

## Original Article

## Evaluation of Nitric Oxide and Interleukin-17 in the Serum of Men During the Fast of Ramadan

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### Abstract

**Background & Objective:** Nitric oxide gas is important in regulating blood vessel dilation, and consequently, blood pressure. Nitric oxide is continuously produced by endothelial cells known as a compound that plays an important role in the cardiovascular system. Also, Interleukin-17 (IL-17) is a pro-inflammatory cytokine that can mediate protective innate immunity to pathogens or contribute to the pathogenesis of inflammatory diseases. In the current study, the plasma levels of nitric oxide and serum level of IL-7 were assessed before and after Ramadan month in fasting people, aimed at the scientific investigation of the possible preventing effects of fasting for cardiovascular disease.

**Materials & Methods:** The present study 61 fasting man (30-60 years old) were selected and blood samples were collected from each one day before and one day after Ramadan month. Serum levels of Nitric oxide and IL-17 were measured in two groups by ELIZA kits.

**Results:** The results of this study shown the serum level of nitric oxide was significantly increased in individuals before and after Ramadan month (from  $16.17 \pm 1.15 \mu\text{mol/L}$  to  $22.17 \pm 1.46 \mu\text{mol/L}$ , respectively) ( $P < 0.0001$ ). Also, the results indicate that serum level of IL-17 was statistically different in two groups (from  $58.25 \pm 10.59 \text{ pg/ml}$  to  $15.98 \pm 4.66 \text{ pg/ml}$ , respectively) ( $P < 0.0001$ ).

**Conclusion:** The results of this study showed that nitric oxide and IL-17 levels decreased during the Ramadan month and so it can be concluded the fasting of Ramadan may have positive effects on cardiovascular and immune factors of the body.

**Keywords:** Fasting, Interleukin-17, Nitric oxide, Ramadan

### Introduction

Based on previous studies it is indicated that fasting leads to some biochemical changes in the body that contribute to restore and promote the

health condition and reduce risk factors and health threats such as obstruction of the arteries. Besides, these changes have shown inevitable effects in the health maintenance of the cardiovascular system and reduce the risk of heart attacks. Since a large number of patients with cardiovascular disease with a related high mortality rate is a major health problem of the world it would be an effective step if fasting based treatment leads to decreasing the losses due to such diseases (1). It is indicated that many

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human diseases are influenced by free radicals which have an intense affinity for reacting with crucial molecules in body cells due to their unpaired electrons. Two important groups of free radical compounds are Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Since oxidants and free radical compounds are highly active and unstable they tend to react with molecules such as proteins, carbohydrates and DNA that leads to oxidative damages. These reactions result in the deregulation of cellular functions and if the damages were severe enough may end up to cell death. Free radicals are generated through several mechanisms. They are produced by losing electron atoms or by obtaining Hydrogen atoms from other chemical compounds. ROS and RNS types of free radicals can cause cancerous cells not only by directly damaging the cellular DNA but also by deregulating proliferation and apoptotic signals of cell cycle (2). Nitric oxide (NO) is known as a radical compound that has an important role in regulating the harmonic movements of the lining of blood vessels and influenced the cardiovascular system (3).

IL-17 is one of the crucial cytokines that has a significant role in occurrence and maintenance of inflammation. IL-17 is released by a subgroup of T lymphocytes called Th17 that is revealed to play a devastating role in several diseases including asthma, cardiovascular disease and autoimmune disorders such as Rheumatoid arthritis, lupus and Multiple Sclerosis, so that many of new therapeutic methods, are designed based on Th17 cells suppression or inhibition of cytokine receptors. In obese patients with a high-fat diet, the increased level of IL-17 shown a significant decrease after taking vitamin diet (a mixture of fruit juice). So the type of diet, could affect the secretion of this cytokine (4). Given that so far there is a scientific source which was measured the exact amount of NO in plasma and IL-17 in serum sample of fasting people, in the current study we tried to investigate the possible effects of fasting on the amount of mentioned biochemical factors and the related effects on the preventing from cardiovascular disease risk and prompt the immune system. This study aimed to found a possible scientific correlation between fasting and the inhibition of cardiovascular and inflammatory disease.

