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

3

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19

20 **Running head:** Ramadan fasting and inflammation.

21

22

23 **Abstract**

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

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

34 **Results:** Decreases were noted for interleukin-6 ($p = 0.02$, $d = 1.4$) and tumor necrosis factor-alpha ($p = 0.01$, d
35 $= 0.7$), with no changes for C-reactive protein ($p = 0.3$; $d=0.1$) in the EG compared to CG group. There were no
36 changes ($P>0.05$) in Δ PV recorded after RIF for either EG ($-0.035\pm 0.02\%$) and CG ($0.055\pm 0.06\%$).

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

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

40

41 **1. Introduction**

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

49 Intermittent fasting (IF) is a frequently used strategy to decrease excess adiposity and circulating pro-
50 inflammatory cytokines while improving overall health[8, 9]. A common approach for undertaking IF involves
51 severe caloric restriction (75-90% of energy needs) for 1-2 days per week. Another form of IF is Ramadan IF
52 (RIF), which is routinely practiced by over 1.8 billion adherents on an annual basis where they undertake
53 one month of RIF in observance of the Islamic obligation (Ramadan) involving abstinence from food and liquids
54 from dawn to sunset [10]. Ramadan is the ninth month of the Islamic lunar calendar and fasting during this
55 month represents one of the five pillars of Islam. The duration of the fasting period commonly lasts from 10 to
56 18 hours and varies according to the season and geographical location [11, 12]. The lunar year is 11 days shorter
57 than the Gregorian year; therefore, Ramadan occurs in a different season every 9 years [13]. The season during
58 which Ramadan occurs affects the duration of each fast because fasting hours are longer in summer than in
59 winter. Ramadan is an extensively studied example of a timed dietary approach to IF [14, 15].

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

71 the number of fasting hours, ethnicity, gender, and the health status and medical history of participants [17, 22,
72 23].

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

83 Thus, we chose to evaluate the effects of RIF on inflammatory (CRP, IL-6, and TNF- α) and biochemical
84 biomarkers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, lactate dehydrogenase
85 (LDH), urea, and creatinine] in males with obesity. We measured these biomarkers at four-time points: 24 hours
86 before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2) and
87 21 days after the end of Ramadan (T3). We hypothesized that RIF would improve circulating pro-inflammatory
88 cytokines and biochemical biomarkers in middle aged males with obesity across all the measured times points.

89

90 **2. Materials and Methods**

91 **2.1. Participants**

92 Fifty-one males were recruited from the students and staff of a regional university, by direct contact as well as
93 via social networks. The inclusion criteria were as follows: body mass index of 30-40 kg/m², no use of drugs or
94 alcohol, lack of regular exercise for at least 6 months before the study, no history of renal, hepatic,
95 cardiovascular diseases or diabetes, and no physical injuries or mobility concerns. The exclusion criteria were as
96 follows: diabetes, cognitive disorders, cardiovascular diseases including uncontrolled hypertension, cancer, liver
97 or renal disease, chronic pulmonary disease, physical disability, or any other contraindications. Moreover, those
98 with evidence of clinical depression were excluded from the study as antidepressant medications can alter pro-
99 inflammatory cytokine concentrations [29, 30]. Thirty-two eligible males with obesity provided informed
100 consent before participating in the study. A physician using a medical health/history questionnaire as well as the

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

104 **2.2. Study Design**

105 This study was conducted during Ramadan 2019 (from May 5th to June 3th) in Tunis, Tunisia. The fasting
106 duration was 15-16 hours per day when the mean daily temperature was approximately 24°C. Participants were
107 allocated (simple randomization) to either an experimental group (EG, n=16, who declared that they would fast
108 for the entire month of Ramadan) or to a control group (CG, n=16, who declared that they would not fast during
109 Ramadan) (**Figure 1**). The EG completed their fasting rituals for the duration of Ramadan (30 days) while the
110 CG continued with their normal daily habits. Participants were fully familiarized with the tests prior to all
111 procedures. Anthropometric measurements and blood samples were collected at four-time points: 24 hours
112 before the start of Ramadan (T0), on the 15th day of Ramadan (T1), the day after the end of Ramadan (T2), and
113 21 days after the end of Ramadan (T3). All measurements were obtained at the same time of day (within ~1
114 hour).

115 **2.3. Dietary habits**

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

121 **2.4. Blood sampling and analysis**

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

131
$$\Delta PV (\%) = 100 \times \left(\frac{[Hb]_a}{[Hb]_b} \times \frac{[1-Ht_b]}{[1-Ht_a]} - 100 \right)$$

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

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

137 Plasma concentrations of IL-6 and TNF- α were determined by enzyme-linked immunosorbent assay (ELISA)
138 using human quantikine ELISA kits. The sensitivity was 5.5 pg/mL for TNF- α and 0.7 pg/mL for IL-6
139 measurements. The activity of CRP was determined using the immunoturbidimetry method (Tina-quant CRP;
140 Boehringer Mannheim, Germany). The intra-assay coefficient of variation for the CRP kit was 1.7%. Activities
141 of AST, ALT, bilirubin, LDH, urea, and creatinine were determined using a multiparametric analyzer (Konelab
142 30TM, Thermo Electron Corporation). The intra-assay and inter-assay coefficient of variation for these
143 biochemical parameters were <10 % and <12%, respectively. The activity of LDH was determined by applying
144 the enzymatic rate method (IFCC). The intra-assay coefficient of variation for the LDH assay was 1.1%.

145 **2.5. Anthropometric measurements**

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

153 **2.6. Statistical analysis**

154 An a priori power analysis (expected SD of residuals = 2 pg/ml for IL-6, desired power = 0.80, and alpha error =
155 0.05) was computed to estimate a statistically significant group-by-time-interaction effect for our primary
156 outcome (IL-6) [33]. The analysis revealed a sample size of $n = 10.8$ per group. To account for potential
157 dropouts, 16 participants were included in each of the two study groups.

