Original Research Article

Effect of Prolonged Intermittent Fasting in Ramadan on Biochemical and Inflammatory Parameters of Healthy Men

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ABSTRACT

Introduction: Ramadan is an Islamic month during which Muslims abstain from eating, drinking and smoking from dawn to sunset. Ramadan is a model of prolonged intermittent fasting. Previous studies have shown that fasting has beneficial effects on human health. However, the results of these studies have been inconsistent. The aim of present study was to investigate the effect of fasting during Ramadan on biochemical parameters and inflammatory markers. **Materials and Methods:** This prospective observational study was conducted in July 2013. Thirty healthy men who were fasting during Ramadan were enrolled in the study. Anthropometric measurements were taken from each subject. Fasting venous blood samples were taken one week before Ramadan, during the last week of Ramadan and four weeks after Ramadan. Serum interleukin-6, high sensitivity C-reactive protein, fasting blood sugar (FBS), insulin, total cholesterol, triglycerides, low-density lipoprotein and high-density lipoprotein levels were measured. **Results:** No significant change was observed in serum total cholesterol, systolic and diastolic blood pressure, interleukin-6 and high sensitivity C-reactive protein. Fasting in Ramadan significantly decreased body mass index ($P< 0.0001$), FBS ($P< 0.0001$), triglycerides ($P< 0.01$), erythrocyte sedimentation rate ($P< 0.01$), insulin ($P< 0.02$), HOMA index ($P< 0.001$) and high-density lipoprotein-cholesterol ($P < 0.0001$). **Conclusions:** This study indicates that fasting during Ramadan has some positive effects on body mass index, serum triglycerides, high-density lipoprotein, FBS, insulin and HOMA index.

KEYWORDS: Ramadan, Fasting, C-reactive protein, blood glucose, High-density lipoprotein

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INTRODUCTION

Ramadan is the ninth month of the Islamic calendar, in which healthy adult Muslims abstain from eating and drinking from dawn to dusk for the entire month. During Ramadan, there are some changes in eating habits such as consumption of more carbohydrates and sweet foods in the form of two large meals at sunrise and sunset [1]. Several studies have reported changes in the metabolic status of fasters including body weight, fasting blood sugar (FBS), lipid profile and hematological parameters [2-4]. Findings of a recent meta-analysis showed that fasting in Ramadan has some positive effects on healthy Muslims, especially among men. According to the mentioned study, fasting caused significant weight loss in men but not in women. Moreover, it significantly decreased total cholesterol, low-density lipoprotein (LDL) and FBS levels. However, no change in high-density lipoprotein (HDL) and triglyceride levels were observed [5]. Interleukin-6 (IL-6) is an inflammatory marker and a pro-inflammatory cytokine regulating an acute-phase response. This cytokine may also have

an effect on insulin sensitivity or glucose metabolism, as it has been shown that IL-6 and C-reactive protein (CRP) levels are elevated in subjects with obesity, insulin-resistance and impaired glucose tolerance [6,7]. CRP is an acute phase protein produced predominantly by hepatocytes under the influence of cytokines such as IL-6 and tumor necrosis factor-alpha. The role of this acute-phase reactant in cardiovascular disease has been highlighted by numerous studies. Serum CRP level is significantly associated with other risk factors for cardiovascular disease [8]. In addition, some studies have shown that high-sensitivity CRP (hs-CRP) is affected by fasting, weight loss, and post-challenge hyperglycemia in non-diabetic subjects [9-12].

Over the past few years, an increasing number of physiological effects have been reported for intermittent fasting (IF) in rodents, monkeys, and humans [13]. Most importantly, IF is thought to decrease mortality rates in patients with cancer and cardiovascular disease [14,15], improve insulin sensitivity [16], and reduce oxidative stress and inflammation [17]. However, inflammatory biomarkers such as IL-6 and CRP have been found to be significantly decreased by short- and long-term IF [18,19]. The purpose of this study was to investigate the effect of fasting during Ramadan on anthropometric parameters (weight, height, BMI, waist circumference) and some of the cardiovascular risk factors including lipid profile, FBS, HOMA index and IL-6 and CRP during fasting and four weeks after Ramadan. These factors are known metabolic risk factors for chronic diseases such as diabetes, cardiovascular disease and some types of cancer.

MATERIAL AND METHODS

Selection of subjects

Overall, 30 men aged 20-35 years who were fasting during Ramadan participated in the study. The subjects were healthy nonsmokers who had stable weight (±4 kg) for at least six months prior to start of data collection. The subjects had no history of chronic or acute diseases. Data collection was carried out in Ramadan 2013, which began on ninth of July. The subjects were fasting in Ramadan for 16 hours a day.

