Original Article

Effects of Intermittent Fasting during Ramadan on Insulin-like Growth Factor-1, Interleukin 2, and Lipid Profile in Healthy Muslims

Abstract

Background: Insulin-like growth factor-1 (IGF-1) and interleukin-2 (IL-2) play an essential role in pathophysiology of several chronic diseases. As a stressor, fasting in Ramadan may increase inflammatory markers such as IGF-1 and IL-2 in Muslims. The aim of this before–after study was to investigate the effects of fasting in Ramadan on IGF-1 and IL-2 levels in individuals. **Methods:** In all, 34 men age 16–64 years were selected out of the overall number of individuals who were ready for fasting entirely throughout Ramadan. A sample of blood was drawn from the contributors before and after Ramadan, and plasma IGF-1, IL-2, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined. To identify differences between the initial and final values of test results of the study for plasma IGF-1, IL-2, and lipid parameters, we used paired sample *T*-test. **Results:** Paired sample *T*-test illustrated a significant decrease in IGF-1 and IL-2 levels after Ramadan fasting compared to before Ramadan. The concentration of TG, cholesterol, and LDL-C levels underwent significant decreases over the period of the study. HDL-C levels did not change significantly during the study. A significant decrease in weight, waist circumferences, calorie, carbohydrate, and fat intake were observed in participants during Ramadan fasting. **Conclusions:** It is concluded that fasting in Ramadan independent of anthropometric measures attenuates inflammation and is beneficiary to health.

Keywords: *Fasting, insulin-like growth factor-1, lipids*

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Introduction

Insulin-like growth factor-1 (IGF-1) plays an essential role in growth and development in humans. This factor directly activates IGF-1 receptor (IGF-1R) and regulates various intracellular signaling pathways. It has been noted that distortion of IGF-1R signaling cascades contributes to a variety of health-threatening diseases such as diabetic retinopathy,[1] age-related macular degeneration,[2] cardiovascular disease, and cancers.[2] The biological activities of IGF-1 have been of strong concern in tumorigenesis.[3,4] In addition, the role of IGF-I in the pathophysiology of vascular complications has been supported by experimental findings.[5]

It has been reported that fasting decreases IGF-1 levels in animals.[6] To stay alive during famine and starvation, one should direct energy expenditure from growth and reproduction toward survival.[7] Therefore, despite elevated GH secretion, starvation and malnutrition lower circulating IGF-I levels.[6]

Among Muslim population, fasting during Ramadan differs from formal fasting. In fact, two whole meals are consumed immediately after sunset and just before sunrise which could be assigned as intermittent fasting,[8] and therefore, it could be assumed the changes in physiology during Ramadan differ from experimental fasting.[9] In addition, in Ramadan the time of serving and the content of the meals, pattern of sleep and being awake, activity, and circadian rhythm are interrupted.[10] In fact, people in Ramadan cope with fasting as a stressor which causes stress response.[11] And inflammatory mediators such as interleukin-2 (IL-2) are produced following this psychological and physiological stress induction.[12] Although some studies indicated positive effect of intermittent fasting on inflammatory markers,[13] it might attenuate the benefits of fasting. To the best of our knowledge, so far, there is no published study on the

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effects of intermittent fasting on IGF-1 and very scarce surveys have reported the changes in serum IL-2 levels during Ramadan in Muslim population.

Therefore, we conducted this study to investigate the impact of fasting in Ramadan on IGF-1 and IL-2 levels in humans. In addition, we measured the changes in lipid profiles during Ramadan.

Methods

Study design and participants

This project was a before–after study. In all, 34 men age 16–64 years were selected out of the overall number of people who were willing to fast in Ramadan. The participants were invited to come to the Persian Gulf Tropical Medicine Research Center in Bushehr University of Medical Sciences between 1 and 30 of July in fasting state through a letter delivered to their homes by the research team. The objectives of the study were explained in the letter. The criteria for inclusion in the study were as follows: willing to fast during Ramadan, being of male gender, and a minimum age of 15 years (cut point for male Muslims to execute their religious duty). Volunteers were excluded if they were smokers, took thyroid drugs, estrogen or medications for hypertension, or showed any clinical signs of dyslipidemia, diabetes mellitus, or hypothyroidism. Potential participants were interviewed and examined by expert physicians and dietitians. At the beginning of the study, a written, signed informed consent was obtained from each participant.

