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Time-restricted feeding influences immune responses without compromising muscle performance in older men

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ABSTRACT

Objective: This study examined the effect of 12 wk of time-restricted feeding (TRF) on complete blood cell counts, natural killer cells, and muscle performance in 20- and 50-year-old men.
Methods: Forty active and healthy participants were randomly divided into young experimental, young control, aged experimental, and aged control group. Experimental groups participated in TRF. Before (P1) and after (P2) TRF, participants performed a maximal exercise test to quantify muscle power. Resting venous blood samples were collected for blood count calculation.
Results: No changes were identified in muscle power in all groups after TRF ($P > 0.05$). At P1, red cells, hemoglobin, and hematocrit were significantly higher in young participants compared with elderly participants ($P < 0.05$). At P2, this age effect was not found in red cells between the young experimental group and the aged experimental group ($P > 0.05$). At P1, white blood cells and neutrophils were significantly higher in young participants compared with elderly participants ($P < 0.05$). At P2, only neutrophils decreased significantly ($P < 0.05$) in experimental groups without significant ($P > 0.05$) difference among them. Lymphocytes decreased significantly in the aged experimental group at P2 ($P < 0.05$), whereas $NKCD16^+$ and $NKCD56^+$ decreased significantly in experimental groups at P2 ($P < 0.05$). TRF had no effect on CD3, $CD4^+$, and $CD8^+$ levels ($P > 0.05$).
Conclusion: TRF decreases hematocrit, total white blood cells, lymphocytes, and neutrophils in young and older men. TRF may be effective in preventing inflammation by decreasing natural killer cells. As such, TRF could be a lifestyle strategy to reduce systemic low-grade inflammation and age-related chronic diseases linked to immunosenescence, without compromising physical performance.

Introduction

Aging is associated with decline of the immune system response, also called "immunosenescence," which is due to multiple factors, such as genetics and lifestyle [1]. Immunosenescence is characterized by a decline in immune response and increases in inflammatory and oxidative profiles [2]. Accordingly, the bone marrow, thymus, and blood undergo changes with age. Several studies found that alterations of immune system begin at an early age; for example, the thymic epithelial space reduction (thymic involution) was detected in late adolescence [3], and its regression has been linked to reduced ability of the immune system to preserve a strong protective response by T lymphocytes [4]. Aging is also associated with decrease in erythropoietic reserve, production of erythropoietin, and other erythropoietic cytokines and an increasing risk of anemia in older adults after 50 y of age [5,6]. Although the number of blood monocytes does not appear to be altered by aging, an increased rate of neutrophil apoptosis [7] and decreased chemotaxis and phagocytosis in macrophages occur in the elderly [2,7]. There are consensus in the literature in that aging does not alter the total number of circulating neutrophils [7], but their chemotaxis and phagocytosis activity is reduced in healthy aged humans [2,7]. However, the most profound effect of stem cell aging is a reduced capacity for

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lymphocyte production and increased myeloid potential, which increases the risk of myeloid leukemia observed in elderly individuals [5]. Indeed, by middle age, stem cells have reduced common lymphoid progenitors, including a decrease in T and B cells [8] with accumulation of myeloid-biased hematopoietic stem cells [9].

The dramatic decline in humoral and cell-mediated responses with aging is mainly a result of alterations in the T-cell compartment. According to Watad et al. [2], aging decreased $CD3^+$ T cells as a result of thymus atrophy and changes at hematopoietic stem cell level. In addition, an overall decrease in the number of naïve $CD4^+$ and $CD8^+$ T cells, an increase in the number of memory T cells [10], and changes in surface expression of T cells, such as the loss of CD28 [11], occur with advanced age. Weng et al. [12] found insufficient proliferation of the lymphocyte T $CD8^+$, whereas naïve T $CD4^+$ differentiated to T helper 17 cells (Th17) in place of Th1 and Th2 cells with advanced age. In addition, natural killer (NK) cells and NK T cells play a central role in providing signals that are required to drive innate and adaptive immune responses. NK cells' and NK T cells' cytotoxicity declines with aging, as well as interferon-γ production by both activated cell types [13].

Early life perturbations to nutrition homeostasis in utero are important determinants of immune responses. They represent risk factors for increased autoimmune diseases and epigenetic inheritance of disease [14–16]. As such, finding a diet strategy to control these "pleiotropic" effects of nutrient imbalance will be a beneficial alternative to fighting several age-related and epigenetically heritable diseases. Emerging evidence suggests that daily periods of feeding and fasting are a dominant determinant of diurnal rhythms in metabolic pathways [17–20]. Hence, any change in these factors will alter the pleiotropic effect of nutrient imbalance. These alterations include gene expression and a variety of clock-controlled genes involved in important biological processes, including immune responses [21,22] and skeletal muscle development [23]. According to Schroder and Esser [23], the internal clock in skeletal muscle can be altered by changing or inverting time of exercise or feeding, independent of the central clock in the brain. In this context, it has been well demonstrated that intermittent fasting (IF) enhances immunity responses in children and patients with deficient immunity [24] and improves inflammation and metabolic responses during rest [25–28]. Moreover, IF promotes resistance to both multiple toxins and longevity extension in bacteria, worms, and rodents [29,30] and reduces inflammation in adult men [30]. Recently, Cheng et al. [31] reported that IF decreases lymphocytes and white blood cell counts in young adults followed by stem-cell–based immune system regeneration on refeeding. In addition, IF alters lipid profile and hematologic parameters in normal-weight young adults or overweight individuals [32–35].

