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Effects of Ramadan intermittent fasting on inflammatory and biochemical biomarkers in males with obesity

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Running head: Ramadan fasting and inflammation.

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Abstract

Background: To determine the effects of Ramadan intermittent fasting (RIF) on inflammatory (C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)) and biochemical markers of liver-renal function (aspartate aminotransferase (AST), alanine amino transferase (ALT), bilirubin, lactate dehydrogenase (LDH), urea and creatinine) in males with obesity.

Materials and Methods: Twenty-eight males with obesity were randomly allocated to an experimental group (EG, n=14) or a control group (CG, n=14). The EG group completed their fasting rituals for the entire month of Ramadan (30 days) whereas the CG group continued with their normal daily habits. Blood samples were collected 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2), and 21 days after the end of Ramadan (T3). Resting plasma volume variation between pre and post-RIF (ΔPV) was calculated.

Results: Decreases were noted for interleukin-6 ($p = 0.02$, $d = 1.4$) and tumor necrosis factor-alpha ($p = 0.01$, $d = 0.7$), with no changes for C-reactive protein ($p = 0.3$; $d=0.1$) in the EG compared to CG group. There were no changes ($P>0.05$) in ΔPV recorded after RIF for either EG (-0.035±0.02%) and CG (0.055±0.06%).

Conclusion: This study demonstrates that RIF improves systemic inflammation biomarkers in males with obesity. Moreover, RIF did not negatively affect biomarkers of liver and renal function.

Keywords: Obese; overweight; inflammation; biochemical biomarkers; Ramadan; fasting.

1. Introduction

Obesity is a key health risk factor characterized by an excessive accumulation of pro-inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6 [1, 2]. CRP and TNF-α are considered important specific biomarkers of vascular inflammation as well as being predictors of atherosclerosis [3, 4], while IL-6 has intrinsic pro-inflammatory activity and increases TNF-α concentrations [5]. Adipose tissue is the main source of these cytokines [6], making it important to implement different therapies to ameliorate excess adiposity and abnormal pro-inflammatory cytokine concentrations [7]; such strategies are likely to reduce the risk of developing metabolic syndrome in populations with obesity [6].

Intermittent fasting (IF) is a frequently used strategy to decrease excess adiposity and circulating pro-inflammatory cytokines while improving overall health[8, 9]. A common approach for undertaking IF involves severe caloric restriction (75-90% of energy needs) for 1-2 days per week. Another form of IF is Ramadan IF (RIF), which is routinely practiced by over 1.8 billion adherents on an annual basis where they undertake one month of RIF in observance of the Islamic obligation (Ramadan) involving abstinence from food and liquids from dawn to sunset [10]. Ramadan is the ninth month of the Islamic lunar calendar and fasting during this month represents one of the five pillars of Islam. The duration of the fasting period commonly lasts from 10 to 18 hours and varies according to the season and geographical location [11, 12]. The lunar year is 11 days shorter than the Gregorian year; therefore, Ramadan occurs in a different season every 9 years [13]. The season during which Ramadan occurs affects the duration of each fast because fasting hours are longer in summer than in winter. Ramadan is an extensively studied example of a timed dietary approach to IF [14, 15].

Several studies reported decreases in body weight following RIF [16]. For instance, a meta-analysis of 35 studies by Sadeghirad et al. (2014) assessed body weight changes in fasting individuals during and after RIF reported fairly small but significant weight loss (-1.24 kg) in both genders, with most of the weight loss being regained within a few weeks after Ramadan [17]. Another meta-analysis of 21 studies that analyzed the effects of RIF on body weight reported a slight but significant reduction in body weight in men, with no significant changes observed in women [18]. A more recent meta-analysis of 70 studies (~3,000 participants) in individuals classified as overweight and obese, demonstrated significant decreases in body fat percentage. This reduction was not observed in normal-weight participants [19]. Nevertheless, body weight and composition returned to near pre-Ramadan levels after 2-5 weeks of follow-up. Moreover, other studies failed to observe significant changes in body weight during RIF [20], while some even described weight gain during this period[21]. The inconsistency in these findings can be explained by varied eating routines, socioeconomic status, differences in

the number of fasting hours, ethnicity, gender, and the health status and medical history of participants [17, 22, 23].