The experimental protocol of the study was approved by the research deputy (DP/8703277/176,14/4/2013). The ethical aspects of the study were also approved by the ethics committee (bpums.res, 2015,32), and the research reported here was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Variable assessment

With a calibrated scale and stadiometer, the weight and height of participants were determined. Heavy outer garments and shoes were removed before height and weight were measured. Body mass index was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Waist circumference was measured with a nonstretchable measuring tape at the midpoint between the lower edge of the rib cage and iliac crests. Waist-to-hip ratio (WHR) was determined as waist circumference divided by hip circumference. For this parameter, waist circumference was defined as the smallest circumference measured at the navel, and hip circumference was defined as the largest circumference measured at the hips and buttocks.

A validated questionnaire (International Physical Activity Questionnaire, Epic-Norfolk) was used to estimate physical activity of participants.[14]

A 24-h dietary recall for 3 nonconsecutive days before Ramadan and during Ramadan was recorded in an interview conducted by trained dietitians. Each food and beverage were analyzed for the content of energy and other nutrients with Nutritionist III software (version 7.0; NSquared Computing, Salem, OR, USA), which was adapted for Iranian foods.

A blood sample was obtained from each participant before and at the end of the month of Ramadan and was transferred to the laboratory of Persian Gulf Tropical Medicine Research Center. Plasma's total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low0density lipoprotein cholesterol (LDL-C), IGF-1, and IL-2 were determined. Venous blood samples were drawn between 8.00 and 9.00 hours and centrifuged at 3000*g* for 15 min at 4°C. Immediately after centrifugation, the serum samples were frozen and stored at –80°C for a period no longer than 6 weeks. Plasma's IGF-1 levels were measured with a commercially available ELISA kit (Mediagnost, Reutlingen/Germany; Catalog No. E20 d/e 100212). The assay range was 42–1050 ng/mL, and the intra- and inter-assay coefficients of variance were less than 6.7% and 6.8%, respectively.

IL-2 levels in serum were measured with an ELISA kit (Bioassay Technology Laboratory, Shanghai Crystal day Biotech Co., Ltd., Shanghai, China; Catalog No. E0094Hu). The range of assess was 5–2000 ng/L. The intra-assay coefficient of variance was less than 10%. Moreover, a value of less than 12% for inter-assay coefficient was reported.

Serum TG was measured with an enzymatic technique for oxidation by glycerol-3 phosphate and detection with phenol aminoantipyrine in an automated Technicon Axon Analyzer. The HDL-C concentration (after precipitation with magnesium chloride) was measured with enzymatic techniques (Pars Azmoon Co., Tehran, Iran). LDL-C concentration was calculated with Friedewald formula.[15]

Statistical analysis

Normality was evaluated using probability plots and Shapiro–Wilk's test. To identify differences between continuous variables at the beginning and at the end of the study, we used paired sample *T*-test. Chi-square McNemar was used to analyze the differences between categorical variables before and after the study. The relationship between the variables was determined by calculating Pearson's correlation coefficient. A value of $P < 0.05$ was accepted as significant.

All statistical analyses were done with an IBM computer and SPSS v. 15 statistical software packages (SPSS Inc., Chicago, IL, USA).

Results

Anthropometric indices and biochemical parameters of the participants are shown in Table 1. Changes in serum

IGF-1 and IL-2 levels correlate significantly with change in weight, waist circumference, WHR, and body mass index or calorie intake [Table 2].

Paired sample *T* test illustrated a significant decrease in IGF-1 levels during the month of Ramadan. IL-2 decreased significantly after Ramadan fasting compared to before Ramadan [Table 3].

The concentration of TG, cholesterol, and LDL-C levels underwent significant decreases over the period of the study. HDL-C levels did not change significantly during the study [Table 3].

Paired sample *T*-test showed a significant decrease in weight and waist circumferences during Ramadan fasting [Table 3].

Calorie, carbohydrate, and fat intake underwent significant decreases during the study [Table 3].

Physical activity levels in study population decreased significantly during Ramadan in comparison with the periods prior to the month of Ramadan [Table 3].

