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ORIGINAL ARTICLE

Naïve, Effector, and Memory T cells Frequency in the Peripheral Blood of Healthy Men Following 4 Weeks of Intermittent Fasting

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ABSTRACT

Background: Intermittent fasting (IF) is a 16/8 fasting model, which is supposed to recruit immune cells to the bone marrow, thus prevents immune exhaustion. The aim of this study was to compare the frequency of naive, memory, and effector T lymphocytes in blood circulation before and after IF.

Methods: Proportion of CD4+CXCR7+45RA+ (naïve T cells), CD4+CXCR7+45RA- (memory T cells), and CD4+CXCR7- 45RA- (effector T cells) cells were evaluated in 18 healthy young men in the week before and on the last day of Ramadan fasting, resembling IF, using flowcytometry.

Results: The percentage of peripheral blood naïve T cells reduced significantly at the end of Ramadan fasting (p=0.001); however, the frequency of memory and effector T cells did not change.

Conclusion: The decreased number of circulatory naïve T cells suggests a protective effect of intermittent fasting on immunosenescence and immune exhaustion. Also, preserved proportion of effector T cells is suggestive of an intact immunocompetence despite 4 weeks of IF.

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Introduction

Fasting and calorie restriction are supposed to affect leukocytes function and homing. Recent studies have shown a correlation between the nutritional state and frequency and function of immune cells, therefore, certain diets have been proposed to reduce inflammatory responses or to augment immune competence (1-3). For instance, symptoms of patients with rheumatoid arthritis, unresponsiveness to standard treatments, were alleviated following long-term fasting periods (4). In recent years, immunosenescence, described as chronic inflammatory state and reduced necessary immune responses, has been noticed and it was observed that various models of calorie reduction could delay immune aging process (5-7).

One of the mechanisms proposed to justify this effect was recirculation of T lymphocytes to the bone marrow, decreased production of new lymphocytes, and prevention of immune exhaustion of these cells (8, 9). Nevertheless, the underlying mechanisms (e.g. chemokine receptor changes, cytokine alteration) and consequences of lymphocyte re-localization (e.g. immunodeficiency, subsets imbalance) have not yet been described (10-12). Noteworthy, the majority of abovementioned findings have been obtained from experimental studies and a few reports from human subjects. Therefore, there is a need to explore the advantages and disadvantages of fasting and calorie restriction in healthy and diseased individuals, in particular, immunological alterations consequent to different models of fasting.

Ramadan fasting described with abstinence from eating and drinking from dawn to dusk during Ramadan month is similar to the pattern of 8/16 intermittent fasting i.e. 16 hours of fasting and 8 hours of eating for 4 weeks. Moreover, it is a voluntary diet in healthy individuals thus a suitable model to investigate the immunological effects of intermittent fasting (IF) in humans (13). In the present study, we aimed to evaluate the frequency of naïve, effector, and memory T lymphocytes in the blood circulation of healthy young men before and on the last days of Ramadan fasting. If any significant correlation is established between IF and immunosenescence, this model of fasting might be considered as a complementary option to prevent immune aging and related disorders.

Materials and Methods

Peripheral blood samples were taken from 18 healthy young males on the days leading to Ramadan and the last day of Ramadan fasting. The participants were living in the university dormitory and had a similar nutritional diet provided by a dietician. This study was conducted from March to April 2024, and according to the calculations, Ramadan fasting of 2024 was very similar to the IF 8/16 pattern. The food intake of the participants was evaluated with Food Frequency Questionnaires (FFQ) and the calorie intake of each participant was calculated. All participants gave informed consent and the study was approved by the Ethics Committee of Tehran University of medical sciences, Tehran, Iran (IR.TUMS.CHMC.REC.1401.023). All subjects gave their informed consent for inclusion before they participated in the study.