Time-restricted feeding (TRF) (e.g., alternate-day fasting, fasting twice a week, or Ramadan fasting) is the most studied form of IF [29]. TRF entails the abstinence from all food during a number of hours and has been found to improve body mass and composition [36–39] and protect normal cells and organs from different toxins [28].

The effectiveness of different TRF forms is strongly debated. In fact, Ramadan fasting (2 meals/d separated by approximately 12 h) and ADF (25%–75% of energy intake) resulted in decreased daily energy intake and greater weight loss, which represents a potential risk for patients with infectious diseases [40] or cancer [41]. However, Adawi et al. [42] concluded that Ramadan fasting resulted in slight alterations to immune responses without negative effects in patients with HIV, cardiac disease, and asthma. Conversely, most studies investigating IF with maintaining normal distribution of the three meals and consumption of 100% of energy needs reported beneficial effects on overall health only in healthy young adults [28]. To the best of our knowledge, limited data are available on the effect of this diet strategy on immunologic parameters in older individuals (>50 y). Moreover, the stress caused by intense exercise (e.g., sprinting) stimulates cortisol and catecholamine production and temporarily weakens the immune system [43]. However, it is unclear whether IF regulates the immune system and body composition at the expense of physical performance. Data on the effect of TRF on muscle power in human are sparse [44]. Thus, the present study sought to investigate the effects of an adapted IF program, based on TRF, on immune parameters, body composition, and physical performance in healthy young and old men.

Methods

Participants reviewed and signed consent forms specifically approved by the Scientific Committee of Higher Institute of Sport and Physical Education. This committee approved the entire study design, which was conducted according to ethical standards of the 1964 Helsinki Declaration.

During the design of the study, statistical power analysis was carried out to calculate sample size requirements. This procedure indicated that nine participants for each of four groups were needed to achieve a statistical power of 80% to detect a small effect ($d = 0.23$) when assessed by three-factor mixed analysis of variance, with a level of significance of 5%. Therefore, 40 healthy men were recruited for participation in the present investigation.

To assess the physical condition of participants, an adapted version of the Baecke questionnaire [45] was used. Participants completed medical history and dietary questionnaires.

The inclusion criteria were as follows: a non-vegetarian or non-vegan diet; not having consumed any ergogenic supplements for the 6 months before the start of the study; not having consumed any medication that could affect the immune system; not involved in food restriction programs; no kidney, liver, and/or heart disease; absence of metabolic syndrome symptoms (e.g., hypertension, impaired fasting glucose); and not having undergone any radioactive procedure in the last year. In addition, participants should have avoided the practice of specific training such as strength or sprint training for 3 mo [46] but should have maintained their regular physical activity of 150 to 180 min/wk. Eligible participants were recruited to this study (Fig. 1).

After recruitment and familiarization, young and older participants were subsequently randomly assigned to an experimental group involving a fast 2 d/wk for 12 wk or a control group. Therefore, four groups existed: young men in the experimental group (YE; 26.90 ± 1.97 y, $n = 10$), young men in the control group (YC; 24.90 ± 1.10 y, $n = 10$), aged men in the experimental group (AE; 51.60 ± 5.87 y, $n = 10$), and aged men in the control group (AC; 53.90 ± 4.09 y; $n = 10$).

Time-restricted feeding 12/12 schedule

YE and AE were asked to fast 2 d separated by 48 h (Monday and Thursday) for 3 mo (February, March, April). The protocol consists of an IF regimen alternating between fasting days and normal feeding days in which participants fast during the daytime and eat at night for 2 d/wk and consume food and liquids ad libitum on the other days [47]. Seven to 8 h of sleep were required for all groups. Contrary to the protocol used by Moro et al. [28] (16/8), the feeding/fasting hours were divided equally, 12 h/12 h, in the present study.

Before the study, the dietitian asked all participants to provide the exact time of their three meals: breakfast, lunch, and dinner. Results suggested that times of breakfast, lunch, and dinner were ≈ 08:00 h, ≈ 13:00 h, and ≈ 20:00 h, respectively.

During the study, the experimental groups (YE and AE) consumed their energy needs in a 12-h period, with their caloric intake divided into three meals consumed as follows: 1) first meal was set between 05:30 h and 06:30 h (before sunrise); 2) second meal was set between 05:30 h and 06:30 h (during sunset); and 3) third meal was set at 20:30 h (night). The 12 h between the first meal and second meal made up the 12-h fasting period. In this period, YE and AE would neither eat nor drink until the end of 12 h. Between the third meal and the first meal, YE and AE could eat and drink ad libitum.

The aim of the TRF design is to fast during daytime (light), meaning between sunrise and sunset, which represents 12 h, and to eat or drink during nighttime. This design is set to disrupt the natural internal clock of cells and especially muscle cells [23], because their working time is set during the daytime. For