

Effect of Acute Maximal Exercise on Circulating Levels of Interleukin-12 during Ramadan Fasting

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Abstract

Purpose: The purpose of this study was to examine the effects of Ramadan fasting on circulating levels of interleukin-12 (IL-12) after a brief maximal exercise.

Methods: Nine subjects performed a Wingate test on three different occasions: (i) the first week of Ramadan (1WR), (ii) the fourth week of Ramadan (4WR), and (iii) three weeks after Ramadan (AR). Blood samples were taken before, immediately and 60 min after the exercise. Plasma concentrations of IL-12 were measured using enzyme-linked immunosorbent assay. Variance analysis revealed no significant effect of Ramadan on P_{peak} and P_{mean} during the three testing periods.

Results: Considering the effect of Ramadan on plasma concentrations of IL-12, analysis of the variance revealed a significant Ramadan effect ($F_{(2, 16)} = 66.27$; $P < 0.001$) as well as a significant time effect ($F_{(2, 16)} = 120.66$; $P < 0.001$). However, no significant (Ramadan \times time) of test interaction ($F_{(4, 32)} = 2.40$; $P > 0.05$). For all measures, IL-12 levels were lower during 1WR and 4WR in comparison with AR ($P < 0.05$). Considering the exercise effects, IL-12 levels measured immediately after the exercise were significantly higher than those measured before and at 60 minutes after the exercise ($P < 0.001$).

Conclusions: These results suggest that an acute intense exercise-induced IL-12 response is modified by daytime fasting and modifications in sleep schedule during Ramadan.

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INTRODUCTION

Interleukin-12 (IL-12) is an immuno-regulatory cytokine^[1] that can activate NK cells, generate lymphokine-activated killer cells (LAKs), and induce interferon- γ (IFN- γ) production and T-cell proliferation^[2]. Interleukin-12 is a heterodimeric pro-

inflammatory cytokine that favours the differentiation of T helper 1 (TH1) cells and forms a link between innate resistance and adaptive immunity. Dendritic cells (DCs) and phagocytes produce IL-12 in response to pathogens during infection^[3].

IL-12 has strong anti-tumour activity and has therapeutic use in cancer patients^[4]. Furthermore, IL-12

has also been shown to inhibit angiogenesis [5] which will be important during exercise.

During physical exercise, many components of the immune system can be affected according to the nature, frequency, and duration of the exercise [6]. Regular exercise has been reported to have several favourable effects on physiological, psychological, and immunological functions [7] and increase the resistance against infections. Vigorous exercise, however, has been reported to have a negative effect on these functions [8].

It has been generally agreed that circulating numbers of neutrophils and NK cells are markedly increased after strenuous exercise [9]. To the best of our knowledge, there was only one study concerning the changes in IL-12 levels in response to a high-intensity exercise challenge. During this last mentioned study, plasma concentrations of IL-12 increased after a modified Wingate test exercise [10]. Thus, the increase of the plasma concentrations of IL-12 during the Wingate test could indicate that the test can be used to modify immunological states [10].

Furthermore, the physical exercise-induced hyper susceptibility to infections can be amplified by sleep deprivation [11] and caloric restriction [12]. Ramadan fasting could be considered as an ideal hypo-caloric diet [13]. In addition, during Ramadan, the normal sleep-wakefulness cycle associated with the solar day is disrupted [14]. Consequently, the modifications mentioned before can be aggravated during this holy month. Thus, it would be interesting to study changes in circulating levels of cytokine after exercise during Ramadan fasting. In this study therefore, we examined the effect of Ramadan on circulating levels of IL-12 after a brief maximal exercise.

