interleukin-1β; IL-6, interleukin-6; LPL, lipoprotein lipase; RIF, Ramadan intermittent fasting; SBP, systolic blood pressure; TG, triglycerides; TNF-α, tumor necrosis factor α; WBC, white blood cells; WC, waist circumference.

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1. Introduction

Excessive caloric intake and subsequent obesity are characterized by a chronic state of inflammation including high circulating proinflammatory cytokine levels, a condition that is described as "low-grade inflammation" [1,2]. In this condition, typically 2- to 3-fold increase in the systemic concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 is observed [3]. This increase in the expression of the proinflammatory cytokines may contribute to the induction of autoimmune diseases such as rheumatoid arthritis [4] and inflammatory diseases including atherosclerosis [5], insulin resistance [6], cardiovascular diseases [7], and tissue damage associated with many types of cancer [8].

Over the past few years, an increasing number of physiological effects of intermittent fasting (IF) have been documented in studies on rodents, monkeys, and humans [9]. Prominent among these are increased lifespan [10], decreased mortality from cancers and cardiovascular diseases [11,12], improved insulin sensitivity [13], and reduced oxidative stress and inflammation [14]. Interestingly, the inflammatory biomarkers such as IL-6 and C-reactive protein were found to be significantly depressed by short and long IF [15,16]. In overweight adults, caloric restriction (CR) has improved the clinical findings for patients with moderate asthma and reduced markers of oxidative stress and inflammation [17] and reduced metabolic disease risk markers [18]. Furthermore, it is noteworthy to point out that previous studies have shown that IF reduces oxidative stress and inflammation in tissues, including the brain [19]. Arumugam et al [19] showed that multiple neuroprotective pathways were activated and inflammatory pathways were suppressed by IF in young mice and that aging impaired the ability of IF to modulate these pathways adaptively. In rats, IF and CR have improved cardiovascular and hormonal responses to stress and substantially affect blood pressure and heart rates [20,21].

One of the 5 pillars of Islam is that healthy adult Muslims must fast from dawn to sunset during the holy month of Ramadan. Over the past 40 years, emerging evidence from epidemiologic studies has supported the health-related benefits of Ramadan IF (RIF), including improved insulin sensitivity [22], decreased atherogenic risk, oxidative stress, and inflammation [16,23]. Furthermore, RIF decreased the levels of some circulating cytokines [16,24]. However, there is no available evidence regarding the effect of RIF on circulating IL-1β, IL-6, and TNF-α and the impact of dietary changes in the people who fast during RIF on proinflammatory cytokines. Therefore, we hypothesized that, compared with nonfasting periods, healthy individuals would show lower levels in the circulating proinflammatory cytokines and immune cells during RIF. To test the hypothesis, we evaluated the anti-inflammatory effect of prolonged (8-24 hours) IF practiced during the holy month of Ramadan in randomly selected healthy volunteers and by using a follow-up model for a cross-sectional study design. The specific objectives of the study were to (i) examine the effect of RIF on the serum levels of the proinflammatory cytokines, namely, IL-1β, IL-6, and TNF-α; (ii) elaborate the effects of RIF on leukocyte differential count, total white blood cells (WBC), anthropometric characteristics, blood pressure, and dietary intake of calorie and antioxidant and energy-yielding nutrients; (iii) determine the correlation between the serum cytokine levels and dietary intakes of calorie, body weight and body fat percentage, systolic (SBP) and diastolic (DBP) blood pressures. Results of the present study are expected to open an avenue for further studies to explore the underlying mechanisms of the anti-inflammatory effect of RIF.

2. Methods and materials

This study was conducted in August to September 2009 over 3 stages: 7 days before Ramadan (T1), after the end of the third week of Ramadan fasting (after 21 days of RIF) (T2), and 1 month after the end of Ramadan fasting month (after 60 days from the start of Ramadan or 30 days from the cessation of Ramadan month) (T3). The range of fasting hours during the month was about 14 to 15 hours a day.