Peripheral blood mononuclear cells (PBMCs) were separated with the Ficoll-hypaque density gradient centrifugation. Flow cytometry was performed with mouse FITC anti-human CD4 conjugated antibody (317407, Biolegend United Kingdom), mouse PE anti-human CCR7 conjugated antibody (353706, Biolegend United Kingdom), and mouse PE/Cy5 anti-human CD45RA conjugated antibody (359119, Biolegend United Kingdom) and analyzed using FLOWJO 10 software. T lymphocytes in different developmental stages were defined as TCD4+CXCR7+45RA+ (naïve T cells), TCD4+CXCR7+45RA- (memory T cells), and TCD4+CXCR7- 45RA- (effector T cells) (14) (Figure 1). SPSS 26 software (SPSS 26.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data were presented as mean±standard deviation (SD). Due to the non-parametric number of the participants and paired variables analysis, the Wilcoxon test was applied to compare the variables before and after fasting. A p value less than 0.05 was considered significant.

Results

The present study included 18 healthy men between 19 and 23 years old (mean±SD: 20±1.2).

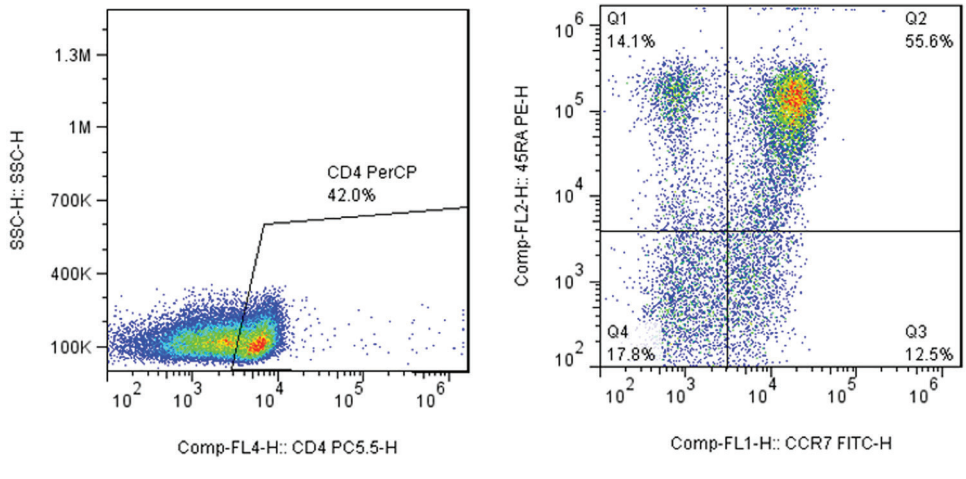

Figure 1: Different types of T cells were defined as naïve T cells: TCD4+CXCR7+45RA+, memory T cells: TCD4+CXCR7+45RA-, and effector T cells: TCD4+CXCR7- 45RA-. The gating method was as follows: Q2 included naive T cells, Q4 included effector T cells, and Q3 included memory T cells.

The women were excluded from the study because they were not allowed to keep fasting during menstrual period. The body mass index (BMI) of participants ranged between 20.2 and 25.3 (mean±SD: 22.3±2.4). Although their BMI decreased at the end of Ramadan, the change was not statistically significant (mean±SD: 21±2.8), (p=0.2). However, the daily calorie intake of subjects reduced significantly during Ramadan fasting [2530+312 vs. 2308+186 (p=0.03)].

The proportion of naive T cells, which was identified as the percentage of the CXCR7+45RA+ population within TCD4+ cells, showed a significant reduction after Ramadan fasting [(mean±SD) 45±8.3 vs. 41±7.8, p=0.001], which was suggestive of naïve T cells recruitment to the bone marrow or secondary lymphatic tissues (Figure 2). The percentage of circulating memory T cells, defined as the CD4+CXCR7+45RA- population, did not show any significant difference between before and after Ramadan fasting [mean±SD: before fasting: 15.7±4.5 vs. after fasting: 16.2±3.7, p=NS)] (Figure 3). The percentages of CD4+CXCR7-45RA- effector T cells in peripheral blood of participants were comparable before and after fasting, suggesting a preserved immune competence against infections [mean±SD: before fasting: 22±4.3 vs. after fasting: 22.1±4.5] (Figures 4).

Discussion

T lymphocytes are a substantial subset of immune cells responsible for initiating and directing immune responses against microbial antigens, cancerous cells, and danger associated molecular patterns (DAMPs) in sterile inflammation. Recent studies suggest that immune cells, especially T cells, are affected by nutritional factors such as glycolysis, fatty acid oxidation, and oxidative phosphorylation. Accordingly, attempts have been made to modify T cells function and frequency by nutritional alterations and calorie restriction (15, 16).