METHODS AND SUBJECTS

Subjects:

Nine male students majoring in physical education participated. They were given a thorough explanation of the protocol before signing an informed consent form. Ethical approval for the investigation was secured from the University Ethics committee. The

mean (\pm SD) age and height of the subjects were 22.1 ± 0.2 yrs, 176.6 ± 2.5 cm, respectively). The criteria for participant inclusion for this study were that each subject kept standard times for eating prior to the commencement of the study (breakfast at 07:00+1:00 h, lunch at 12:00+1:00 h, and dinner at 20:00+1:00 h) and sleeping habits (sleeping between 23:00 and 07:00+1:00 h). Subjects were non-smokers who did not consume caffeine or alcoholic beverages. They observed the Ramadan fast and abstained from food and liquids from approximately 01:30 to 19:35 for 30 days. At the time of the study, the Ramadan month was from September 12th to October 11th. The length of each fasting day was approximately 16 h.

Experimental Design:

During the week before the experiment, all subjects came to the laboratory several times and at different hours of the day to become fully familiarized with the procedure and tests involved so as to minimize learning effects during the experiment.

Then the subjects participated in three weeks of testing, with the experimental protocol comprising three parts: the first week of Ramadan (1WR), the fourth week of Ramadan (4WR), and three weeks after Ramadan (AR). The control trial has been done three weeks after Ramadan because previous studies have shown that anaerobic power [15] and inflammatory and immunological measures [16-17] return to the values observed before Ramadan. In each part, all subjects performed a Wingate test. The tests were performed at the same time of the day (10:00 AM) in order to eradicate the effects of the circadian rhythm of IL-12 and performances in the Wingate test. Before the test body weight was measured to the nearest 0.1 kg by using a Tanita digital scale (Tanita, Tokyo, Japan).

Instructions concerning sleep, diet, and physical activity were given to the subjects prior to experimentation. Before the month of Ramadan, participants were synchronized with a nocturnal rest from 23:00+1 h to 07:00. During the month of Ramadan, the participants had to go to sleep before 01:30 and to wake at 07:00 after a night of uninterrupted sleep. All participants kept the same hours of sleep during the three weeks of the experiment.

Compliance to these rules was assessed using the Bastuji and Jouvret calendar throughout the period [18]. The average sleeping time of the participants was $01:50 \pm 00:20$ h less during the four weeks of fasting than during after Ramadan. During Ramadan, the participants refrained from eating or drinking during the daytime. All meals were eaten at a standard time within the participant's usual schedules and Ramadan customs. There were also dietary restrictions prohibiting any food or drink that could enhance wakefulness, or agents such as alcohol. The participants were required to record their food intakes in a diary over a span of three days for each week of physical testing. The records were analyzed by a nutritionist using a computerized nutrition system, the NUTRISOFT- BILNUT (Vers. 2.01, Paris, France). Throughout the experimental period, participants were requested to maintain their habitual physical activity and avoid strenuous activity during the 24 h before the test sessions. It was easy to control compliance with these directions because all the participants were students who had exactly the same daily schedules in our institution.

Wingate test:

The Wingate test involved a 30 sec maximal sprint against constant resistance. For each participant, the load was determined according to body mass using Bar-Or's [19] optimization tables ($0.087 \text{ kg} \cdot \text{kg}^{-1}$ body mass). Participants were given vigorous verbal encouragement during every test. Seat height was adjusted to each participant's satisfaction and toe-clips were used to prevent the participant's feet from slipping off the pedals. Seat height was recorded and kept the same for each participant throughout the trials. Peak power (P_{peak}) was taken as the highest mechanical power elicited during the test. This index was taken as the highest average power during any 5-second period. Mean power (P_{mean}) was the average power sustained throughout the 30 sec period. The power decrease (Wd) was the difference between the highest and lowest power divided by the highest.

Blood samples:

Blood samples were taken from the vein in the antecubital fossa before, immediately after and 60 min

after the exercise. Hematocrit and hemoglobin concentrations were obtained using an automated blood cell counter (Symex Kx-21N, Japan) as recommended by Thompson and Dixon [20]. Blood samples were centrifuged at $3000 \text{ rpm} \cdot \text{min}^{-1}$ (20 min at 4°C), and plasma samples were separated and stored at -80°C until the measurement of IL-12. Prior to statistical analyses, all data except haemoglobin were corrected for changes in plasma volume using the method of Costill and Fink [21].