2.1. *Subjects*

To maintain sample homogeneity and avoid disparities and different confounding cultural and socioeconomic factors, all participants were chosen from the same living community of medium economic level (Rusaifa city of Zarka governorate, the Hashemite Kingdom of Jordan). A total number of 151 volunteers who visited the Al-Quds Medical Laboratories were recruited. The inclusion criteria necessitated that subjects were fasting at least 21 days of the Ramadan month and were not taking any drug or receiving any medical treatment immediately before or during the study. Those who were taking chronic medications, especially those affecting the inflammatory status such as nonsteroidal anti-inflammatory drugs were excluded before beginning of the study. Of the 151 volunteers, only 50 healthy subjects (21 men aged 18-49 years and 29 women aged 18-51 years) were deprived of the exclusion criteria and completed the 3 stages of the study. All enrolled participants were required to provide a written informed consent before starting the study. The study protocol was approved by Petra University Committee on Human Research Ethics. A questionnaire was administered, in which medical history and socioeconomic information were obtained through a personal interview with each subject by a well-trained staff member. It is noteworthy to mention that female subjects did not fast during their menstrual period because Islamic rules do not permit fasting during the menstrual period. Accordingly, the fasting period in female subjects ranged from 25 to 23 days, whereas for men, it was 30 days.

2.2. *Blood sampling*

Venous blood samples were collected from the enrolled volunteers during the 3 stages or time points. Each subject served as his own control by comparing his/her values before Ramadan (T1) with those during (T2) and after Ramadan (T3). Blood sampling was conducted in the private medical laboratory once a time at T1, T2, and T3 in the same hours

for the 3 time points of the study. It was performed early (first 1-2 hours, 10-12 AM) during the period of investigation (between 10 AM and 4 PM) in each time point. To narrow the time interval for blood sampling and thus to eliminate the effect of day time on blood variables, volunteers were asked to come early (at 10 AM), and blood was first drawn from most of the volunteers according to their arrivals to the laboratory, and then the less time-sensitive assessments (anthropometric and dietary) were subsequently performed during the rest of the investigation period. The obtained sera were separated within an hour by centrifuging the blood samples at room temperature for 5 minutes at 4000 rpm (Sigma 3-18K lab Centrifuge, Osterode am Harz, Germany). Aliquots of the blood samples were directly analyzed for complete blood count cells using Auto Hematology Analyzer (Mindray BC-3000, Shenzhen, China). At each visit, SBP and DBP (mm Hg) were measured using mercury sphygmomanometer, with the subject in a seated position after a 5-minute rest.

2.3. Anthropometric measurements

During each of the 3 visits, anthropometric measurements such as body weight and height (Seca, Hamburg, Germany), waist (WC) and hip (HC) circumferences (nonstretchable measuring tape), and body fat percentage (TANITA body composition analyzer, Tokyo, Japan) were performed by a well-trained staff member. Body weight was measured at the same time to the nearest 0.1 kg; WC and HC, to the nearest 0.01 m; and body height, to nearest 0.01 m. Body mass index (BMI; kg/m^2) and waist-to-hip ratio were the calculated. Volunteers with BMI less than 25 kg/m^2 were considered having normal weight, and those with a BMI of 25 kg/m^2 or greater were considered having overweight [25].

2.4. Assessment of dietary intakes

There were no special nutritional regimens or dietary recommendations given to the subjects during any stage of the study. Instead, a 24-hour food recall was taken from each subject during the 3 stages. Nutrient intakes were calculated using computerized tables from the Food Processor SQL food and diet analysis software system (version 8.4; Esha Research, Salem, OR, USA).

2.5. Determination of cytokine levels

For cytokine measurements, serum samples were aliquoted, coded, and stored at –80°C until being analyzed. Owing to the insufficient volume of the serum collected, TNF-α level was measured in 48 subjects, whereas IL-1β and IL-6 levels were measured in only 40 subjects.

