Effect of Intermittent Fasting during Ramadan on Visfatin, Adiponectin and Tumor Necrotizing Factor-Alpha in Healthy Muslim Individuals

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A R T I C L E I N F O	A B S T R A C T
Article type: Original article	**Introduction:** The aim of this study was to investigate the effect of fasting in Ramadan on visfatin, tumor necrosis factor alpha (TNF-α) and adiponectin level in human. **Methods:** Thirty four men aged 24 to 55 years old were selected from those people who were willing to fast in Ramadan. The blood sample was obtained from each participant in fasting state at the beginning and the end of study to determine serum visfatin, adiponectin and TNF-α. Paired T test was used to identify differences between beginning and the end of the study in serum visfatin, adiponectin and TNF-α. **Results:** Results showed a significant decrease in Visfatin after study. There were no significant changes in adiponectin and TNF-α during Ramadan fasting. **Conclusion:** It is concluded that fasting in Ramadan is beneficial to health and can ameliorate some inflammatory markers in fasting individuals.
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Introduction

Inflammation is primitive in athero-sclerosis (1) and plays an essential role in the development of CVD (2). Recently, growing interest has appeared in the role of inflammatory and anti -inflammatory adipo-cytokines in the progression of CVD (3, 4). Visfatin, as an inflammatory adipocytokine, is involved in many inflammatory diseases (5). Reportedly, Visfatin has been engaged in monocyte adhesion to endothelial cells, (6) and plaque rupture (7) in unstable lesions in atherosclerosis. On the contrary, adiponectin is an anti-inflammatory adipocytokine, (8) which prevents vascular stenosis, (9) insulin resistance, atherosclerosis, (10) renal fibrosis, diabetes nephropathy and albuminuria in animal models (10). In addition, TNF-α induces programmed cell death or apoptosis in Rheumatoid arthritis (RA), (11) Systemic Lupus Erythematosus (SLE) (12) and Ankylosing spondylitis (AS) (13).

Evidences show that fasting is effective in the treatment of rheumatoid diseases, and chronic pain syndromes (14). Fasting is used by Naturopathy to manage treatment of diseases (15). Ayurveda also use fasting to enhance digestive capacity, enthusiasm, and decrease signs and symptoms of diseases (16). However, there are controversies about the period and the rate of calorie restriction in different studies (17). Some studies recommend severe restriction, up to 75-90% of energy needs (18). But prolonged and severed starvation has hazardous effects such

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as heart failure, (19) impaired liver function, (20) cholesterogenesis, (21) autophagy (21) and cachexia (22). It has been hypothesized that intermittent fasting positively affects the inflammatory markers (23). We hypothesize that intermittent fasting might be able to ameliorate some health-threatening factors such as elevated TNF-α or Visfatin without the undesirable side effects of prolonged starvation. However, to the best of our knowledge, so far, there is very scares published study on the effect of intermittent fasting on visfatin and TNF-α during Ramadan. Therefore this study was conducted to investigate the effect of fasting in Ramadan on visfatin, TNF-α and adiponectin level in human.

Material and methods

This study was conducted between 1st and 30th of July in 2015 in Bushehr University of Medical Sciences. Thirty four men aged 16 to 64 years old were selected by simple random sampling from those people who were willing to participate in the study. The participants were invited to present in Bushehr University of Medical Sciences between 8-9 hours on the following morning in fasting state through a letter delivered to their homes by the post. The aims of the study were elucidated in detail in the letter. The inclusion criteria were: willing to fast during Ramadan, male gender and aged 15 years old and more (eligibility criteria for fasting from the religious point of view for male gender). They were excluded if they were using thyroid drug, cigarette, estrogen, medications for hypertension and any clinical signs of hypothyroidism, dyslipidemia, or diabetes mellitus. An expert physician and dietitian interviewed and examined the participants.

At the beginning of study a blood sample was obtained from each participant after 12 hours fasting into tube containing EDTA. Blood sample were centrifuged at 3000 × g for 15 min and the plasma samples were stored in 1.5 ml microtubes at -20° C at the laboratory of Persian Gulf Tropical Medicine Research. At the end of the study once again plasma samples were obtained from each participant and plasma visfatin, TNF-α and adiponectin were measured. A written, signed informed consent was obtained from each participant at the beginning of the study.