Table 1: Characteristics of the individuals (*n*=34); means±SD

Characteristic	Individuals (*n*=34)	
	Mean	SD
Age (years)	35	11
BMI (kg/m^2)	24.79	3.08
WHR	0.908	0.109
Weight (kg)	74.62	10.63
Waist circumference (cm)	91.97	11.71
IGF-1 (ng/mL)	15.35	9.05
IL-2 (ng/L)	772.25	461.94
TG (mg/dL)	143.56	47.64
Chol (mg/dL)	193.66	39.10
LDL-C (mg/dL)	114.30	33.92
HDL-C (mg/dL)	43.79	5.49

SD=Standard deviation, BMI=Body mass index, WHR=Waist–hip ratio, IGF-1=Insulin-like growth hormone-1, IL-2=Interleukin-2, TG=Triglyceride, Chol=Cholesterol, LDL-C=Low-density lipoprotein cholesterol, HDL-C=High-density lipoprotein cholesterol

Table 2: Correlation between changes in anthropometric measures and calorie intake with changes in serum IGF-1 and IL-2 in fasted individuals

	IGF-1		IL-2	
	r	*P*	*r*	*P*
Weight	−0.17	0.45	0.06	0.74
WC	−0.06	0.79	−0.01	0.95
BMI	−0.20	0.38	−0.06	0.75
WHR	−0.24	0.27	−0.06	0.75
Calorie intake	0.17	0.42	0.14	0.45

IGF-1=Insulin-like growth factor-1, IL-2=Interleukin-2, BMI= Body mass index, WC=Waist circumference, WHR=Waist–hip ratio. Pearson's correlation coefficient was used to identify the relationship between variables

Discussion

The findings of this study were indicative of significant reduction in IGF-1 levels over the course of intermittent fasting in Ramadan. It seems that short periods of starvation, even less than 12 h, influence hormonal responses in the human body. In accordance, Azizi reported significant changes in physiologic hormones during Ramadan.[9] Observations indicate that food shortage in various organisms, starting from yeasts and culminating in humans, mediates its impacts through the changes it induce in IGF-1 circulating hormone levels. In this regard, many of the mutations that extend life span act by reduction in IGF-1 levels which springs from suppressed nourishing-signaling pathways.[16] One study which was conducted on mice demonstrated that short-term fasting induces beneficiary effects by reducing circulating IGF-1.[6] In fact, fasting causes the human body to prefer to perform activities for the purpose of survival in comparison with activities such as energy consumption for growth. Therefore, growth hormone–IGF-1 axis adapts with new endocrine situation and IGF-I levels decrease regardless of raised GH secretion.[17] And it might explain the desired impact of intermittent fasting on health-threatening diseases,[18] especially when the role of IGF-1 in human diseases has been clarified.[1-5] However, Bouhle *et al.* found no changes in IGF-1 level in trained athlete men during submaximal exercise during Ramadan fasting.[19] It should be mentioned that the participants in Bouhle *et al.* study were trained athletes with daily exercise schedule. Therefore, it could be concluded that exercise intervenes with the effect of intermittent fasting on IGF-1 level.

Moreover, it is well known that calorie restriction decreases insulin levels.[20] The relationship between insulin level and IGF-1 may explain the association between calorie restriction and reduced IGF-1 production. Edge *et al.* reported that insulin deficiency leads to impaired hepatic IGF-1 synthesis.[21] Insulin regulates the growth hormone receptor (GHR) production and decrement in insulin secretion following fasting which leads to reduction in GHR expression in liver which consequently decreases synthesis of IGF-1.[22,23]

Collectively, we hypothesize that intermittent fasting by above mechanisms, independent of weight reduction, synergistically decreases circulating IGF-1 levels during Ramadan in the Muslim population.

In Ramadan in addition to fasting, several lifestyle changes, including changes in physical activity, time and regularity of feeding, content and calorie of diet, pattern of sleep and being awake, and circadian rhythm[10] which all in all as stressors induce stress response in subjects.[11] However, in this study, Ramadan fasting could decrease IL-2. In accordance with our findings, some studies reported significant decrease in IL-2 level following fasting.[24] In this regard, decrease in cytokine levels during Ramadan

Table 3: Change in variables before and after Ramadan unadjusted and adjusted for age