Samples from the same volunteer were measured in the same assay to reduce the effect of interassay variation on serum cytokine levels. All samples were freeze-thawed once a time. At the end of the study, the 3 frozen samples drawn during the 3 time points (T1, T2, and T3) for each subject were thawed at the same time and then used in the biochemical measurement by using the same kit and personnel and under the same conditions, to eliminate sources of errors, to avoid the negative impact of cycled freezing-thawing on blood proteins and other components, and to avoid cytokine degradation. In addition, different kits were used within the same week. Serum levels of TNF-α, IL-1β, and IL-6 were measured by a sandwich enzyme-linked immunosorbent assay using commercially available matched monoclonal and biotinylated, antibody pairs commercial kits (PeproTech for TNF-α and IL-6, and R&D Systems DuoSet for IL-1β) (Minneapolis, MN, USA). The measurements were undertaken according to the manufacturer's instructions (DuoSet ELISA Development System; R&D Systems). The measurements of cytokines were performed at the specified absorbance and were detected using a microplate reader (BioTek, Winooski, VT, USA). All measurements were performed in triplicates, and concentrations of these mediators were determined using constructed standard curves.

2.6. Statistical analyses

The statistical analyses were performed using the Statistical Package for Social Sciences, version 16.0 (SPSS, Chicago, IL, USA). Results were expressed as means ± SD, and a P value less than .05 was considered statistically significant. Normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill normal distribution (IL-1β, SBP, DBP, and monocytes count) were analyzed by nonparametric tests. Three individuals with outlier values for TNF-α concentration were excluded from TNF-α data analysis because the values were clearly extreme and do not belong to the general distribution. Data were analyzed using the Student t test (either paired or unpaired) and Mann-Whitney and Wilcoxon nonparametric tests, by comparing values during T2, T3, and T1. Correlation tests were performed using the Pearson parametric correlation test for normally distributed variables and Spearman nonparametric test for skewed variables.

3. Results

3.1. Changes in the blood pressure and anthropometric measurements

The mean values of SBP, DBP, body weight, BMI, body fat, WC, and HC obtained during T1, T2, and T3 are listed in Table 1.

Table 1 – Anthropometric and blood pressure characteristics of study subjects before, during, and after RIF (n = 50; mean age, 32.70 ± 9.5 years)

Parameter	T1	T2	T3
BW (kg)	71.82 ± 13.41	70.58 ± 13.20 ***	71.92 ± 13.50
BMI (kg/m^2)	26.30 ± 5.01	25.85 ± 4.91 ***	26.49 ± 4.99
HC (cm)	102.61 ± 9.66	101.90 ± 9.98	101.02 ± 9.67 **
WC (cm)	83.62 ± 11.17	82.69 ± 10.34	82.68 ± 10.37 *
WC/HC ratio	0.81 ± 0.07	0.81 ± 0.07	0.81 ± 0.06
BF (%)	24.12 ± 12.60	20.38 ± 11.32 ***	30.48 ± 11.32 ***
SBP (mm Hg)	112.30 ± 10.01	104.40 ± 9.07 ***	111.82 ± 9.60
DBP (mm Hg)	76.20 ± 8.48	71.60 ± 10.40 **	74.50 ± 14.40

Results are expressed as means ± SD. BF, body fat; BW, body weight; T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
* $P < .05$, when statistically compared with T1 values.
** $P < .01$, when statistically compared with T1 values.
*** $P < .001$, when statistically compared with T1 values.

Compared with prefasting levels, Ramadan fasting resulted in a significant ($P < .001$) decrease in the body weight (70.58 ± 13.20 kg vs 71.82 ± 13.41 kg), BMI (25.85 ± 4.91 kg/m^2 vs 26.30 ± 5.01 kg/m^2), and body fat percentage (20.38 ± 11.32 vs 24.12 ± 12.60). The latter was significantly increased (30.48 ± 11.32), however, after Ramadan (ie, cessation of fasting). In contrast, both WC and HC were slightly but not significantly decreased during Ramadan, and then they were significantly ($P < .05$ and $P < .01$, respectively) decreased after Ramadan, when compared with prefasting (T1). Comparing with prefasting levels (T1), both SBP and DBP were significantly ($P < .001$) decreased during Ramadan (T2) (104.40 ± 9.07 mm Hg vs 112.30 ± 10.01 mm Hg and 71.60 ± 10.40 mm Hg vs 76.20 ± 8.48 mm Hg, respectively) and then returned to the basal values measured 1 month after Ramadan (T3).