Anthropometric measurements

Height and weight of the participants were measured with a stadiometer (Seca stadiometer, model 286 dp, Hamburg, Germany) and a scale (Seca scale, model 755, Hamburg, Germany).

The participants were demanded to remove heavy outer garments and shoes before height and weight measurement. Body mass index was calculated as weight in kilograms divided by the square of the height in meters (kg/m2). Waist circumference was measured at the midpoint between the lower edge of the rib cage and the iliac crests with an un-stretchable measuring tape. Waist-to-hip ratio was determined as waist circumference divided by hip circumference. The smallest circumference measured at the navel, and the largest circumference measured at the hips and buttocks was applied for this measurement (24).

Physical activity

Physical activity of participants was assessed by a questionnaire based on the International Physical Activity Questionair, 2005, Epic-Norfolk.

Dietary intake analysis

Trained dietitians recorded a 24-hour dietary recall for 3 nonconsecutive days from each participant before and during Ramadan. Nutritionist III software (version 7.0; NSquared Computing, Salem, OR, USA) were used to analyze the content of energy and other nutrients of each food and beverage. This software was adapted for Iranian foods.

The protocol of the study was endorsed by the research deputy of Bushehr University of Medical Sciences (DP/8703277/176, 14/4/ 2013). The ethics committee of Bushehr University of Medical Sciences (May 2015) approved the ethical aspects of the study, and the research reported here was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Measurement of serum parameters

Plasma Visfatin was measured by a

commercially available ELISA kit (Bioassay Technology Laboratory, Shanghai crystal day biotech co, LTD, Shanghai, China, Catalog No. E0025Hu). The assay range was 0.5 ng/mL-100ng/mL, and the intra- and inter-assay coefficients of variance were less than 10% and less than 12%, respectively.

To detect Adiponectin in plasma samples, a commercially available ELISA kit (Bioassay Technology Laboratory, Shanghai crystal day biotech co, LTD, Shanghai, China, Catalog No. E1550Hu) was used according to the manufacturer's instructions. The assay range was 0.2 mg/L-60mg/L, and the intra- and inter-assay coefficients of variance were less than 10% and less than 12%, respectively.

Plasma TNF-α was measured by a commercially available ELISA kit (Bioassay Technology Laboratory, Shanghai crystal day biotech co, LTD, Shanghai, China, Catalog No. E0082Hu). The assay range was 3 ng/L-1.52ng/L, and the intra- and inter-assay coefficients of variance were less than 10% and less than 12%, respectively.

Statistical analysis

The distribution of variables was studied with probability plots and the Shapiro-Wilks test. Paired T test was used to identify differences between beginning and the end of the study in serum visfatin, adiponectin and TNF-α.

The relationship between 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 the SPSS v. 15 statistical software packages (SPSS Inc., Chicago, IL, USA) were used to analyze the data statistically.

Results

The characteristics of the participants are shown in Table 1. Bivariate correlation analysis showed no significant correlation between change in serum visfatin, adiponectin and TNF-α level and change in WHR and BMI or calorie intake but there was s significant association between serum visfatin, adiponectin and TNF-α level and age in population study (data not shown).

The paired sample T- test showed 11.79 % significant decrease in Visfatin during Ramadan fasting. TNF-α and adiponectin did not change significantly after Ramadan fasting compared to before Ramadan. (Table 2).

The paired sample T- test showed a 0.5 % significant decrease in weight and waist circumferences during Ramadan fasting. (Table 2).