Variable	Before Ramadan	After Ramadan	*P*
Serum biochemical parameters			
IGF-1 (ng/mL)	15.35±9.05	13.54±8.46	0.037
IL-2 (ng/L)	772.25±461.94	445.64±499.38	<0.001
TG (mg/dL)	143.56±47.64	120.33±42.67	<0.001
Chol (mg/dL)	193.66±39.10	179.25±33.50	<0.001
LDL-C (mg/dL)	114.30±33.92	108.51±34.52	0.038
HDL-C (mg/dL)	43.79±5.49	48.38±9.39	0.002
Anthropometric measures			
Weight (kg)	74.62±10.63	73.93±10.25	0.094
BMI (kg/m^2)	24.79±3.08	24.57±3.05	0.11
WC (cm)	91.97±11.71	91.45±11.59	0.029
WHR	0.908±0.109	0.910±0.115	0.72
Dietary intake			
Calorie intake (calories)	2392.06±664.48	1671.35±568.13	<0.001
Fat intake (g/day)	62.54±22.43	33.34±23.03	<0.001
SFA intake (g/day)	34,65±16.35	16.36±15,85	<0.001
MUFA intake (g/day)	14.20±5.34	9.37±6.483	0.003
PUFA intake (g/day)	11.93±11.31	5.57±10.21	0.034
Carbohydrate intake (g/day)	377.51±141.58	302.90±106.95	0.01
Protein intake (g/day)	87.62±39.87	51.08±19.16	<0.001
Physical activity			
Inactive	26.5% (10)	55.9% (19)	<0.001
Moderately inactive	24.9%(9)	38.2% (13)	<0.001
Moderately active	32.3%(11)	2.9%(1)	<0.001
Active	11.7%(4)	2.9% (1)	<0.001

BMI=Body mass index, WHR=Waist–hip ratio, WC=Waist circumference, IGF-1=Insulin-like growth hormone-1, IL-2=Interleukin-2, TG=Triglyceride, Chol=Cholesterol, LDL-C=Low-density lipoprotein, HDL-C=High-density lipoprotein, SF=Saturated fatty acids, PUFA=Polyunsaturated fatty acids, MUFA=Monounsaturated fatty acids. Paired sample *T*-test and Chi-square were used to identify differences between variables at the beginning and at the end of the study

is due to down-regulation of nuclear factor κB (NF-κB) signaling.[25] Another explanation might be the effect of psychological and physiological stress, in stimulating the secretion of endorphin which could compensate the undesired impact of stress.[26] Mohajeri *et al.* depicted a significant decrease in CXC chemokines concentration during Ramadan[27] which might explain the reason for suppression of inflammatory chemokines during Ramadan.[25]

In this study, the lipid profile including TG, cholesterol, and LDL-cholesterol decreased in the study's participants during Ramadan. Nematy *et al.* reported a significant improvement in coronary heart disease risk factors after Ramadan fasting. They found a significant lowered plasma cholesterol, TGs, and LDL-C levels following the month of Ramadan.[28] Marbut *et al.* in agreement with our study observed a significant decrease in serum cholesterol, serum TGs, and LDL-C levels in addition to a significant increase in serum HDL-C levels at the end of Ramadan.[29] Some studies have indicated an increase in TG level, which attributed to lipolytic effect of prolonged fasting.[30] Moreover, other lines of research have pointed to changes in metabolism of some appoliproteins, which in turn might influence LDL-C metabolism.[31] Furthermore, findings of several studies have been indicative of increases in TG.[32-34] The discrepancies in the finding of various studies concerning the impact of Ramadan fasting on lipid profiles relate to fluctuations in activity levels and feeding patterns as well as different cultural factors between diverse nations during Ramadan. In fact, increased TG concentration reported by some studies in Ramadan could be attributed to consumption of rich carbohydrate meals along with reduced physical activity in this month. Repeatedly, it is reported a trend for consumption of high-sugar meals in Ramadan.[32-34] Accordingly, the association between increased sucrose intake and raised TG level was demonstrated and reported by Albrink and Ullrich.[35] In contrast, based on the findings of this study, the carbohydrate intake in parallel with TG was significantly decreased in Ramadan in comparison to before Ramadan.

In this study, despite significant reductions in dietary energy intake, no significant changes were observed in anthropometric measures and this might be due to the decline in physical activity during the course of the study. Based on our findings, the physical activity levels decreased during Ramadan in comparison to the periods prior to the month of Ramadan [Table 3].

Limitations and strengths

The limitation of this study was that because of the avoidance of participants, the dietary intake analysis was obtained by 24-h dietary recall for 3 nonconsecutive days and not by food weighing record. Dietary intake analysis by food weighing record would validate the finding of this study to a higher degree. The strength of this study was that we measured all variables including dietary intake analysis and physical activity which might influence the serum IGF-1 and IL-2 level, and if they were correlated with serum IGF-1 and IL-2 we used them as covariates in statistical analysis.

Conclusions

It is concluded that fasting in Ramadan independent of anthropometric measures decreases IGF-1, IL-2, and serum lipid levels.

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Conflicts of interest

There are no conflicts of interest.

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