Other than changes in body weight and composition, several health benefits have been credited to RIF including improvements in blood pressure and brain function [19]. However, data on the effects of RIF on pro-inflammatory biomarkers is equivocal, with some studies reporting benefits [24], while others showing no changes [25, 26]. This discrepancy in results may be related to participants' characteristics and study design as well as the length of fasting which differs from one geographical location to the other and from one year to the other [13, 27]. Moreover, to the best of authors' knowledge, only a few studies have explored the effects of RIF on inflammatory biomarkers in individuals with obesity, but with inconclusive findings due to a lack of control groups [24, 28]. Many studies on RIF measured a limited number of pro-inflammatory and biochemical biomarkers but only before and immediately after RIF, with no measurements of biomarkers during and or for a period after RIF.

Thus, we chose to evaluate the effects of RIF on inflammatory (CRP, IL-6, and TNF-α) and biochemical biomarkers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, lactate dehydrogenase (LDH), urea, and creatinine] in males with obesity. We measured these biomarkers at four-time points: 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of Ramadan (T3). We hypothesized that RIF would improve circulating pro-inflammatory cytokines and biochemical biomarkers in middle aged males with obesity across all the measured times points.

2. Materials and Methods

2.1. Participants

Fifty-one males were recruited from the students and staff of a regional university, by direct contact as well as via social networks. The inclusion criteria were as follows: body mass index of 30-40 kg/m^2, no use of drugs or alcohol, lack of regular exercise for at least 6 months before the study, no history of renal, hepatic, cardiovascular diseases or diabetes, and no physical injuries or mobility concerns. The exclusion criteria were as follows: diabetes, cognitive disorders, cardiovascular diseases including uncontrolled hypertension, cancer, liver or renal disease, chronic pulmonary disease, physical disability, or any other contraindications. Moreover, those with evidence of clinical depression were excluded from the study as antidepressant medications can alter pro-inflammatory cytokine concentrations [29, 30]. Thirty-two eligible males with obesity provided informed consent before participating in the study. A physician using a medical health/history questionnaire as well as the

Physical Activity Readiness Questionnaire (PAR-Q) evaluated all these criteria. The study was approved by the Ethical Committee on Human Research of the University of Rennes 2, and the University of la Manouba, Tunisia, and all procedures were carried out in accordance with the Helsinki Declaration.

2.2. Study Design

This study was conducted during Ramadan 2019 (from May 5th to June 3th) in Tunis, Tunisia. The fasting duration was 15-16 hours per day when the mean daily temperature was approximately 24°C. Participants were allocated (simple randomization) to either an experimental group (EG, n=16, who declared that they would fast for the entire month of Ramadan) or to a control group (CG, n=16, who declared that they would not fast during Ramadan) ***(Figure 1).*** The EG completed their fasting rituals for the duration of Ramadan (30 days) while the CG continued with their normal daily habits. Participants were fully familiarized with the tests prior to all procedures. Anthropometric measurements and blood samples were collected at four-time points: 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2), and 21 days after the end of Ramadan (T3). All measurements were obtained at the same time of day (within ~1 hour).

2.3. Dietary habits

All participants were asked to record their normal nutritional habits during the experiment; none of the participants reported using dietary supplements. Specifically, participants were asked to complete a two-day food diary prior to each blood sampling. The amount of nutrients consumed was calculated using the method described by McCance & Widdowson (2002) [31]. Subjects in the CG were instructed to not alter their dietary habits during Ramadan. Other than RIF, no other dietary changes occurred in the EG group.