Measurement of IL-12:

Plasma concentrations of IL-12 were measured using an ELISA (*Enzyme Linked Immuno-Assay*) Kit; Human IL-12 Total ELISA, AbC 261/2, AbCys SA, Paris, France. The intra-assay coefficient of variation was 1.13-7.02%.

Statistical analysis:

All statistical tests were processed using *Statistica* Software (StatSoft, Paris, France). The data were analyzed using analysis of variance (ANOVA). The anaerobic performance parameters were determined using an ANOVA with one factor (Ramadan). The plasmatic parameters were determined using a two-way ANOVA (Ramadan \times time). When appropriate, significant differences among means were tested using the least significant difference Post hoc test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Body Mass and Energy Intake:

The ANOVA showed significant effects for Ramadan on body mass ($P < 0.001$). The body mass was significantly lower in the fourth week of Ramadan in comparison to the other periods of tests. Comparison of mean energy and macro-nutrient intake by the participants during the day in the three different weeks showed significant statistical differences (Table 1).

Wingate test:

Analysis of variance revealed no significant effect of

Table 1: Mean (SD) Values for Daily Nutrient Consumption in the first week of Ramadan (1WR), in the fourth week of Ramadan (4WR) and after Ramadan (AR)

| Parameters | 1WR | 4WR | AR |
|--------------------|---------------|---------------|--------------|
| Body Mass (Kg) | 74.0 (4.2)* | 71.5 (4.4)* | 75.3 (4.4) |
| Energy (Kcal/Kg/d) | 32,9 (3,4)* | 31,8 (3,7)* | 46,3 (4,2) |
| Protein (g/d) | 74.7 (9.08)* | 76.4 (9.2)* | 109.2 (17.2) |
| Protein (%) | 12.2 (0.6) | 13.4 (0.8) | 12.5 (1.1) |
| Fat (g/d) | 79.5 (11.2)* | 71.5 (7.3)* | 117.6 (13.8) |
| Fat (%) | 29.3 (2.0) | 28.2 (1.0) | 30.3 (1.7) |
| Carbohydrate (g/d) | 355.7 (33.7)* | 319.4 (39.8)* | 499.7 (31.5) |
| Carbohydrate (%) | 58.41 (2.5) | 56.1 (2.8) | 57.2 (1.8) |
| Vitamin E (mg/d) | 11.0 (0.2)* | 8.9 (0.1)* | 14.3 (0.1) |
| Vitamin C (mg/d) | 41.4 (1.1)* | 36.2 (0.9)* | 51.7 (1.1) |

*Significant difference in comparison with after Ramadan / SD: Standard Deviation

Ramadan on the P_{peak} and P_{mean} during the three testing periods ($F_{(2,18)}=1,06$; $P>0.05$) (Table2).

Plasma concentration of interleukin-12

The analysis of variance revealed a significant Ramadan effect ($F_{(2, 16)} = 66.27$; $P<0.001$) There was also a time effect ($F_{(2,16)}=120.66$; $P<0.001$). However, no significant (time × Ramadan) of test interaction ($F_{(4,32)}=2.40$; $P>0.05$) (Fig. 1).

Concerning the Ramadan effect, plasma concentration of IL-12 measured at before the exercise in 4WR was lower than during 1WR ($P<0.001$) and AR ($P<0.001$). Moreover, plasma concentration of IL-12 measured at rest was significantly lower during 1WR and AR ($P=0.003$). Immediately after the exercise (T_1), IL-12 levels were lower during 1WR ($P=0.01$) and 4WR ($P<0.001$) in comparison with AR. Moreover, the level of IL-12 immediately after the exercise was significantly decreased in the first week of Ramadan compared to the fourth week of Ramadan ($P<0.001$). Similarly, plasma concentration of IL-12 measured 60 minutes after the exercise (T_2) decreased during 1WR

($P<0.001$) and 4WR ($P<0.001$) in comparison with AR.