When considering the body weight changes during the study, a strong positive correlation ($P < .05$) was found between the body weight and the body fat percentage during the 3 study time points (Table 2).

3.2. Cytokines and immune cells

As revealed in Table 3 and compared with prefasting (T1) values, levels were significantly and substantially ($P < .001$) reduced during Ramadan for circulating IL-1β (3.89 ± 4.84 pg/mL vs 17.84 ± 17.92 pg/mL), IL-6 (67.42 ± 51.25 pg/mL vs 155.85 ± 121.18 pg/mL), and TNF-α (52.22 ± 57.25 pg/mL vs 179.62 ± 129.56 pg/mL), with reduction values of about 78%, 57%, and 71%, respectively. However, IL-6 levels were maintained lower 1 month after Ramadan when compared with the basal levels (T1).

The total counts of leukocytes, granulocytes and, monocytes ($P < .001$) and lymphocytes ($P < .01$) were significantly decreased during Ramadan (T2) in comparison with before Ramadan (T1). Reduction values for WBC were less profound than those of the cytokines, and they were 14.1%, 14.4%, 11.2%, and 29.3%, respectively. Of a particular interest, the count of monocytes was significantly ($P < .01$) increased after Ramadan (T3) (Table 3).

Body weight, fat percentage, and caloric intake are among the most influencing factors that affect the levels of circulating cytokines. When considering the correlation between levels of circulating cytokines and those influencing factors during the 3 time points, we found that IL-1β was negatively ($P < .05$) correlated with body fat percentage, whereas it was positively correlated ($P < .05$) with energy intake during the fasting stage (T2) (Table 4). However, for the 2 other cytokines, no significant correlations were found with any of the influencing factors during the fasting stage (T2), and IL-6 was positively correlated with energy intake during the postfasting stage (T3), whereas TNF-α was positively correlated with energy intake during the prefasting stage (T1). It can be noticed, therefore, that energy intake was the most frequent factor that was positively correlated with the levels of the circulating cytokines (Table 4).

When considering the SBP and DBP, SBP was less intensely correlated with body weight and cytokine levels, whereas DBP was more profoundly correlated with these parameters. As shown in Tables 5 and 6, SBP was positively ($P < .05$) correlated with TNF-α only during the postfasting stage (T3), whereas DBP was positively ($P < .05$) correlated with IL-6, TNF-α, and body weight during the postfasting stage (T3) and with body weight only during the fasting stage (T2).

Interleukin-1β is among the most important cytokines and first studied during Ramadan fasting in our study. Results of IL-1β and its correlation with other cytokines and parameters (Table 7) revealed that it was positively correlated with IL-6 during the prefasting ($P < .001$) (T1) and postfasting ($P < .01$) (T3) stages, whereas it was positively correlated with TNF-α ($P < .05$) only during the postfasting stages (T3). Regarding its correlation with WBCs, IL-1β was positively correlated with total leukocytes ($P < .01$) and granulocytes ($P < .05$) only during the prefasting stages. When considering the age as an influencing factor on IL-1β, results revealed that age was positively correlated with IL-1β during both prefasting (T1) ($P < .05$) and fasting (T2) stages.

3.3. Nutrient intakes

As shown in Table 8, results of nutrient analyses revealed that caloric energy, total protein, total carbohydrates, polyunsaturated fatty acids, *trans*-fat, cholesterol, α-Carotene, vitamin C, and lycopene did not differ significantly during Ramadan (T2) when compared with their prefasting levels (T1). However, water intake and folate ($P < .001$), total fat, saturated and monounsaturated fats ($P < .01$), and β-carotene ($P < .05$) intakes were significantly decreased during Ramadan (T2) when compared with prefasting (T1).