## **Materials & Methods**

All of the subjects had fasted

completely during Ramadan. Blood samples were collected and serum was separated from healthy men twice, one day before the start and finish of the Ramadan and serum samples stored in a  $-20^{\circ}\text{C}$ . Exclusion criteria were included: history of type 2 diabetes, thyroid disease, liver, infectious and heart disease, taking antihypertensive drugs, and alcohol and tobacco (5). The changing amount of Nitric oxide was assessed in plasma samples using the ELIZA kit (Natrix, Cat No: NS15044, UK). This method is a two-step diazotization reaction which the NO derived nitrosating agent, dinitrogen trioxide ( $\text{N}_2\text{O}_3$ ) generated from the acid-catalyzed formation of nitrous acid from nitrite (or autoxidation of NO) reacts with sulfanilamide to produce a diazonium ion which is then coupled to N-(1-naphthyl) ethylenediamine (NED) to form a chromophoric azo product. 50  $\mu\text{l}$  of 6.5 mol/LHCl and 50 ml of 37.5 mmol/l sulphanilic acid were added to 200  $\mu\text{l}$  of supernatant of the deproteinized sample. After 10 min incubation at  $4^{\circ}\text{C}$ , 50  $\mu\text{l}$  of 12.5 mmol/l NED was added. Following 30 min incubation at  $4^{\circ}\text{C}$ , the samples were centrifuged (10000 rpm, 10 min). Absorbance was measured at 540 nm by microplate reader (Biotek, USA).

The serum samples were used to assess the level of IL-17 by ELIZA kit (IBL, Cat No: EK0410, Germany). For IL-17 sensitivity stated by the manufacturer was  $2\text{pg ml}^{-1}$ . The collected data were analyzed by SPSS 16 software. All the data expressed as mean  $\pm$  standard deviation and Statistical analysis (t-test and logistic regression) were used to analyze the results. Statistical significance was set at  $p < 0.05$ .

## **Results**

In the present study the changing amount of the plasma level of Nitric oxide and serum level of IL-17 were assessed in fasting men before and after Ramadan fasting month. The measured amounts of NO and analyzed differences during the fasting period are mentioned in Table 1. Our results showed that the level of NO in the serum of fasting men in fasting with before and after Ramadan was  $16.17 \pm 1.15$  and  $22.17 \pm 1.46$

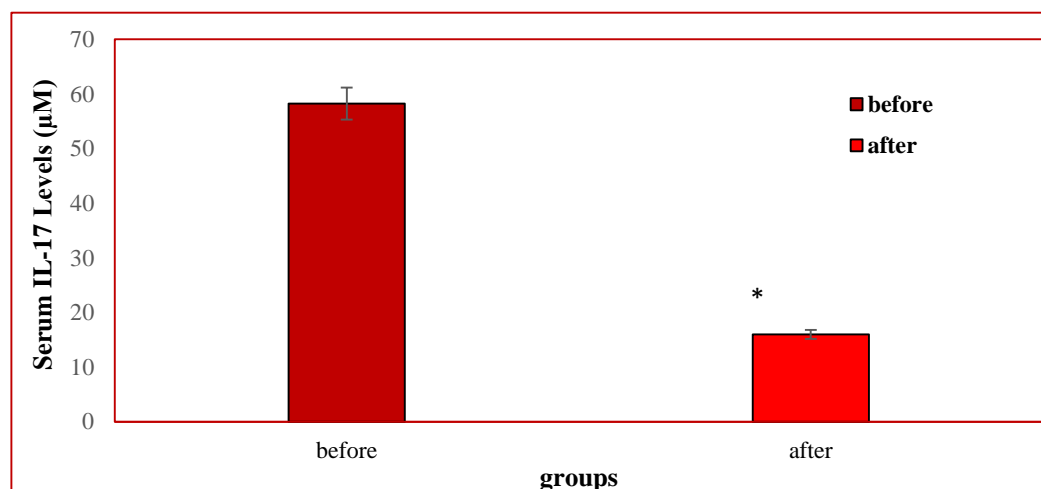
respectively. The level of NO was significantly different between two groups ( $p$ -value =0.01).

The measured amounts of the plasma level of IL-17 showed that the level of IL-17 in the serum of fasting men in fasting with before and after Ramadan was  $58.25 \pm 1.36$  and  $15.98 \pm 1.19$  respectively. The comparison of the plasma level of IL-17 before and after the fasting period showed that the level of IL-17 was significantly different between the two groups ( $p$ -value =0.01) (Chart 1).

perfusion, blood Hypertension (8), inflammation, and oxidative stress (9). Furthermore, various studies have suggested that NO can be signaling molecule as a chain-breaking Antioxidant and inhibit oxidation. NO can terminate chain reactions during lipid peroxidation (10). Nitric oxide has rich chemistry that reacts with radicals such as peroxy radicals. This chain-termination reaction similar to typical donor antioxidants such as vitamin E so peroxy radical ( $LOO\bullet$ ) is removed

**Table 1.** The serum level of NO before and after fasting period.

NO concentrations	Before fasting	After fasting	P. Value
Mean ( $\mu\text{mol/L}$ )	$16.17 \pm 1.15$	$22.17 \pm 1.46$	0.001
Minimum ( $\mu\text{mol/L}$ )	15.88	19.10	0.001
Maximum ( $\mu\text{mol/L}$ )	18.19	23.66	0.001



**Chart 1.** The serum level of IL-17 before and after fasting period. The symbol (\*) represents significant differences ( $p < 0.05$ ). The mean of IL-17 was significantly different between men before and after fasting period.