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

161 between parameters were assessed using Pearson's product-moment correlation coefficient (r) if the group x time
162 interactions were significant. Effect sizes (ES) were determined from ANOVA output by converting partial eta-
163 squared to Cohen's d . Within-group ES were computed using the following equation: $ES = (\text{mean post} - \text{mean}$
164 $\text{pre})/SD$. ES were considered trivial (< 0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0) and very large
165 (2.0-4.0) [34]. The level of significance was set at $p < 0.05$. All statistical analyses were computed using SPSS for
166 Windows, version 24.0 (SPSS Inc., USA).

167 **3. Results**

168 We screened 41 males with obesity during Ramadan 2019 (from May 5th to June 3th) in Tunis, Tunisia. Only 36
169 qualified for baseline evaluation and were subsequently randomized to either the EC or the CG groups; 2
170 participants in the EC group dropped out of the study as they did not fast for all the days of Ramadan. In the CG
171 group, two participants dropped out as they were unable to maintain their normal daily dietary habits (fasted 5 to
172 7 days during this month). No harms or unintended effects were reported by participants. Data are presented for
173 the 28 participants that successfully completed our intervention [(EG, $n=14$, age: 24 ± 3.4 y, height: 173 ± 6 cm),
174 (CG, $n=14$, age: 23.8 ± 3.8 y, height: 171 ± 3.8 cm)] (**Figure 1**). All remaining participants reported a 100%
175 adherence to study procedures. There were no differences in anthropometric and hormonal measurements
176 between the two groups at baseline. Macronutrient values of EG and CG recorded at T0, T1, T2, and T3 are
177 described in **Table 1**. There were no differences in macronutrient intakes between the two groups at any stage of
178 the study.

179 Anthropometric and body composition characteristics of participants are presented in **Table 2**. Participants in the
180 EG experienced significant decreases in body weight (-2.7%, $p = 0.002$, $d = 1.12$), body mass index (-2.8%, $p =$
181 0.006 , $d = 0.73$), body fat percentage (-4.2%, $p = 0.008$, $d = 1.23$), and waist-hip ratio (-6.2%, $p = 0.005$, $d =$
182 1.19) over during Ramadan, with no changes recorded in the CG.

183 Inflammatory and biochemical markers are shown in **Table 3 and 4**, respectively. The EG experienced
184 significant decreases in IL-6 ($p = 0.02$, $d = 1.49$) and TNF- α ($p = 0.019$, $d = 0.78$) concentrations over time.
185 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
186 ($p=0.48$, $\eta^2 =0.03$), 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
187 differ between the two study groups over time

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

190

191 **4. Discussion**

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

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

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

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

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

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

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

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

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

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

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

265

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268 **Conflict of interest**

269 The authors declare no conflict of interest.

270 **Figure legends:**

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

272

273

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452 **Table 1:** Macronutrient values (mean \pm SD) of the experimental group (EG, n=14) and
453 control group (CG, n=14) measured 24 hours before the start of Ramadan (T0), on the 15th
454 day of Ramadan (T1), the day after the end of Ramadan (T2) and 21 days after the end of
455 Ramadan (T3). Data are expressed as means \pm SD.
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Macronutrients	Group	T0	T1	T2	T3
Protein (% kcal)	EG	17 \pm 10	20 \pm 8	19 \pm 6	19 \pm 9
	CG	17 \pm 2	18 \pm 4	19 \pm 8	19 \pm 6
Carbohydrate (% kcal)	EG	48 \pm 7	46 \pm 7	45 \pm 7	47 \pm 11
	CG	47 \pm 7	46 \pm 9	46 \pm 8	47 \pm 8
Fat (% kcal)	EG	35 \pm 3	34 \pm 4	36 \pm 9	36 \pm 6
	CG	36 \pm 3	36 \pm 3	35 \pm 8	34 \pm 5

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Table 2: Anthropometric and body composition characteristics (mean \pm 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 \pm 4.4	96.9 \pm 4.2 ^{\$#}	94.5 \pm 4.5 ^{*#}	95 \pm 4.6 ^{**}	0.001 (0.67)	0.01 (0.21)	0.001 (0.63)
	CG	100.6 \pm 4.6	100.4 \pm 4.6	100.4 \pm 4.7	100.6 \pm 4.7			
BMI (kg/m ²)	EG	33.3 \pm 1.3 [#]	33.1 \pm 1.2 [^]	32.2 \pm 1.2 ^{*#}	32.4 \pm 1.3 ^{**}	0.002 (0.67)	0.2 (0.04)	0.001 (0.65)
	CG	33.7 \pm 2.7	33.6 \pm 2.7	33.6 \pm 2.7	33.7 \pm 2.6			
Body fat percentage (%)	EG	35.2 \pm 1.5 [^]	35.2 \pm 1.5 [^]	33.1 \pm 1.6 ^{**}	33.8 \pm 1.9 ^{**}	0.001 (0.85)	0.95 (0.00)	0.002 (0.85)
	CG	34.3 \pm 1.8	34.4 \pm 1.8	34.3 \pm 1.7	34.4 \pm 1.8			
WHR (cm ²)	EG	0.97 \pm 0.01 [^]	0.95 \pm 0.05 ^{\$}	0.91 \pm 0.04 ^{*\$}	0.87 \pm 0.04 ^{**}	0.08 (0.24)	0.01 (0.68)	0.001 (0.80)
	CG	0.97 \pm 0.03	0.98 \pm 0.02	0.99 \pm 0.01	1 \pm 0.02			

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

Data are expressed as mean \pm SD.

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