Data collection and blood sampling

Venous blood samples were taken from left arm of each subject after 12-14 hours of fasting, one week before Ramadan (9.00-10.00 a.m.), in the last week of Ramadan (4.00-5.00 p.m.) and four weeks after Ramadan (9.00-10.00 a.m.). Blood samples were centrifuged and separated plasma was stored in freezer (at -20˚C) for biochemical analysis. Subjects were weighted in light clothing without shoes using a scale (Seca 803, Germany). Height was measured in upright position without shoes using a mechanical measuring tape (Seca 206, Germany). Body mass index (BMI) was calculated based on the following formula: bodyweight (kg) divided by height (m^2). Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Blood pressure was measured by the Omron upper arm blood pressure monitor in seated position, after a 10-minute rest. Plasma concentrations of total cholesterol, high-density lipoprotein-cholesterol (HDL-C) and triacylglycerol were measured using commercial kits (Pars Azmoon Co., Iran). Low-density lipoprotein-cholesterol (LDL-C) concentration was calculated using the Friedewald equation [20]. Enzyme-linked immunosorbent assay (ELISA) was used for quantitative detection of human IL-6 (Platinum ELISA, eBioscience, Austeria). The IL-6 assay had an analytical sensitivity of 0.9 pg/ml and overall intra-assay coefficient of variation (CV) of 5.2%. In addition, hs-CRP was measured by ELISA

(DRG-Diagnostica, Germany) with intra- and inter-assay CVs of 5.1% and 14.3%, respectively.

Dietary intake assessment

The "three-day food record" was used for assessing dietary intake according to food groups. For this purpose, all subjects were required to record the quality and quantity of food and drinks they consumed in the last 24 hours for three days, two weeks before Ramadan and three days during Ramadan. Before recording foods, exact portion sizes (graduated measures, tablespoon and teaspoon, dinner plate, bowl, cup, slice of breads, etc.) were explained for participants. For estimating overall food consumption, food intake was converted into serving of food within an exchange group [21]. The study was approved by the Ethics Committee of Golestan University of Medical Sciences, Iran.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL). Descriptive data were expressed as mean and standard deviation (SD). Normal distribution and homogeneity of the variances were tested with Shapiro-Wilk test and Kolmogorov-Smirnov test. The normal distribution parameters (weight, BMI, waist circumference, blood pressure, and total cholesterol) were analyzed by one-way repeated measures ANOVA. Friedman test was used for non-normally distributed data. P-values less than <0.05 were considered statistically significant.

RESULTS

The study was performed on 30 healthy males (mean age 29.44 ± 7.4 years), who were fasting during Ramadan for 30 days. As shown in Table 1, mean of weight and BMI decreased significantly in subjects after three weeks of fasting (P< 0.0001). Four weeks after the end of Ramadan, weight and BMI increased significantly (P< 0.0001). Mean ± SD values of all parameters measured in this study are presented in Table 1. Changes in the waist circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were not statistically significant. Mean level of FBS, fasting insulin and HOMA index decreased during Ramadan and increased four weeks after Ramadan fasting. Mean of serum triglyceride (TG) decreased significantly during Ramadan and increased significantly after the Ramadan fasting. Mean level of HDL increased significantly during fasting and decreased significantly after the Ramadan. There was no significant difference in the mean level of total cholesterol and LDL before, during and after Ramadan. The inflammatory markers such as IL-6 and hs-CRP did not change significantly during and after the fasting, while the mean level of erythrocyte sedimentation rate (ESR) decreased significantly during fasting and increased significantly after Ramadan.

Table 1: Clinical and biological parameters at baseline, during three weeks of fasting and four weeks after the end of Ramadan

	Session 1	Session 2	Session 3	P_1 value
Weight (kg)	82.73 ± 14.74	80.43 ± 14.48	81.97 ± 14.86	<0.001*
BMI (kg/m^2)	27.30± 4.26	26/48±4/37	27 ± 4.41	<0.001*
Waist circumference (cm)	96.48 ± 11.38	95.31 ± 10.62	96.73 ± 10.87	NS*
FBS (mg/dl)	98.58 ± 7.04	81 ± 4.97	93.24 ± 7.07	<0.001**
SBP (mmHg)	124.7± 4	121.6± 6	125.2± 4	NS*
DBP (mmHg)	80.3 ± 8	79.2 ± 8	81.3 ± 8	NS*

Total Cholesterol (mg/dl)	164.64 ± 35.34	170.07 ± 37.77	166.66 ± 36.03	NS*
TG (mg/dl)	152.55 ± 64.35	123.83 ± 53.44	143 ± 71.28	0.05**
HDL (mg/dl)	33.83 ± 8.53	47.59 ± 6.70	34.48 ± 7	<0.001**
LDL (mg/dl)	100.52 ± 28.38	95.62 ± 32.86	106.62 ± 28.1	NS**
ESR (mm)	6.21 ± 5.19	4.24 ± 2.59	4.69 ± 4.35	0.03**
CRP (µg/ml)	1.72 ± 1.67	1.99 ± 1.45	2.19 ± 1.94	NS**
IL-6 (pg/ml)	1.09 ± 1.63	0.79 ± 0.26	0.94 ± 1.01	NS**
Insulin (mIU/l)	11.87 ± 6.64	9.03 ± 5.04	16.5 ± 9.09	<0.001**
HOMA	2.94 ± 1.78	1.81 ± 1.04	3.86 ± 2.26	<0.001**

Session 1: baseline/one week before Ramadan, **Session 2**: three weeks into Ramadan, **Session 3**: four weeks after the end Ramadan.