### Discussion

Nitric oxide (NO) is a unique signaling molecule and involved in several physiological and pathological processes. (6). NO is produced by endothelial nitric oxide synthase (eNOS) from L-arginine and stimulates a cGMP-protein kinase G signaling pathway that modulates  $Ca^{2+}$  influx, contractile machinery, cell migration and proliferation (7). Depleted plasma NO and increased levels of plasma oxyhemoglobin in the bloodstream lead to impairment of organ

and product,  $LOO\bullet + NO \rightarrow LOO-N=O$  that is not very reactive, then nitric oxide will serve as a chain-breaking antioxidant. (11). It should also be noted that Nitric oxide has been proposed to act as a pro-oxidant at high concentrations (12) or when it reacts with superoxide ( $O_2^{\bullet-}$ ) forming the highly reactive peroxynitrite ( $ONOO^-$ ) (13). Nitric oxide protects various intracellular macromolecules of the body especially DNA from the toxic effects of these damaging compounds by its directly or indirectly combination with them (14). This study showed

that the amount of the plasma level of Nitric oxide was significantly different between two groups. So that, the amount of nitric oxide in fasting people after Ramadan increased significantly compared to before this month in these people. It seems that in fasting, mechanisms increase the expression and activity of nitric oxide synthase and thus increase the amount of serum nitric oxide. Due to its signaling, increasing nitric oxide reduces the risk of various nitric oxide-related diseases such as cardiovascular disease, hypertension, renal failure, and more. Similar to our study, various studies have suggested that Nitric oxide (NO) is the main vasodilator agent that decreases the development of pulmonary vascular resistance (15), hypertension (8), oxidative stress (11), vasoconstriction, reduced renal perfusion (9), and type 2 diabetes mellitus (T2DM) (7).

Cytokines play an important role in the pathogenesis of various diseases. The increase in pro-inflammatory interleukin (IL)-17 cytokine production is an important marker of diseases and metabolic changes (16). The stimulating secretion of IL-17 is involved in the induction of potentially harmful mediators of inflammation, such as free radical nitric oxide. Moreover, inflammation-related tissue damage in organ-specific autoimmunity could be associated with the IL-17-dependent T-helper cells. Changes of this cytokine could be considered as a useful biomarker of inflammation. (17). Our finding showed that the amount of the plasma level of IL-17 was significantly different between two groups. So that, the amount of IL-17 in fasting people after Ramadan decreased significantly compared to before this month in these people. Interleukin-17 (IL-17) is expressed in the intestine in response to changes in the amount and composition of food. According to various studies, intestinal IL-17 is also regulated by dietary factors, and this has been correlated with abnormal systemic metabolism that occurs in obesity and type 2 diabetes (18, 19). Because it has been seen that the level of IL-17 cytokine increase in obese women (17). So, it seems that in fasting, mechanisms reduce the stimulation of T cells and as a result decrease the amount of serum IL-17. Various studies have shown an increase in IL-17 in diseases such as hypertension (16), insulin resistance (20), atherosclerosis (21), type 2 diabetes (22), Obesity (17), hypothalamus diseases (23). A study aimed to investigate the effects of the fasting in holy month of Ramadan

on the biochemical serum markers of oxidative stress and markers of cell damage in healthy subjects. They indicated that except for a slight decrease in the oxidation of red blood cells, fasting will not change any other biochemical parameters of oxidative stress and cell damage markers in healthy people (24). Another study by S. Asgari and their colleges investigated a correlation between fasting and changes in the serum lipoproteins and fasting blood sugar. The results of the study shown that changed dietary habits in this month have fairly beneficial effects on the plasma lipids. On the other hand, a significant decrease in the Malondialdehyde (MDA) indicated reduced oxidation of LDL cholesterol in this month. Since this factor has an important role in the formation of atherosclerotic plaques it seems that fasting has a preventing effect on the risk of developing cardiovascular disease (25).