The energy, carbohydrate and fat intake

Table 1. Characteristics of the individuals (n=34). Means ± SD

Characteristic NO	individuals (n=34) mean	SD
Age(years)	35	11
BMI (kg/m²)	24.79	3.08
WHR	0.908	0.109
weight (Kg)	74.62	10.63
Waist circumference (Cm)	91.97	11.71
Visfatin (ng/mL)	42.58	21.44
Adiponectin (mg/L)	22.30	10.43
TNF-α (ng/L)	289.92	181.22

BMI: body mass index, WHR: Waist Hip Ratio, igf-1: Insulin like growth hormone-1, TNF-α: Tumor necrosis factor-alpha

Table 2. Change in variables before and after Ramadan unadjusted and age adjusted

variables	Before Ramadan	After Ramadan	Unadjusted P value	Adjusted P value
Visfatin(ng/mL)	42.58±21.44	40.17±20.44	0.045	0.047
Adiponectin(mg/L)	22.30±10.43	21.48±10.50	0.14	0.23
TNF-α(ng/L)	289.92±181.22	277.89±180.58	0.39	0.55
Weight(kg)	74.62±10.63	73.93±10.25	0.094	0.13
BMI(kg/m²)	24.79±3.08	24.57±3.05	0.11	0.42
WC(Cm)	91.97±11.71	91.45±11.59	0.029	0.032
WHR	0.908±0.109	0.910±0.115	0.72	0.94
Energy(Calorie)	2392.06±664.48	1671.35±568.13	0.000	0.001
Fat (gm/day)	62.54±22.43	33.34±23.03	0.000	0.001
SFA (gm/day)	34,65±16.35	16.36±15,85	0.000	0.001
MUFA(gm/day)	14.20±5.34	9.37±6.483	0.003	0.013
PUFA(gm/day)	11.93±11.31	5.57±10.21	0.034	0.034
Carbohydrate (gm/day)	377.51±141.58	302.90±106.95	0.01	0.03
Protein(gm/day)	87.62±39.87	51.08±19.16	0.000	0.002

BMI: body mass index, WHR: Waist Hip Ratio, WC: Waist circumference, TNF-α: Tumor necrosis factor-alpha, SFA: Saturated fatty acids, PUFA: Poly unsaturated fatty acids, MUFA: Mono unsaturated fatty acid

decreased significantly 30.12 %, 19.76% and 46.69 % respectively (Table 2).

Discussion

The data of present study showed a significant decrease in visfatin level in individuals who fasted at least 16 hours per day for one month. Studies with severe fasting failed to change visfatin level in human model (25, 26). Owczarek et al, (2016) supported the existence of an association between nutritional status, inflammation and circulating visfatin level (27). This relationship is in connection with the change of leptin concentration after intermittent fasting (28). Low concentration of leptin is prevalent in energy deprivation in humans (29, 30). The adverse relationship between Leptin and visfatin level is addressed by Tan and et al (31). Chowdhury and et al, (2016) reported a significant lower Systemic concentration of leptin in the afternoon following morning fasting (32). A significant decrease in blood leptin also is reported in fasted individuals during Ramadan (33).

In this study no significant changes were observed in TNF-α sequel fasting. Although in animal model significant decrease were seen after intermittent and prolonged fasting, (34, 35) intermittent fasting in human in correspondence to the present study did not affect the TNF- α (36). It should be mentioned that Ramadan fasting is accompanied by alterations in meal times, meal composition, sleep/wake patterns, circadian rhythms and physical activity (37). Ramadan fasting as a stressor stimulates stress responses in individuals (38). Psychological and physiological stress induces inflammatory markers (39). And this might influence the beneficial effect of intermittent fasting on TNF-α.

The concentration of anti-inflammatory cytokine (adiponectin) was not changed after intermittent fasting during this study. Yannakoulia and colleagues, (2003) in agreement with our findings reported no significant relationship between calorie intake and serum adiponectin level (40). In fact, the concentration of adiponectin compared to other cytokines in the circulation may be too high to be influenced by alteration in intermittent fasting. It has been reported that plasma levels of adiponectin range from 5 to 30 mg/ L in normal weight individuals representing 0.01% of all plasma protein (41) and up to 1000 fold greater than visfatin (Table 1). Furthermore, in animal studies which have demonstrated a significant increase in adiponectin level following intermittent fasting, a calorie restriction was applied concomitant with fasting (42). However, in current study the participant were free to eat after fasting.

Conclusion

The findings of this study revealed that intermittent fasting independent of changes in anthropometric measures decreases visfatin level in human.

Conflicts of interest

We performed this research in the interest of knowledge and declare that we have no conflict of interest.

Acknowledgment

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