2.4. Blood sampling and analysis

Participants were instructed to comply with the following conditions before each blood sample was obtained: 1) avoid using medications for at least 72 h before a blood draw, 2) avoid any strenuous exercise for at least 72 h before a blood draw, and 3) maintain the same diet for 48 hours prior to each blood draw. Blood was collected from an antecubital vein in the morning (between 8 and 9 a.m.) after an overnight fast. Blood sampling and analysis were performed at the laboratory of Biological Analysis, Principal Military Hospital (Tunis, Tunisia). Samples were collected in two pre-cooled 4.9-mL EDTA vacuettes (Klap activator model). Each whole blood sample was analyzed for a complete blood cell count using an automated cell counter (Luna-II^{TM}, Biosystems, France). Hematocrit (Ht) and hemoglobin concentrations ([Hb]) were determined. Plasma volume variation (ΔPV) was calculated according to the formula proposed by Dill and Costill (1974) [32]:

$$\Delta PV\ (\%) = 100 \times [([Hb]_a/[Hb]_b) \times [(1\text{-}Ht_b) / (1\text{-}Ht_a)] \text{ -}100]$$

where 'a' and 'b' refer to fasting state values for Ht and [Hb] before and after training, respectively.

One EDTA vacuette containing freshly drawn blood was immediately centrifuged at 1500 × *g* for 10 min at 4 °C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant was then dispensed into separate 2-mL cryovials and stored at −80 °C for later analysis of inflammation (CRP, IL-6, and TNF- α) and biochemical (ALT, AST, bilirubin, LDH, urea, and creatinine) biomarkers.

Plasma concentrations of IL-6 and TNF-α were determined by enzyme-linked immunosorbent assay (ELISA) using human quantikine ELISA kits. The sensitivity was 5.5 pg/mL for TNF-α and 0.7 pg/mL for IL-6 measurements. The activity of CRP was determined using the immunoturbidimetry method (Tina-quant CRP; Boehringer Mannheim, Germany). The intra-assay coefficient of variation for the CRP kit was 1.7%. Activities of AST, ALT, bilirubin, LDH, urea, and creatinine were determined using a multiparametric analyzer (Konelab 30™, Thermo Electron Corporation). The intra-assay and inter-assay coefficient of variation for these biochemical parameters were <10 % and <12%, respectively. The activity of LDH was determined by applying the enzymatic rate method (IFCC). The intra-assay coefficient of variation for the LDH assay was 1.1%.

2.5. Anthropometric measurements

Body weight were measured on a digital scale (Lumbar, China) with a precision of 0.1 kg. The height was measured with a medical sampler (Race Industrialization, China) with a precision of 0.1 cm. Body mass index was calculated as kg/m^2. Waist circumference was measured as the smallest circumference on the waist area (typically 2.5 cm above the navel) and hip circumference was measured around the widest part of the hips. The waist-hip ratio was calculated using the participant's waist and hip circumferences. Body fat percentage was evaluated using a multi-frequency bioelectrical impedance device (Inbody 720, South Korea) according to the manufacturer's recommendations.

2.6. Statistical analysis

An a priori power analysis (expected SD of residuals = 2 pg/ml for IL-6, desired power = 0.80, and alpha error = 0.05) was computed to estimate a statistically significant group-by-time-interaction effect for our primary outcome (IL-6) [33]. The analysis revealed a sample size of n = 10.8 per group. To account for potential dropouts, 16 participants were included in each of the two study groups.

Data are presented as mean ± standard deviation (M±SD). After confirming the normality of data distribution using the Shapiro-Wilk test, differences within and between groups were calculated using a two-way analysis of variance (ANOVA) with repeated measures. A Newman-Keul's post hoc test was calculated. Correlation

between parameters were assessed using Pearson's product-moment correlation coefficient (r) if the group x time interactions were significant. Effect sizes (ES) were determined from ANOVA output by converting partial eta-squared to Cohen's d. Within-group ES were computed using the following equation: ES = (mean post – mean pre)/SD. ES were considered trivial (< 0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0) and very large (2.0-4.0) [34]. The level of significance was set at $p<0.05$. All statistical analyses were computed using SPSS for Windows, version 24.0 (SPSS Inc., USA).