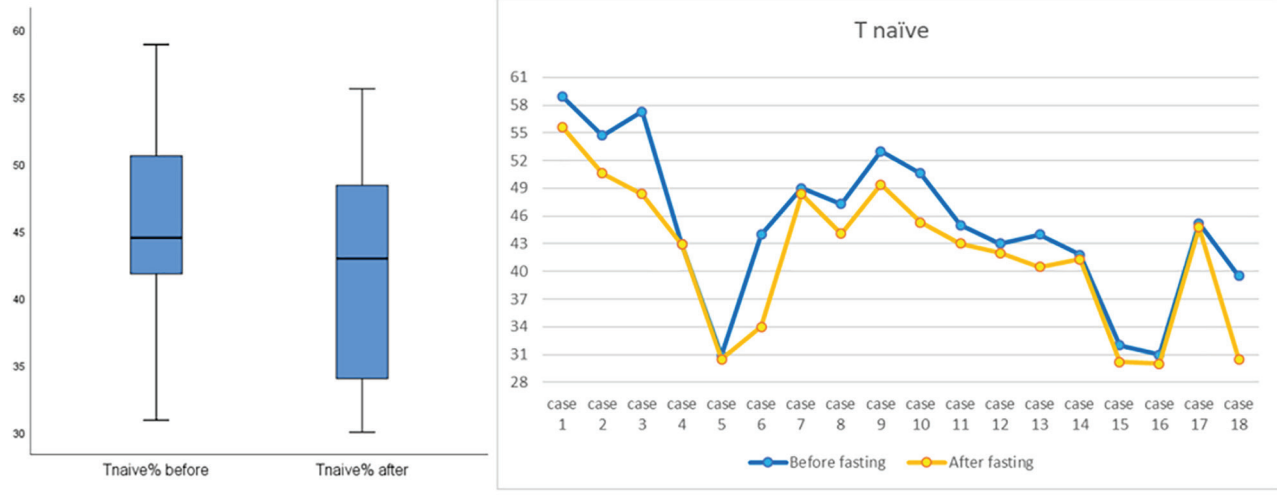

Figure 2: Significant decrease in the percentage of naïve CD4+CXCR7+45RA+ T cell population in the blood circulation after fasting (p=0.001).

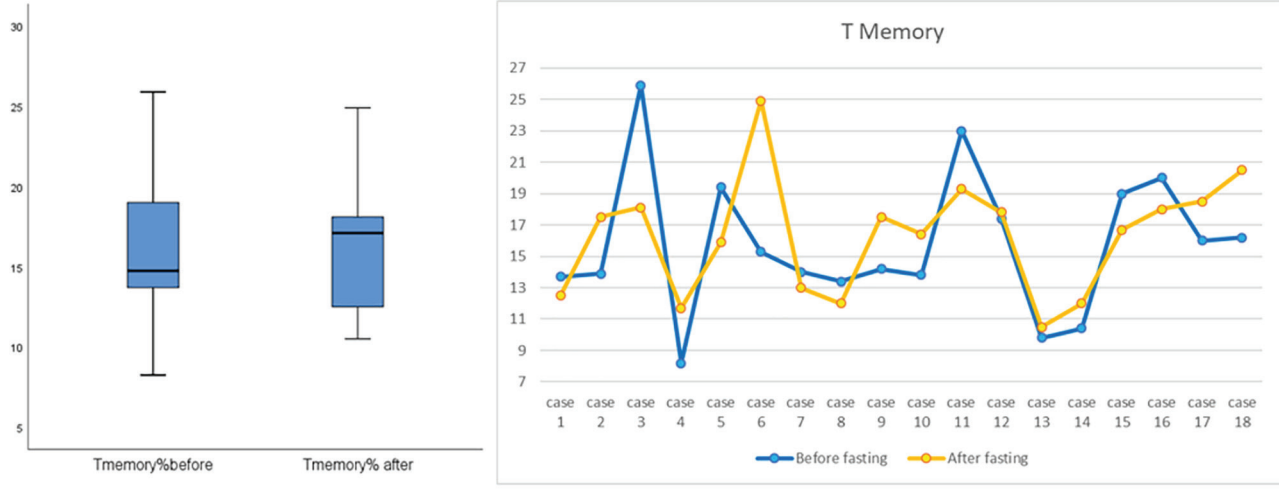

Figure 3: No significant change was noticed in the percentage of circulating memory CD4+CXCR7+45RA- T lymphocytes after Ramadan fasting.