Table 2 – Association between BW and BF percentage before, during, and after RIF

	T1		T2		T3	
	Pearson *r*	*P*	Pearson *r*	*P*	Pearson *r*	*P*
Body fat (%)	0.380 *	.007	0.438 *	.001	0.445 *	.001

Results are expressed as means ± SD. BF, body fat; BW, body weight; T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
* $P < .05$ is considered statistically significant.

Table 3 – Circulating levels of proinflammatory cytokines and immune cells before, during, and after RIF (n = 50; mean age, 32.70 ± 9.5 years)

Biomarker	T1	T2	T3
IL-6 (pg/mL) [a]	155.85 ± 121.18	67.42 ± 51.25 ***	109.6 ± 75.6 *
IL-1β (pg/mL) [a]	17.84 ± 17.92	3.89 ± 4.84 ***	17.59 ± 17.33
TNF-α (pg/mL) [b]	179.62 ± 129.56	52.22 ± 57.25 ***	147.62 ± 120.07
Total leukocytes (10^9/L)	6.74 ± 1.67	5.79 ± 1.42 ***	6.51 ± 1.91
Granulocytes (10^9/L)	4.02 ± 1.37	3.44 ± 1.20 ***	3.70 ± 1.32
Lymphocytes (10^9/L)	2.32 ± 0.71	2.06 ± 0.52 **	2.17 ± 0.86
Monocytes (10^9/L)	0.41 ± 0.15	0.29 ± 0.10 ***	0.57 ± 0.37 **

Results are expressed as means ± SD. T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
[a] n = 40.
[b] n = 48.
* $P < .05$, when statistically compared with T1 values.
** $P < .01$, when statistically compared with T1 values.
*** $P < .001$, when statistically compared with T1 values.

Table 4 – Associations between inflammatory cytokines, and BW, BF%, and EI

		T1			T2			T3		
		BW	BF (%)	EI	BW	BF%	EI	BW	BF%	EI
IL-1β	Pearson ρ	0.046	0.013	0.235	0.220	–0.37*	0.344*	0.092	0.086	0.271
	P	.783	.938	.203	.178	.018	.040	.579	.599	.100
IL-6	Pearson ρ	0.134	0.034	0. 484	0.121	0.053	0. 565	0.018	0.154	0. 459*
	P	.409	.835	.119	.455	.746	. 098	.914	.343	. 012
TNF-α	Pearson ρ	0.100	0.152	0. 797*	0.243	0.210	0. 748	0.101	0.100	0.370
	P	.825	.318	. 041	.107	.186	. 051	.511	.514	.142

BF%, body fat percentage; BW, body weight; EI, energy intake; T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
* $P < .05$ is considered statistically significant.

4. Discussion

It is now well recognized that long-lasting modifications in the circadian distribution of the eating and sleeping schedule during Ramadan fasting result in various changes in metabolism [26]. Significant weight loss and significant body fat reduction were observed during Ramadan and recovered to the baseline values 1 month after Ramadan. Our results were in accordance with previous findings showing a significant decrease in body weight [27,28] and a significant reduction in the body fat [29] during Ramadan. Because energy balance plays a regulatory role in body weight changes, some authors have associated Ramadan-related weight loss with the reduction in the total energy intake [22,28]. In our study, however, there was no significant difference in the total dietary energy intakes; even the difference between T1 and T2 was not a little amount of calories and equals the calorie of a small meal (555 kcal). Weight loss may also be explained by the negative fluid balance [30,31] because water intake significantly decreased among our subjects during Ramadan and then returned to baseline values 1 month after the end of Ramadan. The weight loss observed among our study subjects could be attributed to the reduced body fat because of the positive correlation between the 2 variables. Interestingly, although body fat was significantly increased after Ramadan, WC and HC were significantly decreased in the same time point. This may allow for the possibility of the pattern of body fat redistribution after Ramadan, which differs between males and females.