Concerning the exercise effect during 1WR, 4WR and AR, plasma concentration of IL-12 measured immediately after the exercise was significantly more than those stored at T_0 ($P<0.001$) and these stored at 60 minutes after the exercise ($P<0.001$). However, no significant difference was observed in plasma concentration of IL-12 at (T_0) in comparison with 60 minutes after exercise (T_2) ($P>0.05$).

DISCUSSION

This study was designed to determine an acute intense exercise-induced changes in plasma concentrations of interleukine-12 during Ramadan fasting. The results of this study show that the concentrations of IL-12 increase significantly after the exercise during and after Ramadan.

Table 2: Mean (ES) values for P_{peak} and P_{mean} (n=12) in the first week of Ramadan (1WR), in the fourth week of Ramadan (4WR) and after Ramadan (AR)

| Parameter | 1WR | 4WR | AR |
|-------------------|------------|------------|------------|
| P_{peak} (W/kg) | 9.80 (0.8) | 9.7 (0.3) | 10.2 (0.6) |
| P_{mean} (W/kg) | 7.7 (0.2) | 7.8 (0.2) | 8.1 (0.5) |
| Fatigue index (%) | 42.1 (3.5) | 43.7 (4.2) | 41.4 (4.8) |

ER: Error Standard

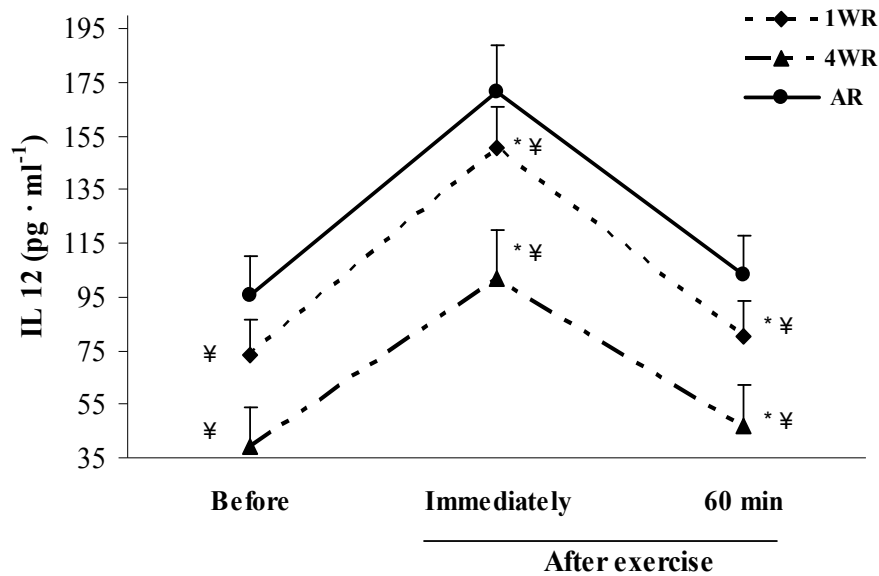


Fig. 1: Effect of brief maximal exercise on plasma concentrations of IL-12. The plasma concentration of IL-12 was significantly increased immediately after a Wingate test during the period of Ramadan and after Ramadan.

* Significant difference with before exercise; ¥ significant difference with after Ramadan

1WR: The first week of Ramadan / 4WR: The fourth week of Ramadan: AR: After Ramadan

The present study showed that performances during the Wingate test were unaffected by Ramadan. These results are in line with those of the Souissi et al [14] study which showed that the negative effects of Ramadan on anaerobic performance are only observed in the afternoon and evening (17:00 and 21:00 h) and not in the morning.