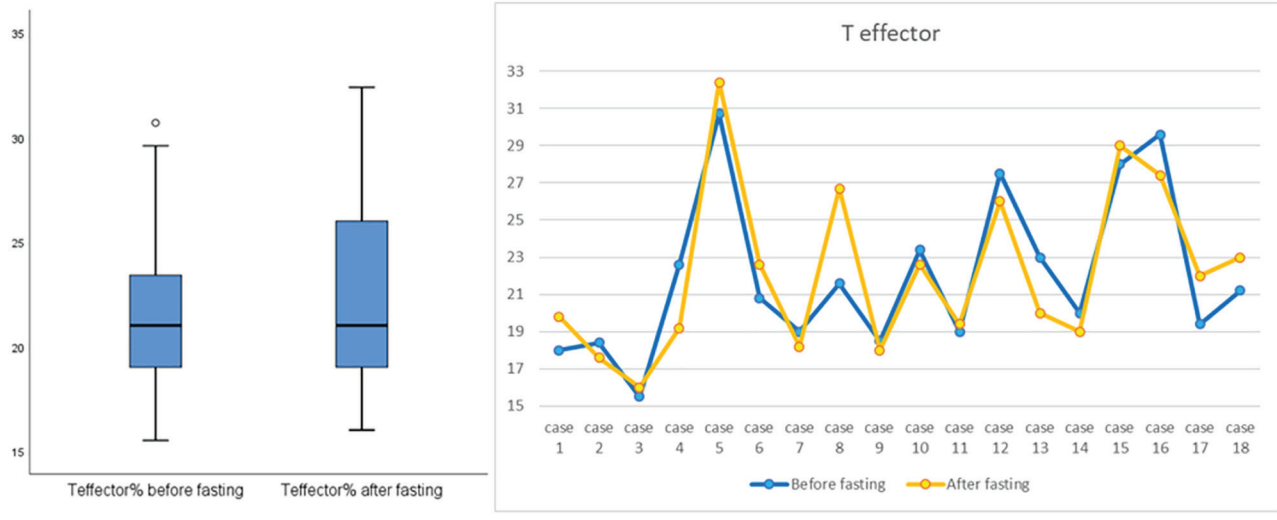

Figure 4: No significant change was observed in the percentage of effector CD4+CXCR7-45RA- T lymphocytes in circulation after Ramadan fasting.

Immune aging or immunosenescence is characterized with reduced lymphocyte function and chronic inflammatory responses, leading to immunodeficiency and autoimmune disorders, respectively (17, 18). The adaptive immune system is the most affected by aging, with a reduction in naive T cells number due to thymus involution, hematopoietic stem cells skewed differentiation toward myeloid lineage, expansion of memory T cells, exhausted T-cell population, and decreased production of IL-7, which is crucial for survival and metabolic homeostasis of naive T cells. Conversely, in older people, the accumulation of senescent cells and the acquisition of senescence-associated secretory phenotype, macrophage activation, oxidative stress, adiposity, and gut dysbiosis promote cytokine production and induce chronic inflammation (19, 20). Considering the potential of fasting to alter lymphocytes hemostasis, researchers have made efforts to use different forms of fasting to regulate T cell function and combat immunosenescence; however, The majority of reported findings are primarily based on animal models (21, 22).

Intermittent fasting encompasses different types, including fasting every other day, fasting two days a week, and daily fasting (8 hours eating/16 hours fasting), which is similar to Ramadan fasting. IF also includes a model of consuming only water for several days (3 to 21 days) followed by 7 days of a normal diet, or consumption of 30-50% of daily food for 4 to 7 days and then a month of normal diet (23, 24). Shushimita *et al.* studied the consequences of fasting on B and T cell dynamics in primary and secondary lymphoid organs of male mice. They found that fasting arrests the development of B cells in bone marrow and inhibits T cell maturation in thymus. Moreover, mature B lymphocytes recirculation increased while, T cells were depleted from spleen and mesenteric lymph nodes and recruited to the bone marrow (25).