Given that TNF-α and IL-6 are well-known inhibitors of lipoprotein lipase (LPL) activity [32], decreased TNF-α and IL-6 levels may lead to increased LPL activity during Ramadan. This is in line with the increased LPL-catalyzed reaction products such as free fatty acids, during Ramadan [33]. Because glucose is less available during fasting, fat is considered a greater substrate for energy production, and accordingly, body fat and body weight are expected to be decreased [34]. This is in line with the insulin hyposecretion during Ramadan [22], favoring a predominant lipolytic state [33], which results in increased fat oxidation, observed during Ramadan [34].

Fasting reduces global cell proliferation rates [11,35], as evidenced in our study by the significant reduction in immune cells proliferation. Indeed, being in the reference ranges, total WBC, lymphocytes, monocytes, and granulocytes were significantly decreased during Ramadan and then returned to the basal values 1 month after Ramadan, except for monocytes, which significantly increased after Ramadan, which could be related to the rebound increase in postprandial activation as meal frequency increases after Ramadan [36]. These results were in accordance with the previous studies on healthy fasting volunteers [25,37,38] who showed a significant decrease in the total number of WBC during Ramadan.

In accordance with the antihypertensive effect of Ramadan fasting in previous studies [22,39,40], the present study also demonstrated decreases in both the SBP and the DBP. All participants were normotensive, which is expected given the fact that only 10 of them were older than 40 years. In fact, the decrease in blood pressure during Ramadan is more probably due to the reduction in body weight [39] or due to the inhibition of catecholamine production [41], which is known to be inhibited during hunger, causing a decrease in the

Table 5 – Associations between SBP and circulating cytokine levels and body weight before, during, and after RIF

Variable	T1		T2		T3	
	Spearman ρ	P	Spearman ρ	P	Spearman ρ	P
IL-1β	0.137	.400	0.017	.915	0.297	.096
IL-6	0.099	.544	0.088	.591	0.195	.199
TNF-α	0.036	.813	0.231	.126	0.351*	.026
BW	0.047	.745	0.208	.147	0.164	.255

Results are expressed as means ± SD. BW, body weight; T1, before Ramadan; T2, during Ramadan; and T3, after Ramadan.
* $P < .05$ is considered statistically significant.

Table 6 – Associations between DBP and levels of circulating cytokine and body weight before, during, and after RIF

Variable	T1		T2		T3	
	Spearman ρ	P	Spearman ρ	P	Spearman ρ	P
IL-1β	0.210	.192	0.040	.809	0.329*	.038
IL-6	0.294	.066	0.007	.966	0.354*	.025
TNF-α	0.028	.853	0.036	.825	0.168	.271
BW	0.134	.353	0.351*	.012	0.305*	.031

Results are expressed as means ± SD. BW, body weight; T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
* $P < .05$ is considered statistically significant.

Table 7 – Associations of IL-1ß with other cytokines, anthropometric measures, WBCs, and age before, during, and after RIF

Variable	T1		T2		T3	
	Pearson ρ	P	Pearson ρ	P	Pearson ρ	P
TNF-α	0.293	.088	0.265	.124	0.429 *	.010
IL-6	0.527 ***	.000	0.035	.228	0.422 **	.007
BMI	0.056	.730	–0.308	.054	0.042	.796
BF%	0.013	.938	–0.372 *	.018	0.086	.599
Total leukocytes	0.414 **	.008	0.006	.972	0.198	.221
Lymphocytes	0.249	.122	0.148	.363	0.109	.502
Granulocytes	0.374 *	.017	0.036	.825	0.177	.276
Monocytes [a]	0.137	.285	0.003	.986	0.120	.188
Age	0.378 *	.016	0.423 **	.007	0.047	.775

Results are expressed as means ± SD. BF, body fat; T1, before Ramadan; T2, during Ramadan; and T3, after Ramadan.
[a] Correlation test was performed using Spearman nonparametric test.
* $P < .05$.
** $P < .01$.
*** $P < .001$.

sympathetic tone and, as a consequence, falling of blood pressure, heart rate, and cardiac output. Our results agree with those that showed that IF has decreased blood pressure in rats [20,21]. Results of the current study are in agreement with those of Unalacak et al [24], who found that Ramadan fasting resulted in a significant reduction in SBP and DBP, as well WBC, TNF-α, and IL-2 and IL-8.