***:** one-way repeated measures ANOVA, **: Non-parametric Friedman Test

Seven participants were excluded from the study for not completing the three-day food record form. Overall, 23 objects completed the 24-hour recall and three-day food record before and during Ramadan. According to the American Dietetic Association exchange list [21], the mean level of starch foods (bread, rice, barely and pasta) decreased from 16.04±4.71 to 10.65±3.61 servings/day in Ramadan (P<0.0001). Consumption of lean and moderate-fat proteins (Chicken, beef, lamb, egg and cheese) decreased from 3.78±1.54 to 3.17±1.97 servings/day (P<0.001). The mean level of milk, vegetable and fruit consumption did not change during Ramadan compared to the baseline values. Intake of simple carbohydrates such as sugar and sugar-added beverage increased significantly from 6.70±5.11 to 9.13±7.16 servings/day (P<0.002).

DISCUSSION

Previous studies have reported inconsistent results for the effects of fasting during Ramadan on physiological and biochemical parameters. The present study aimed to determine the effect of Ramadan fasting on body weight and some biochemical and immunological factors of healthy men. The results of this study showed that fasting significantly reduced weight, FBS, TG and HOMA index. The level of HDL increased significantly during Ramadan. However, SBP, DBP and waist circumference did not change. These finding are consistent with some previous studies. For example, a meta-analysis reported that fasting reduces body weight in the general population, especially in men [5]. However, study of McNeil et al. found no significant difference in body weight of 20 normal-weighted and obese men [22]. Quality of food and eating patterns are changed during Ramadan. In this month, larger meals compensate for lower frequency of intake. It is believed that fasting in Ramadan often leads to reduced energy intake, but a recent review article did not support this hypothesis [23]. Significant reduction in total body weight despite the insignificant change in energy intake may be related to utilization of body fat during fasting in Ramadan [22, 24]. Some animal studies demonstrated that eating one large meal a day decreases weight and body fat percentage [25,26]. Weight loss may also be explained by the abstinence from drinking, and the consequent dehydration that occurs during fasting [27]. Since the total fluid intake in our subjects increased during Ramadan, the negative fluid balance is not the main factor associated with their weight loss. In addition, we found that fasting causes no significant change in the SBP and DBP. This finding is inconsistent with results of previous studies that reported fasting during Ramadan has antihypertensive effects [28-30]. This

inconsistency might be because all participants in our study were normotensive and younger than 40 years. Study of Salahuddin et al. found significant changes in the SBP and DBP of hypertensive subjects, while there was an insignificant decrease in SBP and DBP of control group [31]. In another report, Sayedda et al. stated that mean of blood pressure decreases in the middle of Ramadan, and returns to pre-Ramadan values at the end of the month [32].

In the present study, the level of total cholesterol increased in Ramadan, but this increase was not statistically significant. Some studies claimed that this increase could be related to weight loss [4]. However, other studies have found either no change [31, 33] or decreased level of cholesterol during fasting [34-36], which may be related to consumption of one large meal in 24 hours [37].

It is known that lipid profile is influenced by dietary habits, physical factors, amount and type of dietary fat, and amount of simple carbohydrates in diet [38-39]. Previous studies have shown that eating a large meal per day can change lipid profile [40-41].

Similar to some previous studies, the level of FBS, fasting insulin and insulin-resistance decreased in the subjects after fasting [42-44]. Moreover, regular sleeping patterns are changed during Ramadan. This affects levels of leptin, neuropeptide-Y, insulin, melatonin, steroid hormones, pituitary hormones and thyroid hormones that have essential roles in regulation of energy balance and glucose metabolism [45-46]. The decrease in FBS and HOMA index could be related to the above factors. Some studies showed no significant change in the serum level of glucose [47-48]. However, other studies reported increased FBS levels after fasting in Ramadan [49]. These inconsistencies could be due to differences in dietary habits, calorie intake, the number and duration of fasting days, time of sampling, genetic background and physical activity between these studies. A study reported a significant increase in HOMA-IR in subjects with metabolic syndrome [50], and a significant decrease in insulin and insulin resistance among diabetic men after fasting [48].

Limited number of studies investigated the effect of fasting during Ramadan on pro-inflammatory factors. In our study, the level of ESR decreased during Ramadan, while CRP and IL-6 levels did not change significantly. These findings are consistent with findings of Murat Unalacak [10]. However, some studies demonstrated that IL-6 and CRP levels decrease significantly during fasting in Ramadan [12, 26]

CONCLUSION

Changes in eating patterns during Ramadan have beneficial effects on healthy men. These effects include increase in HDL, weight loss, and decrease in FBS, insulin, HOMA index, and ESR levels. However, the values of these factors (except ESR) returned to baseline values two weeks after Ramadan. In addition, inflammatory factors such as IL-6 and CRP do not change significantly during and after fasting. Future studies with larger sample sizes are required to investigate the impact of dehydration and changes in diet, sleeping patterns and physical activity on healthy individuals who fast during Ramadan.

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