The results of the present study show that plasma concentrations of IL-12 increased after a maximal anaerobic exercise. These results are in line with those of Akimoto et al [10] during a modified Wingate test. The increase of IL-12 concentrations could be explained by the augmentation of the number of monocytes circulating in response to the maximal exercise. In fact, Ostrowski et al [22] showed an increase in the number of monocytes during strenuous, anaerobic exercise.

The increase of the plasma concentrations of IL-12 during the Wingate test observed in the present study could indicate that this test can be used to modify immunological states [10]. The pro-inflammatory functions of interleukin-12, as well as its ability to stimulate innate resistance and to generate a Th1- type

immune response, are essential for resistance to different types of infection. IL-12 is a potent inducer of TH1 responses [23] and it is required for optimal TH1-cell development during the immune response to pathogens [24]. This role of IL-12 has been determined by studying the induction of TH1 responses by recombinant IL-12 in vivo. It is thought that IL-12 directs the generation of the Th1 response via the induction of IFN- γ production, and enhances the cytotoxicity of CD8+ T cells and NK cells [3]. In fact, interferon- γ that is induced by IL-12 has a direct toxic effect on the tumour cells and/or might activate potent anti-angiogenic mechanisms [5].

An important activity of IL-12, acting with IFN- γ and IL-2, is to drive T helper (Th) cell responses toward the Th1 rather than Th2 phenotype [25].

The functions of IL-12 have been studied mainly in terms of lymphocytes, although IL-12 affects other types of cells also. IL-12-induced IFN- γ mediates many of the pro-inflammatory activities of IL-12, whereas the ability of IL-12 to favour a TH1 response exemplifies its function as an immunoregulatory cytokine that bridges innate resistance and adaptive

immunity. Not only is IL-12 a potent inducer of IFN- γ production, but it is also required for optimal IFN- γ production *in vivo* during immune responses, particularly during bacterial or parasitic infections [26,27].

The natural killer (NK) cells show the most pronounced increase after physical exercise [28]. In early studies, it was reported that NK cell activity increased following physical exercise. It is generally agreed that NK cell counts in circulation are increased immediately after Wingate test-type maximal exercise [29].

The decrease of the IL-12 response at the first and the fourth week of Ramadan, before and after the Wingate test, could be, in part, explained by the changes in diet intake during Ramadan fasting such as deficits in vitamin [30]. Indeed, the present study shows significant reduction of vitamins C and E during Ramadan in comparison with the control period. Gleeson et al [31] showed that insufficiencies in vitamins lead to a decrease in the resistance to infections. Similarly, Germano [32] showed that vitamin D deficiency induced a decrease in pro-inflammatory cytokines in particular IL-12.

Partial sleep deprivation can be expected to occur in Ramadan. Recently, Chennaoui et al [16] showed that the Ramadan fasting period is associated with change in sleep habits and increased sleepiness, which may induce inflammatory disturbances. Thus, in this study, repeated partial sleep deprivations can be to the origin of the decrease in plasma concentrations of IL-12 during Ramadan. These results are in line with those of the Dimitrov et al [33] study that showed that sleep deprivation was associated with a decrease in the number of DC producing IL-12, which are a main inducer of Th1 responses. Dinges et al [34] observed that a total deprivation of sleep showed an

increase of the non-specific response immunity (the number of phagocytes, the number of NK cells and the activity of the NK cells). Furthermore, Lange et al [35] showed that sleep increases the production of interleukine-12 (IL-12) by the monocytes and inhibited that of interleukine-10 (IL-10), inducing a rhythmicity in the production of these two cytokines.

As we said before, IFN gamma is a potent indicator of a Th1 response which reflects the *in vivo* function of the altered production of IL-12 by Ramadan fasting. In our study, we would rather measure the plasma level of INF- γ but it is the limitation of the results in our study.

CONCLUSION

In conclusion, our result shows that maximal exercise-induced changes in plasma concentrations of IL-12 during Ramadan are lower than that of after Ramadan. Future studies are needed to explain how Ramadan can modify the IL-12 level after exercise.

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Conflict of interests: None

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