Likewise, Takakuwa *et al.* observed a significant increase of T cells including both naïve CD8+ T cells and naïve CD4+ T cells in the bone marrow of fasted mice. In addition, immature hematopoietic cells remained in a quiescent state, but retained colony-forming capacity. These results suggested that some lymphoid cells and immature hematopoietic cells could survive starvation and preserve their function (26, 27). Similar results were obtained by Nagai *et al.* that demonstrated migration of lymphocytes from Payer's patches (PP) to bone marrow subsequent to fasting and back to PPs by refeeding. The significant migration of naive B cells from PPs to the bone marrow was attributed to upregulated expression of CXCL13 by stromal cells. Of note, a considerable number of germinal centers and IgA+ B cells were lost via apoptosis, and antigen-specific IgA responses were impaired (28). Nonetheless, our previous research demonstrated reduced amounts of serum IgA but unchanged salivary IgA levels in healthy men after Ramadan fasting (29). This discrepancy might be due to the different types of fasting investigated in two studies. It appears that the duration of fasting affect the intensity of immunological alterations; however, this needs further investigation.

It was shown that memory T cells leave the secondary lymphoid organs and migrate to the bone marrow in response to calorie restriction. This finding was attributed to the release of significant amounts of glucocorticoids and chemokine receptors in the bone marrow, mainly CXCR4 and Sphingosine-1-phosphate (S1P) (30). In another experiment, it was observed that following calorie restriction, the number of circulating monocytes decreased, and

inflammatory condition improved. Moreover, the activation of the energy-sensitive sensor 50-AMP-activated protein kinase (AMPK) in hepatocytes reduced the chemokine CCL2 via peroxisome proliferator-activated receptor alpha (PPARa) and diminished the release of monocytes from the bone marrow. Furthermore, this study demonstrated that despite reduced number of circulating monocytes, the defense against microbial agents remained unaffected (31).

Additionally, a 2-year study of young adults who reduced their caloric intake by 14% demonstrated that caloric restriction reduced the number of circulating lymphocytes and monocytes; but there was no change in vaccine responses. Immune responses to infectious diseases were also comparable with the control group (32). The present study also showed that the percentage of naive lymphocytes in circulation decreased significantly after 4 weeks of Ramadan fasting, which is in line with the results of previous studies and suggests a protective effect for fasting against immunosenescence and immunological exhaustion. However, unlike some previous studies, no considerable change was observed in the percentage of circulating memory T lymphocytes, maybe due to the small number of study subjects. The percentage of effector T lymphocytes also remained unchanged, indicating the preservation of immune defense against infectious agents. These and other findings imply an immunoregulatory role for fasting and calorie restriction by inhibition of chronic inflammation while maintaining anti-microbial defense (33, 34).

It appears that in response to the reduced calorie intake, the bone marrow protects the vital components of the immune system by recruiting them from the blood circulation and lymph nodes (35). This also reduces the production and differentiation of new immune cells thus prevents immunosenescence and immune exhaustion. Despite the small number of cases, the results of the present study corroborated the previous findings about the potential of fasting and calorie restriction in regulating immune responses while maintaining the immunocompetence. Nonetheless, further studies, especially in human subjects are warranted to establish fasting as an alternative for treating autoimmune or hypersensitivity disorders. In addition, it is necessary to discern physiochemical alterations in primary and secondary lymphoid organs subsequent to the calorie restriction.

Conclusion

Following 4 weeks of Ramadan fasting, the percentage of circulating naive T lymphocytes decreased significantly, suggesting the protective effect of fasting against immune exhaustion and probably immunosenescence. However, the percentages of memory and effector lymphocytes remained unchanged, indicating a preserved immunocompetence.

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Authors' Contribution

SA: Study design, manuscript revision, NS: Statistical analysis, MHA: Sample provision, ZM: Draft preparation, SS: Sampling and data provision, HM and MS: Flow cytometry test, MHN: Ggrant and supervision.

Conflict of Interest

The authors declare that there is no conflict of interest.

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