Interestingly, we found a positive association between SBP and TNF-α level after Ramadan. Similarly, a significant positive association was found between DBP and both IL-6 and IL-1β after Ramadan. This is in consistent with the reported decrease in the plasma IL-6 level in association with the decreased SBP and DBP during Ramadan [42].

Unexpected negative correlation was found between IL-1β levels and body fat percentage ($r = –0.372$, $P = .018$) during Ramadan. This is in contrast with previous findings linking adipose tissue to increased cytokine production, including IL-1β, IL-6, and TNF-α [43].

More than 80% of cytokine production by adipose tissue originates from nonfat cells, of which macrophages are the major constituents [43]. Further, weight loss is associated with a reduction in macrophage infiltration of adipose tissue [44], and macrophages are mainly derived from monocytes. Accordingly, it could be postulated that the decreased cytokine levels may be related to the decrease in the number of monocytes during Ramadan, as reported in this study. An indirect support of this notion is the postprandial activation of monocytes expressing TNF-α and IL-1β in response to meals [45] and the sustained increase in the monocyte secretion of TNF-α to more than 8 hours after each meal [36] and by the elevation of the number of monocytes expressing TNF-α within adipose tissue after a fat meal. Interestingly, the present study showed a significant positive correlation between IL-1β and total WBC ($r = 0.414$, $P = .008$) and granulocytes ($r = 0.374$, $P = .017$), supporting the correlation between the cytokine level and the fasting-induced reduction in the number WBC.

The reduction in the cytokine level during Ramadan fasting could also be attributed to the decreased oxidative stress during Ramadan [46] and the decreased reactive oxygen species, which plays an important role in activating the transcriptional factor nuclear factor κB [47], which is responsible for the expression of proinflammatory cytokines including IL-1β, IL-6, and TNF-α [47], a matter that requires to be further investigated. Given that the antioxidant nutrients such as vitamin C, vitamin E, and carotenoids are involved in cytokines expression and secretion via activation of the nuclear factor κB [47] and that our volunteer's diet during Ramadan (T2) was poor in these antioxidant nutrients including β-carotene and lycopene, the reductive effect of Ramadan fasting on the levels of proinflammatory cytokines

Table 8 – Dietary intake of different macronutrients and micronutrients by study subjects before, during, and after RIF (n = 50; mean age, 32.70 ± 9.5 years)

Nutrient	T1	T2	T3
Energy (kcal)	2516.6 ± 2144.98	1961.6 ± 1165.69	2079.0 ± 1446.04
Water (mL)	1585.4 ± 884.10	1296.66 ± 905.88 ***	1250.2 ± 673.21
Total protein (g)	83.09 ± 77.80	86.58 ± 192.15	68.39 ± 58.31
Total carbohydrates (g)	370.87 ± 362.20	285.84 ± 150.72	285.98 ± 216.22
Total fat (g)	74.46 ± 51.02	52.49 ± 29.87 **	71.78 ± 68.64
Saturated fat (g)	17.50 ± 14.61	12.34 ± 8.33 **	15.15 ± 12.89
Monounsaturated fat (g)	23.12 ± 20.55	14.03 ± 11.50 **	24.43 ± 47.11
Polyunsaturated fat (g)	9.84 ± 10.07	7.19 ± 5.91	8.83 ± 7.58
Trans-fat (g)	0.086 ± 0.19	0.14 ± 0.53	0.19 ± 0.52
Cholesterol (mg)	205.39 ± 236.48	193.73 ± 373.51	130.98 ± 172.18
α-Carotene (μg)	79.53 ± 358.77	87.83 ± 347.93	255.71 ± 865.43
β-Carotene (μg)	616.09 ± 1142.22	368.23 ± 852.11 *	892.65 ± 2195.08
Vitamin C (mg)	59.95 ± 52.42	74.72 ± 85.95	87.67 ± 150.20
Folate (μg)	505.25 ± 667.14	246.71 ± 216.63 ***	358.31 ± 445.81
Lycopene (μg)	353.23 ± 852.78	284.20 ± 693.36	575.17 ± 213.50