3. Results

We screened 41 males with obesity during Ramadan 2019 (from May 5th to June 3th) in Tunis, Tunisia. Only 36 qualified for baseline evaluation and were subsequently randomized to either the EC or the CG groups; 2 participants in the EC group dropped out of the study as they did not fast for all the days of Ramadan. In the CG group, two participants dropped out as they were unable to maintain their normal daily dietary habits (fasted 5 to 7 days during this month). No harms or unintended effects were reported by participants. Data are presented for the 28 participants that successfully completed our intervention [(EG, n=14, age: 24± 3.4 y, height: 173 ± 6 cm), (CG, n=14, age: 23.8 ± 3.8 y, height: 171 ± 3.8 cm)] ***(Figure 1).*** All remaining participants reported a 100% adherence to study procedures. There were no differences in anthropometric and hormonal measurements between the two groups at baseline. Macronutrient values of EG and CG recorded at T0, T1, T2, and T3 are described in ***Table 1***. There were no differences in macronutrient intakes between the two groups at any stage of the study.

Anthropometric and body composition characteristics of participants are presented in ***Table 2***. Participants in the EG experienced significant decreases in body weight (-2.7%, $p = 0.002$, $d = 1.12$), body mass index (-2.8%, $p = 0.006$, $d = 0.73$), body fat percentage (-4.2%, $p = 0.008$, $d = 1.23$), and waist-hip ratio (-6.2%, $p = 0.005$, $d = 1.19$) over during Ramadan, with no changes recorded in the CG.

Inflammatory and biochemical markers are shown in ***Table 3 and 4***, respectively. The EG experienced significant decreases in IL-6 ($p = 0.02$, $d = 1.49$) and TNF-α ($p = 0.019$, $d = 0.78$) concentrations over time. Concentrations of AST ($p=0.27$, $\eta^2= 0.05$), ALT ($p=0.42$, $\eta^2 =0.01$), bilirubin ($p=0.40$, $\eta^2 =0.06$), creatinine ($p=0.48$, $\eta^2 =0.03$), CRP ($p = 0.3$; $\eta^2 =0.02$), LDH ($p=0.66$, $\eta^2 =0.03$), and urea ($p=0.32$, $\eta^2 =0.05$) did not differ between the two study groups over time

Some significant correlations were noted between anthropometric changes and inflammatory markers ***(Table 5)***. The Δ of TNF-α correlated with Δ of body weight ($r=-0.649$, $p=0.008$) and with Δ of BMI ($r=-0.683$, $p=0.007$).

4. Discussion

The main finding of our study is RIF improves body composition and inflammatory biomarkers in middle aged males with obesity without harmful effects on their hepatic or renal function. The improvements in body composition (weight, body mass index, body fat percentage, and waist-hip ratio) occurred later in Ramadan (30-day) but not in the middle of Ramadan and remained unchanged for 21 days following RIF. Furthermore, decreases in IL-6 and TNF-α occurred in the middle of Ramadan and remained unchanged until the end of Ramadan, and 21 days thereafter. To the best of our knowledge, our study is the first to assess the effects of RIF on body composition, inflammatory, and biochemical biomarkers at four different time points: 24 hours before the start of Ramadan, on the 15th day of Ramadan, the day after the end of Ramadan, and 21 days after the end of Ramadan.