In the study of S. Ahmadi about the metabolic and clinical effects of fasting on patients with type 2 diabetes, was found that fasting has beneficial effects on diabetic patients who have a diet or consuming hypoglycemic drugs. However, these patients should have a healthy diet, especially in terms of daily intake of cholesterol and saturated fat (26). A study by B. Larijani found that fasting serum glucose concentration reduced after Ramadan fasting month. It seems that fasting in Ramadan does not have any serious adverse effects on blood glucose in healthy subjects. (27) By study of M. Zare was indicated that fasting is completely safe for healthy subjects and it could be useful for preventing the increase in cholesterol and other blood fats, reduce uric acid levels and better maintaining the protein in the body (28). In another study by M. Rashidi was shown that during fasting period relatively large amounts of metabolic changes have occurred in the concentration of blood glucose, blood levels of fatty acids and biomolecule oxidative reactions (29). In another study by Tokmedashi investigation of the changing amounts of plasma nitric oxide and the products of plasma lipid peroxidation in fasting period, indicated that fasting leads to a significant change in the amounts of NO and MDA level in the plasma and changing of these two factors affects the risk of cardiovascular disease (30, 31). Also, a study conducted by L. Peluso was indicated that in obese patients with a High-fat diet, the increased level of IL-17 shown a significant decrease after

taking vitamin diet. Also, a High-fat diet leads to increase in the amount of other inflammatory cytokines such as Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-6 (32). In another study by N. Tajic et al. in Tehran, they found that in obese women, weight loss due to a change in diet leads to a significant reduction in serum levels of IL-6 and IL-18 (33). Finally, it seems that in fasting period with increased plasma level of NO and decrease of IL-17 level, the risk of dangerous diseases such as blood hypertension, cardiovascular, kidney and another disease was reduced and it can be beneficial for individuals.

### **Conclusions**

Although effects of fasting in Ramadan month on various health and physiological aspects were studied and its impact on medicine has been proved, its effects on the plasma concentration of NO and serum level of IL-17 was not studied yet. One of the important effects of a low-calorie diet on the body is its effects on the cardiovascular and immune system. On the other hand, during Ramadan for 30 days and with its own specific rules of fasting that people are allowed to consume food for limited hours, is extremely different from common decreased caloric intake.

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### **Conflict of Interests**

Authors declare no conflict of interest.

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## مقاله پژوهشی

## بررسی میزان نیتریک اکساید و اینترلوکین-۱۷ در سرم مردان روزه دار در ماه رمضان

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## چکیده

**زمینه و هدف:** اکسید نیتریک در تنظیم اتساع رگ های خونی و در نتیجه فشار خون از اهمیت برخوردار است. اکسید نیتریک به طور مداوم توسط سلولهای اندوتلیال بصورت ترکیبی تولید می شود که در سیستم قلبی عروقی نقش مهمی دارد. علاوه بر این، اینترلوکین-۱۷ (IL-17) یک سیتوکین ضد التهابی است که می تواند از ایمنی محافظت کننده ذاتی در برابر عوامل بیماری زا یا عوامل ایجاد بیماری های التهابی کمک کند. در مطالعه حاضر، سطح پلاسمایی نیتریک اکسید و سطح سرمی IL-7 قبل و بعد از ماه رمضان با هدف بررسی پیشگیری های احتمالی روزه داری برای بیماری های قلبی عروقی در افراد روزه دار بررسی شد.

**مواد و روش ها:** مطالعه حاضر ۶۱ فرد ناشتا (۳۰-۶۰ ساله) انتخاب و نمونه خون از هر فرد یک روز قبل و یک روز پس از ماه رمضان جمع آوری شد. سطح سرمی اکسید نیتریک و IL-17 در دو گروه با کیت های ELIZA اندازه گیری شد.

**نتایج:** نتایج این مطالعه نشان داد که سطح سرمی اکسید نیتریک در افراد قبل و بعد از ماه رمضان به طور معنی داری افزایش یافته است (از  $16/17 \pm 1/15$  میکرومول بر لیتر تا  $22/17 \pm 1/46$  میکرومول بر لیتر (به ترتیب)) ( $P < 0.001$ ). همچنین، نتایج نشان می دهد که سطح سرمی IL-17 در دو گروه از نظر آماری تفاوت معنی داری داشت (از  $58/25 \pm 10/59$  میکرومول بر لیتر تا  $15/98 \pm 4/66$  میکرومول بر لیتر (به ترتیب)) ( $P < 0.001$ ). **نتیجه گیری:** نتایج این مطالعه نشان داد که سطح نیتریک اکسید و اینترلوکین-۱۷ در ماه رمضان کاهش یافته است، بنابراین نتیجه گیری می شود که روزه داری ماه رمضان می تواند اثرات مثبتی بر روی عوامل قلبی و عروقی و ایمنی بدن داشته باشد.

**کلمات کلیدی:** روزه داری، اینترلوکین ۱۷، اکسید نیتریک، ماه رمضان.

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