Results are expressed as means ± SD. T1, before Ramadan; T2, during Ramadan; T3, after Ramadan.
* $P < 0.05$, when statistically compared with before T1 values.
** $P < .01$, when statistically compared with before T1 values.
*** $P < .001$, when statistically compared with before T1 values.

could not be ascribed to these elements but, rather, to the practice of IF and CR.

High-density lipoprotein (HDL) levels have been found to be increased during Ramadan fasting and maintained for at least 1 month after Ramadan [27,40], explaining, in our study, the sustained decrease in the IL-6 levels month after Ramadan. Although not statistically significant, TNF-α levels were also lowered 1 month after Ramadan. In addition, TNF-α interferes with lipid homeostasis and activates proatherogenic processes, including reduction of HDL cholesterol and increase of low-density lipoprotein cholesterol [48]. Although increased HDL levels during RIF were attributed to changes in dietary patterns [49], findings from our study raise the hypothesis that decreased IL-6 and TNF-α level might be the most probable explanation for the previously reported antiatherogenic effect of Ramadan fasting [23]. The ability of RIF to reduce the levels of cytokinesIL-6 and TNF-α upon fasting may drive a speculation that RIF could be beneficial for those with from rheumatoid arthritis, a disease that had been reported to be characterized by an increased activity of the aforementioned cytokines [50], a mater that requires to be clinically investigated. Finally, because the 3 studied cytokines were found to be up-regulated in diabetes and reported to be significantly depressed in diabetic rats upon CR [51], it is worth to elaborate the impact of Ramadan fasting on diabetic people who practice Ramadan fasting, although those people are exempted from this worship and not allowed to fast.

This study maintains a few limitations. A potential limitation of this study was that, first, the protocol used in the present study may probably fail to give a better comparison between parameters in view of the prolonged investigation duration, which lasted from 10 AM to 4 PM for some, not all, of the subjects. Furthermore, it would be advisable to collect samples at 2 different time points each day (early morning and late afternoon) to elaborate the effect of fasting on 2 different periods of the day. Second, because both female and male subjects were included, the interruption of fasting during the menstrual period (5 ± 2 days) seems to affect the study results. However, data from female subjects showed a similar reduction in study parameters to that of male subjects. Third, the relatively small number of volunteers and, lastly, the lack of a control nonfasting group deprived the study from the ability to compare the effect of fasting to nonfasting, thus making it difficult to attribute the changes in cytokines and immune cells to the absolute effect of fasting.

Overall, our findings support the hypothesis that IF practiced during Ramadan by healthy subjects can effectively reduce inflammatory processes as evidenced by significantly reduced levels of leukocytes and circulating proinflammatory cytokines (IL-1β, IL-6, and TNF-α). On the other hand, RIF effectively reduced body weight, body fat, and WC, and HC. From the results of the present study, it can be found out that although caloric intake was not significantly restricted upon fasting, cytokine levels were significantly reduced, indicating that practicing IF could be beneficial for health even it is not associated with significant CR. Furthermore, energy intake was the factor most frequently correlated with the changes of cytokines before, during, and after fasting, a matter that necessitates the significance of energy intake on the inflammatory process. This impact of Ramadan fasting on cytokines provides biologically plausible mechanisms that may explain how Ramadan fasting may beneficially affect lipid and carbohydrate homeostasis as well as autoimmune diseases such as rheumatoid arthritis. The present study opens the door for future studies aiming to explore eventual mechanisms mediating this anti-inflammatory effect of RIF. It is suggested that further studies are to be done to elaborate the impact of RIF on patients with rheumatoid arthritis.

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