Obesity increases pro-inflammatory cytokines and acute-phase proteins such as TNF-α, IL-6, or CRP [35]. Weight loss by caloric-restriction is a key recommendation for decreasing the complications of obesity [36]. Hence, RIF as a form of intermittent fasting, (caloric restrictions involving a restricted feeding period based on religious and spiritual traditions) can decrease systemic inflammation in individuals with obesity and chronic diseases [9, 24]. In agreement with our results, a previous systematic review and meta-analysis reported that TNF-α and IL-6 were the most impacted biomarkers following RIF [9], while another study reported that RIF reduced circulating concentrations of the pro-inflammatory cytokines (IL-6 and TNF-α) before, during, and after Ramadan in overweight participants [37]. The concentrations of IL-6 and TNF-α on day 15 of Ramadan were lower compared to pre-Ramadan concentrations [28]. A study by Unalacak et al. reported that RIF reduced TNF-α (but not CRP) concentrations in healthy and individuals with obesity [24]. Animal studies suggest that IF inhibits the nuclear factor κB (NF-κB) signaling pathway [38]. NF-κB is an important pro-inflammatory transcriptional regulator that regulates the expression of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α.

Weight loss following RIF improves the inflammatory biomarkers in adipose tissue by reducing inflammation-related gene expression in adipocytes and macrophages [39]. Calorie restriction and weight loss decreases activation of NF-κB, a major pro-inflammatory signaling pathway that regulates the expression of pro-inflammatory cytokines such as IL-6, and TNF-α[40]. Weight loss decreases CRP concentrations[41]. On the other hand, the most of the cytokines produced by adipose tissue originates from non-fat cells, of which macrophages are key components[42]. Weight loss lowers macrophage infiltration of adipose tissue; macrophages are mainly derived from monocytes[43]. Therefore, it is likely that improving inflammatory

biomarkers by decreasing cytokine concentrations may be related to a decreased number of monocytes following RIF-induced weight and fat loss[37].

The reduction in the circulating concentrations of pro-inflammatory cytokines during RIF could also be attributed to decreased oxidative stress [25]. RIF improved the gene expression of antioxidant defense enzyme systems including mitochondrial transcription factor A (TFAM), superoxide dismutase (SOD2), and nuclear factor erythroid 2-related factor 2 (Nrf2) [44]. These changes could also explain the reduction in circulating pro-inflammatory cytokines at the end of Ramadan as reported in previous studies [9, 44] and our study. Moreover, decreased reactive oxygen species could also alter the activation of NF-kB signaling during RIF [45]. Another possible mechanism for the anti-inflammatory effects of RIF could be related to its association with mental health and mood improvement since mental health is also associated with improvement in systemic inflammation[46].

The results of our study indicate a positive correlation between weight loss, BMI, and TNF-α concentrations following RIF. Some potential mechanisms that could account for weight and BMI decreases associated with lower circulating TNF-α concentrations involve calorie restriction following RIF [47]. Findings from animal and human studies suggest that dietary restrictions which causes weight loss are related to improvement in TNF-α concentrations and gene expression [48, 49] that may be mediated by NF-kB signaling [50].

Our study suggests that RIF is a relatively convenient means of achieving weight loss over a short period, and therefore may be a useful tool for weight loss strategies [51]. However, the effects of RIF on body weight is controversial. Although RIF decreases meal frequency, some studies reported either increased energy intake [52] or comparable energy intake before, during, and after RIF occurrences [53]. However, a meta-analysis of thirty-five studies suggests that RIF causes significant (1.24 kg) weight loss [17]. This analysis also indicated that most of the weight loss was regained within a few weeks after RIF [17]. Our study indicates that RIF resulted in a significant weight loss at days 15 (-0.6 kg) and 30 (-3kg or 3.1%). However, 17% (not statistically significant) of the weight loss was regained within 21 days after the end of RIF.

We report a 2.1% decrease in body fat percentage, which can lower carbohydrate oxidation and increase fat oxidation during RIF [54]. Decreases in body fat percentage are generally anticipated during RIF [19], but this is not always the case as some studies report no changes in body fat percentage [55], while others reported non-significant changes [56]. These discrepancies may be related to the different characteristics of the participants in terms of age, sex, dietary intake, physical activity, and the number of hours of fasting.

