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Research paper

The effects of Ramadan fasting on serum concentrations of vaspin and omentin-1 in patients with nonalcoholic fatty liver disease

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ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most frequent liver disease. Vaspin may modulate the inflammatory process, insulin resistance, and NAFLD. Omentin-1 is independently associated with hepatocyte ballooning. There is evidence of improvement in the adipokines level and histological hepatic steatosis following energy intake restriction and weight loss. The aim of the study was to evaluate the effects of Ramadan fasting on various circulating adipokines in NAFLD patients.

Methods: This study was conducted on 83 NAFLD patients, 42 cases who declared that they would fast during the month of Ramadan fasted and 41 controls who decided not to fast during Ramadan. Anthropometric parameters including weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR) and body fat percentage were measured before and after Ramadan. Dietary intake and physical activity levels were assessed by 24-h recall questionnaire and International physical activity questionnaire-short form (IPAQ-S), respectively. Serum adipokines including vaspin and omentin-1 were measured using commercial ELISA kit.

Results: Patients who fasted displayed a lower mean hip circumference and body mass index and considerable changes in serum vaspin and omentin-1 (p-value < 0.05). The stepwise multivariate linear regression model revealed that the changes in weight, WHR, and dietary intakes were significant predictors of changes in serum adipokines.

Conclusion: There is a potential for fasting in improving several anthropometric indices in NAFLD patients. Additionally, fasting during Ramadan resulted in decreased levels of adipokines including vaspin and omentin-1. Studies with longer follow-up periods are required.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, is a major public health concern. NAFLD is characterized by accumulation of liver fat exceeding 5% in the absence of significant alcohol intake, viral infection or any specific etiology of the liver [1].

The increased prevalence of obesity and sedentary lifestyle is associated with the increased prevalence of NAFLD [2,3].

Adipose tissue-derived inflammatory molecules contribute to obesity-related complications [4]. The main mechanisms involved in the epidemiological relationship between visceral fat mass and increased metabolic risk has been attributed to adipokines, which are predominantly expressed and secreted from visceral adipose tissue [5]. Several effects on insulin resistance have been attributed to vaspin (visceral adipose tissue-derived serine protease inhibitor) [6], additionally, the serum level of vaspin has been directly associated with aminotransferase concentrations as well as hepatocyte ballooning degeneration [7,8]. Vaspin is involved in the inflammatory process [7,9], and plays a mediating role in resting metabolic rate [10]. Omentin-1, which has the most expression in visceral adipose tissue, is reported to be an independent predictor of hepatocyte ballooning [7] and increased level of omentin-1 and vaspin has been observed in NAFLD [7,8].

The dietary and lifestyle modifications and proper weight management are the first lines treatment of hepatic fat accumulation and prevention of NAFLD. According to the evidence, weight loss and

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energy restriction (ER) could reduce histological steatosis (intrahepatic fat content), serum enzymes concentrations and improve the adipokines level [11]. Weight loss has been associated with a significant decline in levels of vaspin [12,13]. Muslims fast one month a year based on their religion during which they do not eat, drink or smoke from dawn until sunset every day, which may result in changes in metabolism. According to the season and latitude, fasting during Ramadan lasts from eight to 18 h [14]. In this regard, marked lower concentrations of omentin-1 have been found in pregnant women due to Ramadan fasting [15]. Moreover, the significant effects of Ramadan fasting on glycemic status, blood pressure and inflammatory cytokines including IL-6 and hs-CRP; have been recently reported [16]. There is limited evidence for the effectiveness of dietary strategies to weight loss such as fasting with respect to these two adipokines. Our objective, hence, was to investigate the impacts of fasting during Ramadan on anthropometric indices and adipokines concentrations in patients with NAFLD.

2. Methods

2.1. Subjects

This prospective observational study was carried out in a group of patients with NAFLD. A total of eighty-three NAFLD patients referred to the hospital in Jahrom, Iran, were recruited. In all patients, NAFLD diagnosis was established by an ultrasonography scan of the liver compatible with NAFLD chronic and elevation of liver enzymes. All patients had documented prior history of NAFLD as determined by a biopsy and carried out within the last two years.