Previous studies demonstrated that RIF did not adversely affect the liver and renal function in individuals with obesity [24, 57, 59] which we confirmed, but other studies offered different conclusions. For instance, Nasiri et al. reported that RIF increased ALT, AST, ALP, and bilirubin concentrations, although these changes were within the normal range and non-significant, but nonetheless, the authors offered caution about such changes [58]. Our findings indicate no differences in renal and liver biomarkers during and after RIF, which is supported by most studies on liver and renal biomarkers following RIF [59].

Our study has some limitations. Although physical activity levels usually decrease during RIF [10], we did not measure the levels of physical activity before, during, and after RIF. Physical activity influences inflammatory and biochemical biomarkers. Additionally, we performed a per-protocol analysis which may have biased our findings. Although this type of analysis better reflects the effects of intervention when performed optimally, the clinical applicability of this per-protocol effect is limited if the effect in the intervention compliers differs substantially from the effect in other cohorts [60].

Our study indicates that RIF improves body composition and biomarkers of systemic inflammation, and that these changes persisted for three weeks after Ramadan. Moreover, RIF did not adversely impact liver and renal biomarkers, suggesting the approach is not only efficacious but safe to perform.

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Conflict of interest

The authors declare no conflict of interest.

Figure legends:

Figure 1. Flow diagram of study design and participant allocation.

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Table 1: Macronutrient values (mean ± SD) of the experimental group (EG, n=14) and control group (CG, n=14) measured 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of Ramadan (T3). Data are expressed as means ± SD.

Macronutrients	Group	T0	T1	T2	T3
Protein (% kcal)	EG	17 ± 10	20 ± 8	19 ± 6	19 ± 9
	CG	17 ± 2	18 ± 4	19 ± 8	19 ± 6
Carbohydrate (% kcal)	EG	48 ± 7	46 ±7	45 ± 7	47 ± 11
	CG	47 ± 7	46 ± 9	46 ± 8	47 ± 8
Fat (% kcal)	EG	35 ± 3	34 ± 4	36 ± 9	36 ± 6
	CG	36 ± 3	36 ± 3	35 ± 8	34 ±5

Table 2: Anthropometric and body composition characteristics (mean ± SD) of the experimental group (EG, n=14) and control group (CG, n=14) measured 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of Ramadan (T3).

Variables	Group	Phases				p values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
Body weight (kg)	EG	97.5 ± 4.4	96.9 ± 4.2 $^{\$\#}$	94.5 ± 4.5 $^{*\#}$	95 ± 4.6 $^{*\#}$	0.001 (0.67)	0.01 (0.21)	0.001 (0.63)
	CG	100.6 ± 4.6	100.4 ± 4.6	100.4 ± 4.7	100.6 ± 4.7			
BMI (kg/m^2)	EG	33.3 ± 1.3 $^{\#\$}$	33.1 ± 1.2 $^{\wedge\$}$	32.2 ± 1.2 $^{*\#}$	32.4 ± 1.3 $^{*\#}$	0.002 (0.67)	0.2 (0.04)	0.001 (0.65)
	CG	33.7 ± 2.7	33.6 ± 2.7	33.6 ± 2.7	33.7 ± 2.6			
Body fat percentage (%)	EG	35.2 ± 1.5 $^{\wedge\$}$	35.2 ± 1.5 $^{\wedge\$}$	33.1 ± 1.6 $^{*\#\$}$	33.8 ± 1.9 $^{*\#\wedge}$	0.001 (0.85)	0.95 (0.00)	0.002 (0.85)
	CG	34.3 ± 1.8	34.4 ± 1.8	34.3 ± 1.7	34.4 ± 1.8			
WHR (cm^2)	EG	0.97 ± 0.01$^{\wedge\$}$	0.95 ± 0.05 $^{\$}$	0.91 ± 0.04 $^{*\$}$	0.87 ± 0.04 $^{*\#\wedge}$	0.08 (0.24)	0.01 (0.68)	0.001 (0.80)
	CG	0.97 ± 0.03	0.98 ± 0.02	0.99 ± 0.01	1 ± 0.02			

Data are expressed as means ± SD.

BMI: BMI; WHR: Waist to hip ratio; ES: effect size. * *Significant from T0.* $^{\#}$ *Significant from T1,* ^ *Significant from T2,* $^{\$}$ *Significant from T3.*

Table 3. Inflammation markers of the experimental group (EG, n=14) and control group (CG, n=14) measured 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of Ramadan (T3).

Markers	Group	Phases				p values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
CRP (mg/L)	EG	18.02 ± 1.7 $	18.03 ± 1.7 $	18.2 ± 1.8 $	18.7 ± 1.7 *#^	0.002 (0.66)	0.6 (0.008)	0.3 (0.13)
	CG	17.6 ± 1.7	17.9 ± 2.1	17.7 ± 2.2 $	18.3 ± 2.5 ^			
IL-6 (pg/mL)	EG	49.1 ± 4.2 #^$	46.2 ± 3.5 *	44 ± 3.1*	45.5 ± 3.2*	0.001 (0.5)	0.2 (0.04)	0.006 (0.39)
	CG	48.2 ± 4.8	47.9 ± 5.1	47.4 ± 5.5	48 ± 5.1			
TNF-α (pg/mL)	EG	4485.7 ± 575 #	4078.5 ± 629 *	4028.5 ± 610.7	3971.4 ± 693.2	0.001 (0.53)	0.86 (0.001)	0.024 (0.32)
	CG	4192.8 ± 880 #^	4021.4 ± 893 *	4000 ± 842.1*$	4157.1 ± 809.2 ^			

Data are expressed as mean ± SD.

CRP, high-sensitivity C-reactive protein; IL-6, Interleukin 6; TNF-α, tumor necrosis factor alpha. * Significant from T0. # Significant from T1, ^ Significant from T2, $ Significant from T3.

Table 4: Biochemical markers (AST, ALT, Bilirubin, LDH, Urea and creatinine) (means ± SD) of the experimental group (EG) and control group (CG) measured 24 hours before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of Ramadan (T3).

Variables	Group	Phases				p values (ES)		
		T0	T1	T2	T3	Time	Group	Group x Time
AST (UI/L)	EG	28.9 ± 7.2	28.5 ± 6.7	27.9 ± 6.9	28.8 ± 6.8	0.003 (0.42)	0.76 (0.003)	0.27 (0.14)
	CG	29.4 ± 5.9	29 ± 7.5	26.3 ± 7.6	26.8 ± 8.3			
ALT (UI/L)	EG	69.3 ± 5.3 #^$	68.4 ± 4.7 *^$	65.6 ± 4.9 *#$	67.4 ± 4.6 *#^	0.001 (0.60)	0.14 (0.08)	0.42 (0.1)
	CG	66.8 ± 6	64.8 ± 5.6	62.9 ± 6.3	64.2 ± 6.1			
Bilirubin (mg/dL)	EG	14.5 ± 1.9	14.3 ± 1.8	13.9 ± 2	14.5 ± 2	0.002 (0.54)	0.95 (0.00)	0.4 (0.11)
	CG	14 ± 2.4	14.3 ± 2.2	14.3 ± 2.1 $	14.8 ± 2.2 ^			
LDH (UI/L)	EG	62.9 ± 17.9 ^	62.3 ± 17.6	59.4 ± 17 *	60.9 ± 17.2	0.002 (0.44)	0.1 (0.09)	0.66 (0.06)
	CG	69.8 ± 15.1	71.3 ± 12.6	70 ± 10.7	71.6 ± 11.4			
Urea (mmol/L)	EG	6.8 ± 1.2 $	6.7 ± 1.1 $	6.8 ± 1.2 $	7.2 ± 1.3 *#^	0.001 (0.49)	0.2 (0.06)	0.32 (0.13)
	CG	7 ± 1	7.4 ± 0.8	7.3 ± 0.6	7.8 ± 0.5			
Creatinine (mg/L)	EG	13.7 ± 2.4	13.9 ± 2.5 ^	13.2 ± 2.2 #	13 ± 2.2	0.06 (0.26)	0.5 (0.01)	0.48 (0.09)
	CG	14 ± 1.4	13.9 ± 1.3	13.7 ± 1.2	13.8 ± 1.7			

Data are expressed as mean ± SD.