The inclusion criteria were age between 20 and 50 years old and decision to fast for more than 20 days during the Ramadan. Exclusion criteria included smoking, kidney diseases, diabetes and malignancy, pregnancy and lactation, menopause. The range of fasting hours during the month was about 15 to 16 h a day. Patients in the fasting group were fasting for more than 20 days during the period from January 18, 2015 to July 17, 2015. All patients were monitored by weekly phone calls; and were motivated to continue the practice. The average call duration was 5 min. Approval was obtained from the ethical committee of Jahrom University of Medical Sciences with the number IR.JUMS.REC.1394.158 and written informed consent was obtained from all patients.

2.2. Anthropometric measurements

Anthropometric indices were measured in light clothing and without shoes. Body weight and height were measured in the fasting state using calibrated scale (Seca, Hamburg, Germany) to the nearest 0.1 kg and a stadiometer attached to the scale with a precision of 0.1 cm, respectively. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest and hip circumference (HC) was measured at the widest part of the hip region. Waist-to-hip ratio (WHR) was calculated as waist circumference (cm) divided by hip circumference (cm). To assess body fat percentage body composition analyzer (InBody720, Biospace, Korea) was used. The subjects were asked to step on the foot electrode in barefoot and hold the handles and the procedure was held, as mentioned in the manual [17].

To control for the confounding effects of physical activity, the International physical activity questionnaire-short form (IPAQ-S) was completed by each patient; [Available from http://www.ipaq.ki.se/scoring.pdf]. High physical activity was defined as follows: a) vigorous-intensity activity on at least 3 days/week achieving a minimum total physical activity of at least 1500 MET-min/week or b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-min/week. Moderate score was defined as having a) 3 or more days of vigorous-intensity activity of at least 20 min per day or b) 5 or more days of moderate-intensity activity and/or walking of at least 30 min per day or c) 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum total physical activity of at least 600 MET-min/week. Those individuals who did not meet the criteria for categories 2 or 3 are considered to have a 'low' physical activity level [13]. Dietary intakes including energy and macronutrients were assessed by 24-h recall questionnaire and were analyzed by Nutritionist IV software (version 3.5.2, The Hearst Corporation, San Bruno, CA).

The grading of NAFLD was scored according to the NAFLD activity score (NAS) based on Brunt et al. [18]. A threshold of 5% of hepatocytes showing steatosis was required for the diagnosis of NAFLD [18]. This scoring system is the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores. Additionally, NAS has reasonable inter-rater reproducibility that is useful for studies of both adults and children with any degree of NAFLD.

2.3. Sample collection and laboratory assessment

Subjects attended the laboratory after 12 h fasting, in two stages: 3 days before Ramadan and 3 days after end of this month.

Approximately 10 ml of venous blood samples were obtained from all participants, and serum and plasma were immediately separated by centrifugation at 4 °C for 10 min at 2500 r.p.m. and were frozen at − 80 °C until analyzing. Serum adipokines including vaspin and omentin-1 were measured using commercial ELISA kit (East BioPharm).

2.4. Statistical analysis

Data were analyzed using SPSS Program version 22.0 [SPSS, Inc., Chicago, Ill]. Continuous variables are expressed as mean ± SD and categorical variables as frequency and percentage. Paired *t*-test, independent sample T-test, Wilcoxon, and Chi-squared test were used when appropriate. All significant tests were 2-tailed, and P values < 0.05 were considered statistically significant.

3. Results

Demographic characteristic and physical activity levels of patients are shown in Table 1. There were no significant differences in mean age, grades of NAFLD and physical activity levels between the two groups at baseline ($p > 0.05$; Table 1).

The mean (± SD) ages of the patients in the fasting and non-fasting groups were 37.59 ± 7.06 and 35.80 ± 7.33 years, respectively. The

Table 1
Demographic characteristic and physical activity levels of study subjects.

Characteristics	Group		the
	Fasting (n = 42)	Non-fasting (n = 41)	
Age (year) *	37.59 ± 7.06	35.80 ± 7.33	0.19
Sex**			
Male (%)	25 (59.5%)	32 (78.0%)	0.06
Female (%)	17 (40.5%)	9 (22%)	
	42	41	
NAFLD grade			
Grade 1(%)	31 (73.8%)	32 (78.0%)	0.39
Grade 2	7 (16.7%)	8 (19.5%)	
Grade 3	4 (9.5%)	1(2.4%)	
Physical activity			
Light (%)	19 (45.2%)	16 (39.0%)	0.62
Moderate (%)	18 (42.9%)	17 (41.5%)	
Severe (%)	5 (11.9%)	8 (19.5%)	

*Independent *t*-test, mean ± SD;**Chi-squared test. P-value < 0.05 is significant.