AST, aspartate Aminotransferase; ALT, Alanine Aminotransferase; LDH, Lactate Dehydrogenase.* *Significant from T0.* # *Significant from T1,* ^ *Significant from T2,* $ *Significant from T3.*

Table 5: Relationship between variations of anthropometric characteristics and inflammatory markers

		Δ Weight			Δ BMI			Δ Waist/Hip		
		T1-T0	**T2-T0**	**T3-T0**	**T1-T0**	**T2-T0**	**T3-T0**	**T1-T0**	**T2-T0**	**T3-T0**
IL-6 (pg/mL)	**T1-T0**	P=0.708 r=-0.11	P=0.467 r= -0.21	P=0.467 r= -0.21	P=0.708 r= -0.11	P=0.467 r= -0.21	P=0.467 r= -0.21	P=0.726 r= -0.103	P=0.685 r= -0.119	P=0.66 r=0.127
	T2-T0	P=0.592 r =0.157	P=0.613 r= 0.148	P=0.998 r= 0.001	P=0.592 r= 0.157	P=0.613 r= 0.148	P=0.988 r= 0.001	P= 0.312 r=-0.291	P=0.21 r= -0.357	P= 0.188 r=-0.374
	T3-T0	P=0.658 r = 0.13	P=0.908 r= 0.034	P=0.807 r= 0.072	P=0.658 r = 0.13	P=0.908 r= 0.034	P=0.807 r= 0.072	P=0.379 r=-0.255	P=0.358 r=-0.266	P=0.097 r=-0.461
TNF-α (pg/mL)	**T1-T0**	P=0.113 r=-0.443	P=0.585 r=0.16	P=0.342 r=0.275	P=0.113 r=-0.443	P=0.585 r=0.16	P=0.342 r=0.275	P=0.584 r=-0.161	P=0.66 r=-0.129	P=0.486 r=-0.203
	T2-T0	**P=0.011*** **r=-0.653**	P=0.608 r=-0.15	P=0.939 r=0.023	**P=0.012*** **r=-0.675**	P=0.608 r=-0.15	P=0.939 r=0.023	P=0.102 r=0.455	P=0.122 r=0.433	P=0.058 r=0.517
	T3-T0	**P=0.008**** **r=-0.649**	P=0.782 r=-0.81	P=0.77 r=0.083	**P=0.007**** **r=-0.683**	P=0.782 r=-0.81	P=0.77 r=0.083	P=0.146 r=0.409	P=0.128 r=0.426	P=0.051 r= 0.53
CRP (mg/L)	**T1-T0**	P=0.409 r=-0.24	P=0.114 r=-0.441	P=0.545 r=-0.177	P=0.409 r=-0.24	P=0.114 r=-0.441	P=0.545 r=-0.177	P=0.771 r=0.086	P=0.333 r=0.279	P=0.096 r=-0.462
	T2-T0	P=0.814 r=0.069	P=0.691 r=-0.117	P=0.943 r=0.021	P=0.814 r=0.069	P=0.691 r=-0.117	P=0.943 r=0.021	P=0.239 r=0.377	P=0.177 r=0.382	P=0.264 r=0.321
	T3-T0	P=0.195 r=-0.368	P=0.256 r=-0.325	P=0.887 r=-0.042	P=0.195 r=-0.368	P=0.256 r=-0.325	P=0.887 r=-0.042	P=0.246 r=0.332	P=0.258 r=0.324	P=0.436 r=0.436

Δ: delta variations, CRP, high-sensitivity C-reactive protein; IL-6, Interleukin 6; TNF-α, tumor necrosis factor alpha. * Significant correlation between parameters.

Figure 1. Flow diagram of study design and participant allocation.

